The development of seed treatment products based on the new fungicide ipconazole

M J Tomkins, S J Maude, T Archer, K M Littlewood and D Jackson

Chemtura Europe Ltd, Evesham, UK

Email: Malcolm.Tomkins@chemtura.com

Summary

Ipconazole is a new broad-spectrum fungicide belonging to the triazole group of SBI fungicides. It has activity against pathogenic fungi in the major groups of Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes, and is active as a seed treatment against major seed-borne and early soil-borne pathogens on a range of crops. Ipconazole 15 ME has been developed on small grain cereals in Europe, and results presented illustrate its high activity against loose smut (*Ustilago nuda*) in barley, common bunt (*Tilletia caries*) in wheat, leaf stripe (*Pyrenophora graminea*) in barley, and seedling blights (*Fusarium* spp./*Microdochium nivale*) in wheat. Ipconazole 15 ME also demonstrates a very high level of crop selectivity, and does not adversely affect seed germination or crop emergence.

Introduction

Seed treatment continues to increase in importance as a first step in sustainable crop protection in global agriculture. Whilst this market is, in many ways, driven by the use of insecticides, there is also a need for the development of new and effective fungicides to partner the seed-treatment insecticides on a wide range of crops. It is against this background that the fungicide ipconazole was discovered and developed. Ipconazole was first patented by Kureha Chemical Corporation, and the seed treatment uses have since been licensed for global development to Chemtura Corporation. It is one of the more recent additions to the triazole group of fungicides, with an SBI demethylation (DMI) mode of action at the cytochrome P450 site. Ipconazole controls target pathogens by both protectant and curative activity as it is both a contact and systemic fungicide. It has a broad spectrum of activity relative to some earlier triazoles and controls fungal pathogens in all classes except Oomycetes. Ipconazole is very selective, being safe to seed of both monocot and dicot crops. The selectivity and efficacy profiles of ipconazole fit it for use as a seed treatment on a wide range of crops; it is already registered in Japan, Latin America and USA, and has recently received provisional or full approval in several European countries, with the UK being the RMS for the EU.

This paper describes the development of the 15 ME (microemulsion) formulation of ipconazole on wheat and barley in Europe and illustrates its activity against the major seed-borne pathogens of wheat and barley.

Materials and methods

Ipconazole (1RS,2SR,5RS;1RS,2SR,5SR)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1,2,4-triazol-=1-ylmethyl) cyclopentanol (IUPAC) was discovered and developed as a rice and wheat seed treatment in Japan by Kureha Chemical Corporation (Tateishi *et al.*, 1998). Chemtura Corporation has since undertaken extensive formulation evaluation work in USA and Europe, culminating in the development of a range of stable commercial products, one of which is ipconazole 15 g/l ME (microemulsion). This product is being targeted at the cereal seed treatment market in Europe, and its formulation development and physical-chemical properties are described elsewhere in these Proceedings (see Poster: The development of an ipconazole microemulsion formulation for seed treatment. R M Clapperton, K M Littlewood). The ME technology gives a very low viscosity product which can be easily and accurately delivered to seed through existing commercial treatment equipment. Ipconazole 15 ME has a favourable toxicology profile, and is not classified.

The ipconazole ME product was applied to seed using a laboratory-scale batch treater such as the Rotogard R300, mostly pre-diluted with water. In most of the trials described, the rate of use was the label rate: 100 ml/100 kg on wheat (delivering 1.5 g a.s.) and 133 ml/100 kg on barley (delivering 2 g a.s.). Commercial seed treatment formulations of standard fungicides were applied in the same equipment for use as references in the trials.

Efficacy evaluations were done in small-plot field trials, mostly with a plot size of 1.4–2 × 6–12 m and four replications, using seed infected with the relevant pathogen. All carried natural infections except for common bunt, where spores of *Tilletia caries* were mixed with the wheat seed (2 g/kg seed) prior to chemical treatment. Control of soil-borne common bunt was assessed in trials where the plots were inoculated with a spore/sand mix prior to sowing the wheat seed. Efficacy against *Fusarium* spp. and *M. nivale* was assessed soon after emergence (crop stage BBCH 12–13) by counting numbers of emerged plants per m^2 to give a measure of seedling blight damage. Leaf stripe symptoms were assessed on barley at BBCH 51–59 by counting infected tillers per plot. Loose smut symptoms were assessed in barley by counting infected ears at BBCH 60–69. Bunt symptoms were assessed by sampling mature ears of wheat (BBCH 73–92) and counting the number of healthy and infected ears to calculate the percent infection.

