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A literature review and new observations on the use of diflubenzuron for control of locusts and grasshoppers throughout the world

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Abstract

The insect growth regulator diflubenzuron (Dimilin®) is used to control locusts and grasshoppers on rangeland and croplands worldwide. Ingestion of diflubenzuron by immature insects results in disruption of chitin formation and deposition that affects the cuticle and the molting process. Symptoms of diflubenzuron intoxication include: death, physical abnormalities (such as loss of limbs), lethargy and cessation of feeding. Field trials with diflubenzuron on grasslands are reported in this paper and support previously published work showing it to be effective in broadcast treatments or alternating swaths and barriers. Affected insects were observed 3 d after application but maximum control of populations occurred by 14 d. Low grasshopper population counts the year after application suggested fewer eggs hatched from adults exposed to diflubenzuron. This bears out results of earlier laboratory and small-scale experiments. Diflubenzuron applications to rangeland did not seriously affect the populations of nontarget arthropods.

Keywords

Acrididae, diflubenzuron, pest management, barrier treatment, nontargets

Review

Locust plagues were described in the Old Testament of The Holy Bible (King James Version) as follows: ". . . and when it was morning, the East wind brought the locusts. And the locusts went up over all the land of Egypt . . . very grievous were they . . . for they covered the face of the whole earth, so that the land was darkened; and they did eat every herb of the land, and all the fruit of the trees . . . and there remained not any green thing in the trees, or in the herbs of the field, through all the land of Egypt" (Exodus 10: 13-15). This description of the magnitude of a locust outbreak and the damage inflicted is as accurate today.

In the Western USA, it has been estimated that grasshoppers can devour 25% of the available rangeland fodder, leading to losses valued at US$400 million (Hewitt and Onsager 1983, DeBrey *et al.* 1993). In 1999, 220,000 ha of cereals were destroyed in Kazakhstan by swarms of Italian locust (*Calliptamus italicus* L.) migrating from rangeland (Latchininsky 2001). As a result, 8 million ha were sprayed in 2000 for locust control at a cost of US$21 million. In 2001, infestations of Mormon crickets (Tettigoniidae, *Anabrus simplex* Haldeman) in the State of Utah caused damage to crops worth US$25 million (Janofsky 2001), and locust outbreaks in China infested 8 million ha (Anonymous 2001).

The organochlorine insecticide dieldrin was the standard for locust control. The practice was to control moving bands of hoppers by treating strips of vegetation in their path. However, dieldrin was banned 10 y ago with other organochlorines because of persistence and bioaccumulation in the food chain. Organophosphates, pyrethroids and carbamates were then used for locust control. More recently Insect Growth Regulators (IGR) such as diflubenzuron (a benzoyl urea), and the insecticide fipronil (a phenylpyrazole) have been used.

The benzoyl urea chemistry was discovered by researchers at Philips-Duphar B.V. to have insecticidal activity, based on interference with chitin deposition (van Daalen *et al.* 1972). Diflubenzuron was the first analog to be commercialized in 1975. Its common name is 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea. The registered trademark for diflubenzuron formulations used for locust and grasshopper control is Dimilin®.

Diflubenzuron is considered an IGR because of its effects on the endocuticle (Mulder & Gijswijt 1973), and specifically on chitin deposition (Post & Vincent 1973, Post *et al.* 1974), that renders larvae unable to complete the molt process. This results in death. Hunter and Vincent (1974) reported diflubenzuron inhibited chitin biosynthesis in *Locusta migratoria capito* (Sauss.), resulting in abnormal cuticle formation. The protein matrix of the cuticle was not affected. Ker (1977) showed similar effects on the cuticle of the desert locust, *Schistocerca gregaria* Forskål. Mathur & Saxena (1995) found that diflubenzuron promoted the synthesis of compounds that interfered with the deposition of chitin.