Selectivity and seed safety was evaluated in field trials and in laboratory tests using healthy seed. Speed of emergence was assessed visually at BBCH 10, and then final plant emergence was assessed by counting seedlings in pairs of 0.5 or 1.0 m row lengths at five locations per plot at BBCH 12–13. Laboratory tests were conducted according to ISTA Rules in rolled paper towels, with germination being assessed after 4 and 7 days' incubation at 20°C with an 8 h photoperiod. This period was preceded by a pre-chill incubation at 5°C to break dormancy in winter cereals.

Results

Control of bunt of wheat

a) Seed-borne bunt
Trials were conducted over several seasons in Europe against soil-borne bunt, and data from six trials in the UK are shown in Table 1. Ipconazole 15 ME at the UK label rate gave 99.9–100% control and was comparable with the prothioconazole standards, and this robust level of control has been repeated across the EU.

Table 1 Untreated infection levels of seed-borne bunt and control (%) by seed treatment

Treatment	Rate g a.s. per 100 kg	E06/13-3	EC06-SAC	XAC 1475	E06/33-1	E06/33-2	E06/33-3
Untreated infection (%)	–	17.6	12.4	16.8	3.3	10.1	16.7
Ipconazole	1.5	99.9	100	100	100	100	99.6
Prothioconazole	10	99.0	100	100	–	–	–
Prothioconazole + fluoxastrobin	5.625/5.625	–	–	–	100	99.4	100

Table 2 Untreated infection levels of soil-borne bunt and control (%) by seed treatment

Treatment	Rate g a.s. per 100 kg	UK 06/1	UK 06/2	France 05/1	France 05/2	France 05/3
Untreated infection (%)	–	26.6	15.2	61.0	49.7	13.3
Ipconazole	2	99.4	99.9	99.7	99.8	100
Prothioconazole	10	99.2	99.5	–	–	–
Product A	5/5/50	–	–	100	100	–
Product B	3/2/70	–	–	–	–	88.6

Product A = Fludioxonil + difenoconazole + anthraquinone
Product B = Tebuconazole + triazoxide + imidacloprid

b) Soil-borne bunt

Infection from soil-borne spores of common bunt can be relatively important in dry autumns in France and the eastern part of the UK, and a summary of five trials carried out with ipconazole in these countries in 2005 and 2006 is given in Table 2. Infection was very successful, with symptom expression ranging from 13.3 to 61%. Ipconazole at 2 g a.s. per 100 kg seed gave excellent control of this disease: control ranged from 99.4 to 100%, and was equivalent to prothioconazole and fludioxonil/difenoconazole standards and more effective in one trial than tebuconazole/triazoxide/imidacloprid.

Control of seedling blight of wheat

The effect of *Fusarium* spp. and *M. nivale* on wheat plants and suppression of attack by seed treatments is a complex subject. The trials reported here are limited to the effects of seed-borne inocula on seedling emergence, and to the improvement in that emergence by the use of seed treatments.

Table 3 Field plot emergence counts (plants per m row) for Fusarium-infected (*M. nivale* and *Fusarium* spp.) winter wheat

Treatment	**Rate g a.s. per 100 kg**	**E06/ 05 -3R**	**E07/ 15 -2H**	**E07/ 25 -2R**	**E08/ 27 -2**	**E08/ 28 -2**
Percentage seed infection:						
M. nivale	–	39	25	21.5	31	76
Fusarium spp.	–	0	65.1	70.5	24	0
Untreated	–	8.8	7.6	6.8	9.5	2.6
Ipconazole	1.5	14.9	11.1	9.5	13.8	8.1
Carboxin/thiram	60/60	19.6	–	–	15.6	10.8
Prothioconazole + fluoxastrobin	5.63/5.63	–	12.9	11.3	–	–
LSD ($P = 0.05$)	–	–	1.38	1.48	1.88	1.88

Trials were conducted in the UK in 2006, 2007 and 2008 with a range of seed stocks infected with either pure *M. nivale* or a mixed infection of several species of *Fusarium* plus *M. nivale* as shown in Table 3. Ipconazole at 1.5 g gave good improvements in numbers of emerged plants, but its effect was less uniform than that of the best standard carboxin/thiram. There is some evidence that the activity of ipconazole is stronger against seed-borne *Fusarium* spp. than against *M. nivale*, and this is borne out by the use of ipconazole on maize where its activity against *F. moniliforme* is very good.

Control of loose smut of barley

Many trials have been carried out to prove the efficacy of ipconazole against loose smut, and data from five trials from the UK and France in 2005 and 2006 are summarised in Table 4.

Ipconazole at 2 g a.s. per 100 kg seed gave a very high and uniform level of control of this important disease, which requires systemic activity to limit the growth of mycelium from the inoculum carried inside the embryo of the seed. Ipconazole was equal to the fludioxonil/tebuconazole/cyproconazole standard and superior to prothioconazole and carboxin/thiram, and meets the level of performance needed for it to be used for retrieval in multiplication seed in the UK.

Control of leaf stripe on barley

Trials with ipconazole across the EU have shown that it does have activity against this important seed-borne pathogen, but the level of this activity is moderate compared with modern standards. This will be sufficient to obtain a partial control claim on EU labels and this will support the use on barley. Further development of a mixture of ipconazole + imazalil has therefore continued in order to provide a new seed treatment product which will give full control of leaf stripe as well as loose smut. Imazalil is a well known seed treatment fungicide

Table 4 Ear infection by loose smut in winter barley and its control (%) by seed treatment

Treatment	Rate g a.s. per 100 kg	E05/ 18 -3 UK	XAC 1475 UK	AP/10193/ CT 2 UK	AF/8396/ CT2 France	D27 ITS BS France
Untreated infection	–	2.4%	20.8/m^2	2.6%	2.9%	8.5%
Ipconazole	2	100	99.8	98.6	100	100
Tebuconazole	3	100	–	–	–	–
Carboxin/thiram	60/60	–	93.1	91.6	–	–
Prothioconazole	10	–	97.7	95.8	–	–
Fludioxonil + tebuconazole + cyproconazole + anthraquinone	2.5/ 3/ 5/ 50	–	–	–	100	100

Table 5 Percentage of normal germination of winter wheat and barley at the final assessment in paper towel tests before and after storage of seed, mean of 12 tests

Treatment	Storage period (months)					
	Wheat			Barley		
	0	6	12	0	6	12
Untreated	95.8	90.4	89.8	91.2	85.2	84.0
Ipconazole label rate	96.0	91.4	93.8	91.2	84.8	86.8
Ipconazole 2N label	95.2	91.8	94.2	90.6	83.8	87.6

for leaf stripe control, and trials in recent years with this mixture have shown that a rate of 2/5 g a.s. per 100 kg will give sufficiently high and uniform levels of control.

Seed safety and crop selectivity

These parameters are vital when considering the development of any new seed treatment, and are particularly important for a triazole fungicide, as this class of chemistry can also have plant growth regulation effects on emerging seedlings, particularly under adverse field conditions.

Ipconazole 15 ME has shown excellent crop safety on a range of cultivars of winter wheat and winter barley, and evaluation of this new fungicide at the label rate and twice the label rate in many field trials with healthy as well as infected seed has not indicated any reduction in speed of emergence nor final stand. Those field trials have included late drilling in difficult seed beds, and it seems evident that ipconazole has good crop safety under a wide range of conditions.

The excellent selectivity of ipconazole has been confirmed in laboratory seed safety tests, and typical data from rolled paper towel tests are presented in Table 5. This shows that the germination of seed treated at 2N rates and stored for up to 12 months was not adversely affected by ipconazole: the germination of untreated seed had decreased slightly over this period, as is usual, but the germination of seed treated with ipconazole is often higher than that of untreated seed.

Discussion

The broad-spectrum, systemic fungicidal activity of ipconazole, linked to its excellent seed safety, evident in early-stage evaluations, have proved to be key benefits of the products developed in Europe for cereal seed treatment.