The cuticle of orthopteran nymphs that have ingested diflubenzuron may be weakened and ruptured by increased pressure of the hemolymph during the molting process (Grosscurt & Jongsma 1987). Nonlethal effects such as slow movement, uncoordinated jumping, loss of legs, decreased feeding and malformed wings have also been associated with diflubenzuron (Mariy *et al.* 1981, Walgenbach *et al.* 1988, Kepner 1989, Jepson & Yemane 1992, Coppen & Jepson 1996a, Wakgari 1997, Suleimenova *et al.* 1998, Yskak & Komissarova 1999, Kambulin 2000). Clarke *et al.* (1977) and Bouaichi & Chihrane (2001) showed that diflubenzuron also interfered with the formation of the peritrophic membrane in the midgut of *L. migratoria* and *S. gregaria*. Rao & Mehrotra (1987) found that diflubenzuron reduced chitin deposition in wings of adult *S. gregaria* exposed as 5th instars.

Jepson & Yemane (1992) found that mortality was related to the length of time diflubenzuron was in the gut. It has also been shown that diflubenzuron prolongs the duration of instars (Coppen 1994), but it does not accumulate in the insects (Coppen & Jepson 1996b). Concentrations of 10 mg l^{-1} and above in the diet completely inhibited molting of 2nd instars of the migratory grasshopper [*Melanoplus sanguinipes* (Fabricius)] (Elliott & Iyer 1982). When they fed these grasshoppers treated wheat (*Triticum* spp.) seedlings for 8, 12 and 20 d , LC_{90} values of 10.0, 2.4 and 0.8 mg l^{-1} were obtained. Exposing nymphs to treated foliage for only 1 d resulted in a higher LC_{90} value. Due to its mode of action, impact on population numbers of locusts and grasshoppers is noticeable approximately 3 d after treatment (Cooper *et al*. 1995, Suleimenova *et al*. 1998, Yskak & Komissarova 1999), but maximum control is approached 10 to 14 d after treatment (Bomar *et al*. 1992; Cantangui *et. al*. 1993a; Bouaichi *et al*. 1994a; Cantangui 1994a, 1994b; Foster *et al*. 2000; Kambulin 2000).

Vincent & Clarke (1985) determined that diflubenzuron greatly reduced the amount of chitin deposited in the cuticle of newly emerged adult *L. migratoria*, as well as affecting the amount and nature of cuticular protein. When adults of Colorado potato beetle [(*Leptinotarsa decemlineata* (Say)] were fed potato (*Solanum* spp.) leaves sprayed with diflubenzuron at 1000 mg l^{-1} up to 10 d after emergence from pupae, their elytra failed to harden normally. It was also shown that stabilization of protein differed in soft and hard cuticles (Grosscurt 1978). In soft larval cuticles, no distinct deposition of proteins took place after diflubenzuron treatment. However, in the hard cuticle of elytra, distinct mesocuticular layers containing proteins were still deposited after diflubenzuron treatment. The production of ketocatechols, which are involved in tanning of the structural proteins, was partially inhibited, and it was concluded that interference of diflubenzuron with this process is a secondary effect (Grosscurt & Andersen 1980). As with the 10-d hardening period in *L. decemlineata*, chitin deposition in newly emerged adults of several locust species can last 2 to 3 w (Neville 1975) and a weak, soft cuticle would make adults more vulnerable to parasites and predators.

Production and hatch of eggs may be affected when adult females feed on diflubenzuron residues. Although the number of egg pods laid per treated female and the number of eggs per pod were not affected, there was a decrease in the number of locust (*S. gregaria*) eggs that hatched (Mariy *et al*. 1981, Bouaichi & Chihrane 2001). Kepner (1989) found that *M. sanguinipes* females, which had fed on diflubenzuron residue, laid fewer egg pods, although the number of eggs per pod was not altered. He also noted both female and male grasshoppers had shorter life spans after feeding on diflubenzuron. Effects on female *Oxya japonica* Willemse, fed in the laboratory on a diet treated with diflubenzuron, were: reduced fecundity, reduced egg hatch, and reduced female life span (Lim & Lee 1982). Suleimenova *et al*. (1998) and Evdokimov *et al*. (1999) noted that adult locusts (mainly *C. italicus*) lost their ability to lay eggs when they had fed on diflubenzuron-treated foliage in the field.