Ipconazole 15 ME is the first in a range of products being developed by Chemtura based on ipconazole, and is being registered and introduced across Europe as Rancona™. Dose–response trials defined the use rate on wheat to be 1.5 g a.s. per 100 kg seed, and the data presented in this paper demonstrate the full control of seed-borne common bunt given by ipconazole 15 ME at this rate. This rate, equivalent to 100 ml of formulated product per 100 kg seed, has also been shown to improve crop establishment of winter wheat by giving protection against seedling blight caused by seed-borne *Fusarium* spp. and *M. nivale*. The same product but at the slightly higher rate of 2 g a.s. (133 ml of formulated product) also gives full control of soil-borne common bunt, even at high infection levels.

The use rate of ipconazole at 2 g a.s. on winter barley has given complete, or almost complete, control of loose smut, and this activity is linked with partial control of leaf stripe for the ipconazole 15 ME product.

The ipconazole 15 ME product has been shown to be very safe to wheat and barley seed even at high rates and after storage of treated seed, and it is very selective on crops in the field. Ipconazole 15 ME will therefore be a valuable addition to the range of seed treatment fungicides for small grain cereals in Europe.

This will be followed by the introduction of an ipconazole/imazalil ME product specifically for barley seed treatment and giving full control of both loose smut and leaf stripe.

Other solo ipconazole products are registered in USA, Canada and Latin America. Mixtures with co-fungicides, including metalaxyl, which expand the spectrum of ipconazole to suit crops such as maize, peanuts and soybeans, are now registered in the USA and Argentina.

Acknowledgements

The authors wish to thank colleagues in Chemtura Europe Ltd and CRO companies for their help in providing the formulated products and generating the trials data presented in this paper.

References

Tateishi H; Saishoji T; Suzuki T; Chida T (1998) Antifungal properties of the seed disinfectant ipconazole and its protection against 'Bakanae' and other diseases of rice. *Annals of the Phytopathological Society of Japan* **64**, 443–450.

Spinosad: an effective, organic seed treatment insecticide for certain vegetable crops

K W Dorschner[1], A G Taylor[2], B A Nault[3] and D B Walsh[4]

[1]IR-4 Project Headquarters, Rutgers University, 500 College Road East, Princeton, New Jersey, 08540, USA; [2]Department of Horticultural Sciences, Cornell University, NYSAS, 630 West North Street, Geneva, New York, 14456, USA; [3]Department of Entomology, Cornell University, NYSAS, 630 West North Street, Geneva, New York, 14456, USA; [4]Department of Entomology, Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, Washington, 99350, USA

dorschner@aesop.rutgers.edu

The IR-4 Project (Interregional Research Project No. 4) is a publicly funded program in the United States that assists growers of specialty crops to gain registrations for pest control products. Assistance from IR-4 is essential and necessary when the economic incentive for the registrant companies precludes the companies from obtaining the registrations themselves. This is often the case for small acreage specialty crops in the United States. The costs associated with GLP data generation and the fees required to submit a tolerance petition to the US Environmental Protection Agency are simply too high to justify the investment when the expected returns from the registration are considered. Without the assistance of the IR-4 Project, many specialty crop growers would be unable to use the newest, safest pesticides on the market. IR-4 helps growers produce an abundant, affordable and safe crop for domestic consumption and export markets.

Spinosad insecticide (formulated as Entrust® from Dow AgroSciences) is a well known and effective organic insecticide that has been registered for several years in the United States for the control of many important foliar pests. It is extremely safe and approved for use on all food commodities.

The potential for spinosad as a seed treatment in the USA first became apparent when it was tested against onion maggot (*Delia antique*) in 2001 by Cornell researchers Alan Taylor and Brian Nault. Soil drenches of chlorpyrifos, the standard control material, were far less effective in preventing seedling loss than the spinosad seed treatment. Spinosad seed treatment was also numerically superior to seed treatment with cyromazine.

Nault and Taylor repeated their onion work in 2002 and 2003 and continued to observe encouraging results. Unfortunately, Dow AgroSciences was not convinced there was commercial potential. The registrant also had very little experience with seed treatments and this exacerbated the situation. Realising the potential of this technology for onion growers, the researchers came to IR-4 for registration assistance in August 2003.

A dialog between the researchers, IR-4 and Dow AgroSciences was established, the goal being to encourage the registrant to pursue registration of spinosad as a treatment on onion and perhaps other crops.