Optimal characteristics of an insecticide for locust and grasshopper control would be extended activity on plants and low mammalian and environmental toxicity (Coppen 1999). Diflubenzuron has 4 w or more residual activity against locust and grasshoppers in rangeland (Scherer & Rakotonandrasana 1993; Cantangui *et al*. 1993a; Bouaichi *et al*. 1994a; Catangui *et al*. 1994a, 1994b; Foster *et al*. 2000; Kambulin 2000; Royer *et al*. 2001). Further, diflubenzuron has low toxicity to mammals, birds, fish, and earthworms (Marx 1977, Booth *et al*. 1987, World Health Organization 1996). Although it is toxic to aquatic microcrustaceans (Hansen & Garton 1982, Lahr *et al*. 2000, Lahr *et al*. 2001), populations recover rapidly due to dissipation of diflubenzuron in water (Schaefer & Dupras 1976, Booth *et al*. 1987, Knuth & Heinis 1995, Mabury & Crosby 1996, World Health Organization 1996, Lahr *et al*. 2000). Effects on aquatic microcrustacea depend on their developmental stage when exposed (Touart & Rao 1987).

Databases have been compiled comparing toxicity of different pesticides on terrestrial nontarget beneficial invertebrates (Theiling & Croft 1988, Murphy *et al*. 1994). Diflubenzuron was found to be less toxic and more selective than synthetic pyrethroids, carbamates, and organophosphates. Similarly, many rangeland field trials in the USA (Catangui *et al*. 1993b), Madagascar, Senegal, and Kazakhstan have shown low effect of diflubenzuron on nontargets. Extensive assessment of nontarget beneficial insects in Madagascar between untreated grassland and spray barriers of diflubenzuron has been reported (Tingle 1996, 1997a, 1997b). One location was sprayed in 1993 and monitored for 2 y, while another was sprayed and monitored in 1994. Barrier swaths that were 50 m wide were treated with 93 g ai ha^{-1}[grams of active ingedient per hectare], which gave a protected area rate of approximately 8 to 9 g ai ha^{-1}. Only certain Lepidoptera and Acrididae were affected in this work (Tingle 1996). Only four of 23 taxa exhibited any changes in relative abundance between sprayed and unsprayed areas 3 mo after spraying (Tingle 1997a). None of the four taxa showed effects of diflubenzuron lasting over 1 y. Although spiders (Tingle 1997a, 1997b) and certain parasitic wasps (Tingle 1997a) did occur in lower numbers in sprayed areas a year after spraying, it was concluded that diflubenzuron had less impact on a wider range of beneficial insects than conventional insecticides (Tingle, 1996). Others have noted this in Senegal, Russia, and Kazakhstan (van der Valk & Kamara 1993, Kamara & van der Valk 1995, Evdokimov *et al*. 1999, Yskak & Komissarova 1999, Kambulin 2000). Effects on parasitic wasps (and other parasites and predators) could be a result of reduced available hosts. Recent research showed diflubenzuron effects were not transferred when nontarget insects consumed affected grasshoppers in contrast to fipronil (Lockwood *et al*. 2001). Diflubenzuron does not affect the adult flea beetle, *Aphthona* spp., which is a biological control agent for the noxious weed, leafy spurge (*Euphorbia esula*), in rangeland in the USA (Foster 2001).