Dow AgroSciences did agree to support the registration of spinosad against onion maggot in 2006 via a research effort coordinated by IR-4. IR-4 quickly pushed for registration on nine crop groups with maggot pests, not just onion. However, the ambitious proposal lacked sound efficacy data to back it up. A national efficacy program, coordinated by IR-4, was set up in 2007 and 2008 to address this deficiency as well as collect additional confirmatory data for onion maggot, the original and still main target pest.

Efficacy data were generated during 2007 on the following crops: onion, green onion, cabbage, kale, corn, peas, beans, carrot and melon. In general, spinosad was confirmed to be an effective seed treatment against onion maggot and also seedcorn maggot (*Delia platura*). Commercial levels of control were not seen for cabbage maggot (*Delia radicum*) or carrot rust fly (*Psila rosae*). We were surprised to see some early control of onion-infesting thrips with the spinosad seed treatment, but the level of control was not considered commercially significant and it was not observed in all trials.

The data collected in 2008 focused on refining seed treatment rates and finalising the pest control spectrum. Trials were established for cabbage, turnip, parsnip, onion, bunching onion, shallots, dry beans, snap beans, field corn, sweet corn, cucumber, peas and pumpkin. These trials once again demonstrated commercial control of seedcorn maggot and onion maggot. Carrot rust fly and cabbage maggot were eliminated as targets, essentially confirming the 2007 results. Cabbage maggot was not controlled even at rates 7.5× higher than rates effective for onion maggot and seedcorn maggot control.

During the summer of 2008, IR-4 requested the US Environmental Protection Agency (EPA) to outline the process for seed treatment registration on the target crops (eventually narrowed down to carrot, bulb vegetables, cucurbit vegetables, legume vegetables, sweet corn and field corn). Much to our surprise and consternation, EPA was concerned about the potential for violative spinosad residues in the crops at harvest.

Not to be thwarted at this point, IR-4 coordinated with the researchers to provide crop samples from their 2008 efficacy plots for spinosad residue analysis. Crop samples of cucumber, onion, sweet corn, field corn, carrot (tops and roots), dry bean and snap bean were collected at commercial maturity and shipped frozen to Dow AgroSciences for spinosad analysis.

Currently the analysis is not complete; however, preliminary numbers appear to be encouraging. Most crops are free of spinosad residues, and in the cases where residues were detected, they were very low, even so low as to be unquantifiable. Once the analysis is completed and a report written, IR-4 will present the data to EPA for their consideration. It is hoped that some uses may be approved in time for the 2009 use season. All target crops should be registered in time for 2010 planting.

This is a fine example of how researchers can identify innovative control solutions for growers and then stay involved in the process until the technology is made commercially available. Great research can only have an impact when it is put into practice.

Neonicotinoid seed treatments for early-season management of cucumber beetles in cucurbits

T Kuhar[1], H Doughty[1], G Brust[2], J Whalen[3], C Welty[4], B Nault[5] and A Taylor[6]

[1]Virginia Tech University, 33446 Research Drive, Painter, VA 23420, USA; [2]Central Maryland Research and Education Center – UMF, University of Maryland, 2005 Largo Road, Upper Marlboro, MD 20774, USA; [3]University of Delaware, Newark, DE 19717-1303, USA; [4]Ohio State University, 2501 Carmack Road, Columbus, OH 43210-1065; [5]NYSAES, Cornell University, 525 Barton Laboratory, Geneva, NY 14456, USA; [6]NYSAES, Cornell University, 103 Hedrick Hall, Geneva, NY 14456, USA

E-mail: tkuhar@vt.edu

Introduction

In North America, the striped cucumber beetle, *Acalymma vittatum* (Fabr.), and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber, are serious pests of cucurbit crops, especially cucumbers, muskmelons and squash. Adult beetles emerge in the spring and aggregate on cucurbit plants. Female beetles oviposit in the soil at the base of plants where larvae subsequently feed on roots, occasionally reducing plant vigour and yield. However, it is the adults that cause the most severe damage by feeding on foliage and by transmitting the bacterial wilt pathogen, *Erwinia tracheiphila*, which they harbour within their bodies. Young plants are most susceptible to beetle feeding injury and bacterial transmission. Most plant mortality occurs at the cotyledon and first true leaf stage. Cucurbit plants typically can overcome the feeding injury inflicted by cucumber beetles past the second and third true leaf stage.