Early field trials with diflubenzuron showed control of rangeland grasshoppers using bait and foliar sprays (Romijn & Sissoko 1990, Foster *et al*. 1991, Catangui *et al*. 1993a, Jech *et al*. 1993). Barrier applications, where diflubenzuron or other benzoyl ureas were applied to swaths several meters wide in front of migratory bands of locust nymphs, also effectively controlled locusts (Scherer & Rakotonandrasana 1993, Bouaichi *et al*. 1994b, Cooper *et al*. 1995). A recent control tactic has been developed for rangeland that utilizes grasshopper mobility. Strip treatments (Royer *et al*. 2001) and Reduced Agent Area Treatment (RAAT) (Lockwood and Schell 1997) have provided more economical control of grasshoppers by alternating application of active ingredient in swaths with untreated swaths, thus reducing the area treated with insecticide and minimizing effects on nontargets. The extent to which the active ingredient can be reduced, while still obtaining effective grasshopper control, varies with active ingredient (Lockwood & Schell 1997). Application of diflubenzuron in a RAAT program has been found to be very effective (Foster *et al*. 2000, Lockwood *et al*. 2001).

The purpose of the present paper is to present additional research work carried out with diflubenzuron for the control of locusts and grasshoppers on different types of grasslands throughout the world since 1990.

Materials, Methods and Results

Formulations.—The following formulations are referred to in this paper:
Dimilin 25W (WP-25): 25% water dispersible powder.
Dimilin 80WG (WG-80): 80% water dispersible granule containing 800 g ai kg^{-1}.
Dimilin OF-6: ultra-low-volume oil dispersible concentrate containing 60 g ai l^{-1}. This is a 'ready-to-use' formulation normally applied without further dilution.
Dimilin SC-48: water-based suspension concentrate containing 480 g ai l^{-1}.
Dimilin 2L: water-based suspension concentrate containing 240 g ai l^{-1}.
Dimilin 2F: oil-based dispersible concentrate containing 240 g ai l^{-1}.
Decis flow SC5: a water-based suspension concentrate containing 50 g ai deltamethrin l^{-1}.
Decis 2.5% EC: an emulsifiable concentrate containing 25 g ai deltamethrin l^{-1}.
Kinmix 5% EC: an emulsifiable concentrate containing 50 g ai beta-cypermethrin l^{-1}.
Sevin XLR PLUS: a latex-based emulsifiable concentrate containing 480 g ai carbaryl l^{-1}.

Glossary.—
Crop Oil Concentrate (COC): an emulsified mineral oil (83% oil: 17% emulsifier) included in tank mixes to reduce aerial drift and/or enhance plant canopy penetration.
Broadcast/blanket treatment: application to 100% of an area of land.
Barrier treatment: strip (1 or more sprayer swaths) application in front of migrating locust nymphs.
RAAT: 'Reduced Agent Area Treatment' using reduced active ingredient application in swaths alternating with equal or unequal untreated swath areas.
Protected area: treated plus untreated swath areas.

Analysis.—Control percentages were calculated using Henderson's formula (Henderson & Tilton 1955). Fisher's protected least significant difference (LSD) post-ANOVA test (SAS/STAT 1990) was used to compare differences in insect densities or control percentages. Using the residuals from the ANOVA, a test for normality was done with the Shapiro-Wilk Statistic (SAS 1990). When the data appeared not to be normally distributed, they were transformed using $\log_{10}$ values. Differences are considered statistically different when $p < 0.05$, unless otherwise indicated.

1991 – Guernsey, Wyoming, USA.—This study was established in cooperation with the University of Wyoming and conducted by Dr. Jeffrey Lockwood. The objectives were to assess control of rangeland grasshopper populations and potential effects on nontarget arthropod populations using an aerial application of Dimilin 25W at 17.5 and 52.5 g ai ha^{-1} and carbaryl (Sevin XLR PLUS) at 750 g ai ha^{-1}. Blue grama grass (*Bouteloua gracilis*) and clover (*Melilotus* spp.) were the dominant plants and comprised 40 and 15% of the ground cover respectively. Bare ground accounted for 25% of the area.

The trial consisted of sixteen 16.2-ha plots. The four furthest from the farmstead were assigned the carbaryl treatment because of the land owner's concerns regarding risk of toxicity to humans. The remaining 12 were randomly assigned as untreated controls and treatments of Dimilin 25W. Thus the experiment was conducted using a stratified block design with randomization across 12 of the blocks and four replicates per treatment. Carbaryl and Dimilin 25W were broadcast-applied in total volumes of 4.68 and 2.34 l ha^{-1}, respectively, using 18.3-m swaths.

Grasshopper populations were estimated using 27 sampling sites, each of 0.093 m^2 per plot (DeBrey *et al.* 1993). The location averaged 13.2 grasshoppers per m^2 before spraying. Nymphs of *Aulocara elliotti* (Thomas) and *Melanoplus* spp. were the main species of grasshopper present. The majority of nymphs were 2nd to 4th instars at application. The densities of nontarget arthropods were based on 100 sweep-net captures (50 high and 50 low) in each plot, using a 30.5-cm diameter net. These sampling procedures were conducted the day prior to the aerial applications and 7, 14 and 21 d after treatment.

Population densities of grasshoppers in the plots treated with Dimilin 25W did not differ significantly from those in the untreated plots prior to treatment. However, pretreatment densities in plots treated with carbaryl were significantly greater than in the control plots (Table 1). Grasshopper densities in all treated plots were significantly lower than in the untreated plots at 7, 14, and 21 d after treatment. Numbers in the treated plots did not differ significantly from each other. Twenty-one d after application both rates of Dimilin 25W gave > 98% control of grasshoppers and carbaryl gave 83% control.

The population densities of nontarget arthropods did not differ significantly among plots prior to treatment (Table 2). No treatment differences for Homoptera, Hymenoptera, Coleoptera, Hemiptera, Lepidoptera, or Neuroptera were determined at any of the sampling times. At 1 w after treatment, there were significantly more Diptera in the plots with carbaryl and the high rate of Dimilin 25W than in the untreated plots. By 3 w after treatment, the plots treated with either rate of Dimilin 25W had significantly greater numbers of Diptera than either the untreated plots or those treated with carbaryl. One and 2 w after treatment, there were significantly fewer spiders (Araneae) in the plots treated with the low rate of Dimilin 25W compared to the untreated, the high rate of Dimilin 25W and

Table 1. 1991 – Guernsey, Wyoming, USA. Grasshopper population counts taken 7, 14 and 21 d after treatment (DAT). Dimilin 25W applied by air as a broadcast treatment (100% coverage) at 17.5 and 52.5 g ai ha^{-1}, and carbaryl applied at 750 g ai ha^{-1}.

Treatment	Untreated	Dimilin 25W		Carbaryl
Rate (g ai ha^{-1})		17.5	52.5	750
DAT	Mean number m^{-2}			
Pretreatment	10.8a	15.6ab	10.4a	19.9b
7	24.3a	1.3b	2.3b	3.9b
14	17.6a	0.2b	0.5b	3.4b
21	19.6a	0.0b	0.2b	3.3b

Mean counts within a sampling period with different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test.

Table 2. 1991 – Guernsey, Wyoming, USA. Nontarget arthropod counts taken 7, 14 and 21 d after treatment (DAT). Dimilin 25W applied by air as broadcast treatment (100% coverage) at 17.5 and 52.5 g ai ha^{-1}, and carbaryl applied at 750 g ai ha^{-1}.

	Treatment	Untreated	Dimilin 25W		Carbaryl
	Rate (g ai ha^{-1})		17.5	52.5	750
Order	DAT	Mean number per 100 sweeps			
Homoptera	Pretreatment	13	43	50	12
	7	41	62	74	51
	14	100	181	117	80
	21	20	70	50	44
Diptera	Pretreatment	29	79	63	39
	7	22a	30ab	52bc	77c
	14	13	17	22	31
	21	2a	12b	10b	4a
Hymenoptera	Pretreatment	14	17	16	14
	7	16	6	11	14
	14	10	12	15	26
	21	2	10	12	6
Coleoptera	Pretreatment	2	4	10	6
	7	14	8	12	4
	14	6	2	8	4
	21	3	2	4	1
Hemiptera	Pretreatment	15	30	36	25
	7	26	28	27	22
	14	11	20	15	13
	21	2	2	24	3
Lepidoptera	Pretreatment	2	1	1	0
	7	2	1	2	1
	14	3	1	1	0
	21	0	1	0	0
Neuroptera	Pretreatment	0	2	0	1
	7	0	0	0	0
	14	0	1	0	0
	21	0	0	0	0
Araneae	Pretreatment	6	14	14	18
	7	20bc	5a	15ab	30c
	14	20b	8a	16b	14ab
	21	1ab	2b	4c	0a