For decades, growers have used foliar applications of insecticides (often pyrethroids) to control cucumber beetles. Multiple sprays were often needed to achieve effective control. Since the late 1990s, the systemic neonicotinoid insecticides, imidacloprid and thiamethoxam, have provided growers with an alternative to foliar insecticide sprays. Transplant drenches, at-planting soil drenches, and drip irrigation injections of imidacloprid or thiamethoxam have been quite efficacious against cucumber beetles, offering plant protection for up to 56 days after planting (McLeod, 2006). A recently considered alternative to using soil-applied neonicotinoids on cucurbit crops has been to treat the seeds directly, an approach that has achieved much success in other crops such as corn and beans. The purpose of this study was to examine the efficacy of neonicotinoid seed treatments for the control of cucumber beetles in cucumber (*Cucumis sativus*) and pumpkin (*Cucurbita maxima*).

Materials and methods

Field experiments were conducted in summer 2008 at six locations in the eastern United States (Table 1). Seeds of 'Vlaspik' cucumber and 'Gladiator' pumpkin were treated in the laboratory of Alan Taylor (Cornell University NYSAES, Geneva, NY*)*. A film coating method was used with Disco A and water (1:1) binder. All treatments including the control received the fungicide Thiram Technical grade (98.5% tetramethylthiuram disulfide) at 2.57 g a.i./kg.

Table 1 Locations and planting information where cucurbit seed treatment experiments were conducted in 2008

Location	Crop	Plot size and no. of reps	PD
Freemont, Ohio	Cucumber	1 row × 7.6 m, 4 reps	6 Jun
Columbus, Ohio	Cucumber	4 rows × 6.1 m, 4 reps	24 Jun
Painter, Virginia	Cucumber	2 rows × 6.1 m, 6 reps	27 May
Georgetown, Delaware	Cucumber	4 rows × 6.1 m, 4 reps	9 Jun
Geneva, New York	Pumpkin	2 rows × 6.1 m, 6 reps	28 May
Upper Marlboro, Maryland	Pumpkin	5 rows × 15.2 m, 5 reps	5 Jun

All experiments included the insecticide seed treatments: thiamethoxam (Cruiser™, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.75 mg a.i./seed, and chlothianidin + imidacloprid (Sepresto™, Bayer CropScience LP, Research Triangle Park, NC) at 1 mg a.i./seed. At most locations, soil insecticide treatments were also applied for comparison and included: thiamethoxam (Platinum™ 2FS, Syngenta Crop Protection) at 584.36 ml of product/ha, and imidacloprid (Admire Pro™ 4.6F, Bayer CropScience LP) at 511.3 ml of product/ha. Soil insecticides were applied at-planting in-furrow with a backpack sprayer that delivered 400 to 500 l/ha.

Potential phytotoxicity of the seed treatments was evaluated by assessing seed germination or cotyledon emergence in the field. Seed germination was evaluated in the laboratory by wrapping at least 10 seeds per treatment in a moistened paper towel. All field experiments were arranged in a randomised complete block design. Plot sizes and number of replications varied by location (Table 1). At various plant growth stages from cotyledon to 4-leaf stage, attempts were made at least weekly to count numbers of live and dead cucumber beetles or other insect pests per 10 plants per plot and evaluate beetle feeding injury (defoliation and percentage of killed seedlings) in the field. Cucumber beetle feeding injury was rated on a 0–3 scale (see Table 3 footnote). In addition, cucumber beetle toxicity assays using excised leaves

Table 2 Results of pumpkin neonicotinoid seed treatment field efficacy trial, Upper Marlboro, Maryland, 2008

	Mean no. live beetles/plant			% dead plants
Treatment	**13 Jun (7 DAP)**	**18 Jun (12 DAP)**	**23 Jun (17 DAP)**	**27 Jun (24 DAP)**
Control	10.7 a	12.3 a	2.2 a	15.2 a
Thiamethoxam	1.5 b	0.5 b	0.2 a	1.3 b
Imidacloprid + clothianidin	2.1 b	0.8 b	0.2 a	0.0 b

Means in a column followed by the same letter are not significantly different ($P \leq 0.05$, orthogonal contrasts).

from the field plots were conducted at two to four different plant growth stages depending on location. Assays consisted of field-collected cotyledons or leaves placed on moistened floral foam along with five to 10 cucumber beetles (striped or spotted) per container. Beetles were field-collected from untreated cucurbits spatially separated from the experimental plots. Leaf bioassays were replicated at least four times per treatment. Beetle mortality was recorded at 72, 96 or 120 h. All data were analysed using analysis of variance procedures. Mean separation tests included Fisher's protected LSD, Tukey's HSD, or orthogonal contrasts depending on location (each cooperator analysed his/her own data).

Results

Seed germination and stands

Germination tests at two of the locations revealed >98% germination of all treatments. Stand counts were highly variable across locations, and generally did not reveal differences from the insecticide seed treatments except at the Delaware location, where the imidacloprid + clothianidin seed treatment and the thiamethoxam in-furrow spray treatment had a significantly reduced stand in comparison with the control plots or other treatments. Also, at the Maryland location, the thiamethoxam seed treatment had a significantly reduced stand (40%) compared with the other treatments. However, replanted seeds of this treatment had nearly perfect emergence.

Cucumber beetle control in the field

Natural cucumber beetle densities were low across the region in 2008, and at only one of the six locations (Upper Marlboro, Maryland) were beetle counts sufficiently high to obtain useful data. At this location, striped cucumber beetles started to feed at the cotyledon to 1-leaf stage about 8 days after planting. All treatments were effective in killing beetles and reducing

Table 3 Results of cucumber neonicotinoid seed treatment field efficacy trial, Freemont, Ohio, 2008

	4-leaf stage (25 DAP)	
Treatment[1]	**Mean no. live beetles/plant**	**Leaf feeding injury (0–3 rating scale[2])**
Control	0.2	0.6 ab
Imidacloprid (IF)	0.2	0.4 bc
Thiamethoxam (IF)	0.3	0.7 a
Imidacloprid + clothianidin (ST)	0.4	0.5 abc
Thiamethoxam (ST)	0.2	0.3 c

[1] ST, seed treatment; IF, in-furrow application at planting.
[2] Cucumber beetle leaf feeding injury scale: 0 = no injury; 1 = <10% of leaf injured; 2 = 10–50% of leaf injured; 3 = >50% of leaf injured.
Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Fisher's LSD).

Table 4 Mean percentage mortality of striped cucumber beetle after 120 h placed on excised cucumber leaves during the cotyledon, 1-leaf and 2-leaf stages from a field experiment in Columbus, Ohio, 2008

Treatment[1]	**Cotyledon**	**1-leaf stage**	**2-leaf stage**
Control	25	10	10
Imidacloprid (IF)	75	25	15
Thiamethoxam (IF)	80	30	30
Imidacloprid + clothianidin (ST)	75	40	25
Thiamethoxam (ST)	65	10	20

[1] ST, seed treatment; IF, in-furrow application at planting.

damage (Table 2). At the 4–5-leaf stage (24 DAP) the untreated control had approximately 15% of the plants killed by beetle feeding, while the seed treatments had few to no plants killed. In addition, at the Freemont, Ohio location there was a significant difference in beetle feeding injury on leaves despite a relatively low beetle population (Table 3). By the 4-leaf stage, the thiamethoxam seed treatment had significantly less beetle feeding injury than the control.

Residual toxicity of leaves against cucumber beetle

Excised leaf bioassays showed toxicity of the seed treatments against cucumber beetles at all locations. At the Columbus, Ohio location, all treatments had a higher percentage mortality of beetles compared with the untreated check at the cotyledon stage (Table 4), but by the 1-leaf stage (16 DAP) none of the treatments were effectively killing beetles.

In excised leaf bioassays conducted 20 days after planting at the Virginia location, both seed treatments provided significant beetle mortality, whereas the in-furrow applications did not (Table 5). In addition, the thiamethoxam seed treatment appeared to be more toxic than the imidacloprid + clothianidin seed treatment at 20 DAP, although not statistically significant.

Table 5 Mean percentage mortality (after 96 h) of striped cucumber beetles placed on excised cucumber leaves 20 days after planting at Painter, Virginia, 2008

Treatment[1]	**16 Jun (20 DAP)**
Control	5.0 b
Imidacloprid (IF)	10.0 b
Thiamethoxam (IF)	15.0 b
Imidacloprid + clothianidin (ST)	65.0 a
Thiamethoxam (ST)	95.0 a

[1] ST, seed treatment; IF, in-furrow application at planting.
Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Fisher's LSD).