Mean counts of orders within a sampling period followed by different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test.

carbaryl-treated plots. Three weeks after treatment, the number of spiders found in plots treated with the low rate of Dimilin 25W was similar to the untreated, and the highest number was found in the plots treated with the high rate of Dimilin 25W. However, the overall number of spiders was much lower than found in previous counts.

1997 – Huinca Renanco, Argentina.—Two field trials were placed in pastureland of the Huinca Renanco area of La Pampa Province. Terrain was flat and vegetation consisted of a variety of grass and broadleaf species (*e.g. Phalaris, Bromus, Lolium, Lotus, Melilotus*). Treatment blocks at both trials were: an untreated control; diflubenzuron (Dimilin 80WG) at 60 g ai ha^{-1}; deltamethrin (Decis flow SC5) at 7.5 g ai ha^{-1}. Treatments were broadcast, applied by air with spray volumes of 3 and 5 l ha^{-1} at Sites 1 and 2 respectively. Treatment plots at Site 1 were 2 ha, and at Site 2 were 10 ha. Grasshopper numbers were sampled with sweepnets at 9 locations per plot with 3 sweeps at each location. Predominate grasshopper species

Table 3. 1997 – Huinca Renanco, Argentina. Grasshopper population counts taken at 1, 3, 7 and 10 d after treatment (DAT). Dimilin 80WG applied as broadcast treatment (100% coverage) at 60 g ai ha^{-1} and deltamethrin applied at 7.5 g ai ha^{-1}. Aerial application volumes at Site 1 and 2 were 3 and 5 l ha^{-1} respectively.

Treatment	Untreated	Dimilin 80WG	Deltamethirin
Rate (g ai ha^{-1})		60	7.5
DAT	Mean number per 3 sweeps		
SITE 1			
Pretreatment	6.1	6.4	5.9
1	6.4a	6.2a	0.9b
3	6.3a	5.1a	1.0b
7	6.4a	3.8b	1.9c
10	5.6a	1.6b	1.1b
SITE 2			
Pretreatment	9.1a	3.7b	7.4a
1	6.4a	2.4b	2.7b
3	8.8a	1.7b	2.7b
7	11.3a	0.9b	1.9b
10	9.6a	0.2b	2.0b

Mean counts within a sampling period for a site followed by different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test. Analyses for Site 1 were done on transformed numbers; however, actual sweep means are shown.

present included *Dichroplus pratensis* (Bruner), *D. bergii* (Stål), *D. punctulatus* (Thunberg) and *Rhammatocerus pictus* (Bruner). At Site 1, the population consisted of approximately 20% adults with the remainder late instars. At Site 2, the population was almost entirely mid to late instars.

Deltamethrin caused significant reduction of grasshopper populations 1 d after treatment in both trials and remained effective through the duration of the 10-d sampling period (Table 3). Dimilin 80WG exhibited efficacy starting 7 d after treatment at Site 1, which had a mixed population of nymphs and adults. Maximum control was achieved by day 10. At Site 2 the grasshopper population in the block treated with Dimilin 80WG initially had fewer nymphs than the untreated block or that treated with deltamethrin. Population reduction started 1 to 3 d after treatment and 95% control was achieved after 10 d; deltamethrin reduced the population by 73% after 10 d. At Site 1 after 10 d, respective population reductions were 75 and 81%.