Table 6 Mean percentage mortality (after 96 or 72 h) of striped cucumber beetles placed on excised cucumber leaves collected at the cotyledon and 4-leaf stages at Georgetown, Delaware, 2008

Treatment[1]	**Cotyledon stage 19 Jun (10 DAP)**	**4-leaf stage 30 Jun (21 DAP)**
Control	0.0 b	0.0 b
Imidacloprid (IF)	n/a	62.5 a
Thiamethoxam (IF)	n/a	75.0 a
Imidacloprid + clothianidin (ST)	80.0 a	68.8 a
Thiamethoxam (ST)	85.0 a	81.3 a

[1] ST, seed treatment; IF, in-furrow application at planting.

Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Tukey's mean separation test).

In excised leaf bioassays conducted at the Delaware location, both seed treatments provided significant beetle mortality at the cotyledon stage and at the 4-leaf stage 21 DAP (Table 6). The two in-furrow insecticide applications were also efficacious at 21 DAP.

In pumpkin excised leaf bioassays conducted at the New York location, both seed treatments provided significant beetle mortality at the 2-leaf stage and at the 4-leaf stage 26 DAP (Table 7). By the 7-leaf stage (33 DAP), none of the treatments effectively killed beetles. The thiamethoxam seed treatment was significantly more toxic than the imidacloprid + clothianidin seed treatment at 19, 26 and 33 DAP. These results were consistent with numerical differences noted with cucumber seed treatments at 20 and 21 DAP at the Virginia and Delaware locations, respectively.

Table 7 Mean percentage mortality of striped cucumber beetle after 72 h placed on excised pumpkin leaves during the 2-leaf, 4-leaf and 7-leaf stages from a field experiment in Geneva, New York, 2008

Treatment[1]	**2-leaf stage 16 Jun (19 DAP)**	**4-leaf stage 23 Jun (26 DAP)**	**7-leaf stage 30 Jun (33 DAP)**
Control	1.7 c	0.0 c	3.3 bc
Imidacloprid (IF)	24.6 bc	11.4 c	1.7 c
Thiamethoxam (IF)	41.1 b	67.4 a	8.1 bc
Imidacloprid + clothianidin (ST)	44.1 b	11.7 c	1.7 c
Thiamethoxam (ST)	68.0 a	47.4 b	11.5 ab

[1] ST, seed treatment; IF, in-furrow application at planting.

Table 8 Results of cucumber neonicotinoid seed treatment and in-furrow application efficacy trial, Painter, Virginia, 2008

Treatment[1]	Mean no. tobacco thrips/10 plants (16 DAP)
Control	36.5 a
Thiamethoxam (IF)	13.5 b
Imidacloprid (IF)	10.3 b
Imidacloprid + clothianidin (ST)	1.8 b
Thiamethoxam (ST)	0.5 b

[1] ST, seed treatment; IF, in-furrow application at planting.
Means followed by the same letter are not significantly different (*P* ≤ 0.05; LSD).

Thrips control in the field

Seed treatments may also provide control of other insect pests in addition to beetles. At the Virginia location, tobacco thrips, *Frankliniella fusca*, were present on leaves with an average of 37 thrips per 10 plants in the control plots at the second true leaf stage (16 DAP). Although this insect may not be considered a serious pest of cucurbits, it should be noted that all treatments significantly controlled tobacco thrips compared with the untreated control, with the highest efficacy obtained from the seed treatments (Table 8).

Discussion

In summary, the results of our experiments conducted on cucumbers and pumpkins demonstrated that the neonicotinoid seed treatments thiamethoxam at 0.75 mg a.i./seed and the combination of imidacloprid + clothianidin at 1.0 mg a.i./seed were consistently efficacious against cucumber beetles. Control extended up to the 4-leaf stage or about 20–26 days after planting. Thiamethoxam seed treatment appears to have a longer active residual than imidacloprid + clothianidin. Neonicotinoid seed treatments offer growers an effective new method of combating cucumber beetles and other insect pests that may attack plants early in their development. Seed treatments have the added benefits of less insecticide input in the environment and limited insecticide exposure for the applicator as compared with in-furrow or foliar applications.

References

McLeod P (2006) Use of neonicotinoid insecticides to manage cucumber beetles on seedling zucchini. *Plant Health Progress* 10. doi:10.1094/PHP-2006-1020-01-RS