1998 – Volgograd, Ilovlinsky, Russia.—A.P. Sazonov and O.N. Naumovich of the Russian Plant Protection Institute conducted and reported results for this 1998 trial in the Volgograd region of the Ilovlinsky district in Russia. Trials on pastureland consisted of treatments with broadcast coverage of Dimilin 25W at 35 and 60 g ai ha^{-1}. In another trial, strips 20 m wide were treated with Dimilin 25W at 35 g ai ha^{-1}, and between them strips 60 m wide were not treated, giving 25% coverage or an effective rate of 9 g ai ha^{-1}. Deltamethrin (Decis EC) at 12.5 g ai ha^{-1} was included as the standard broadcast treatment. All treatments were applied in a spray volume of 18 l ha^{-1} using an OM 630-2 boom sprayer. These treatments and an untreated control were arranged in a complete

Table 4. 1998 – Volgograd, Ilovlinsky, Russia. *C. italicus* population counts and percent control taken at 3, 6, 9, 12, and 15 d after treatment (DAT). Dimilin 25W ground-applied as broadcast treatment (100% coverage) at 35 and 60 g ai ha^{-1} and at 35 g ai ha^{-1} as 20-m strips separated by 60-m untreated strips (25% coverage). Deltamethrin ground-applied as broadcast treatment at 12.5 g ai ha^{-1}.

Treatment	Untreated	Dimilin 25W			Deltamethrin
Rate (g ai ha^{-1})		35	60	35	12.5
% Coverage		100	100	25	100
DAT	Mean number per m^2				
Pretreatment	72.2	67.8	76.1	63.1	81.2
3	70.7	58.3	59.3	58	4
6	68.8	48.4	37.5	51.6	5.3
9	66.8	18.5	6.1	29.1	18.7
12	54.9	2.5	1.9	6.7	23.2
15	51.7	1.7	1.5	5.8	25
		% Control			
3		12a	20b	6a	95a
6		25c	48b	14c	93a
9		71b	91a	50c	74b
12		95a	97a	86a	62b
15		96a	97a	87a	56b

Means for percent control for a sampling period followed by different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test.

randomized block design with two replicates. Total area covered by the trial was 15 ha. Ninety-nine percent of the locust population at application consisted of 2nd instar Italian locust (*C. italicus*). Pretreatment population densities and densities 3, 6, 9, 12, and 15 d after treatment were determined by a visual count of live insects in areas of approximately 0.25 m^2.

Deltamethrin provided approximately 95% control of Italian locust 3 d after application; however numbers started increasing 9 d after treatment and by 15 d the level of control was <60% (Table 4). The broadcast application of Dimilin 25W at 35 g ai ha^{-1} resulted in slower control than 60 g ai However, both treatments attained maximum control of ≥95% 12 d after application. The test plots, which received 25% coverage of Dimilin 25W, gave slower control than the comparable broadcast application, reaching maximum control of ≥86% after 12 d.

1999 – Almaty, Kazakstan.—N.Y. Evdokimov and A. Dinasilov of the Kazakh Agrarian University conducted this trial in the Almaty Region of the Balkhash District and reported the results. The flora consisted of common reed (*Phragmites* spp.) in fallow rice fields (*Oryza sativa*). Five to 50% of the ground was bare. Dimilin SC-48 was applied as a broadcast treatment at 7 and 9 g ai ha^{-1}. On other plots Dimilin SC-48 was applied as 50% coverage on 40-m strips at 18 and 30 g ai ha^{-1} alternating with 40-m untreated strips, giving 9 and 15 g ai per protected ha. Beta-cypermethrin (Kinmix 5% EC) at 15 g ai ha^{-1} was used as a standard broadcast treatment. Applications were made with a backpack sprayer, applying 20 l spray volume per ha. Treatment blocks were 0.2 ha with four replicates. The population of Asian locust (*L. migratoria*) at application was 90% 3rd to 5th instars and 8% adults. Population density was estimated at 3, 7 and 10 d after treatment by making counts in areas of 0.25 m^2.

Beta-cypermethrin provided approximately 95% control of the Asian locust from 3 to 7 d after treatment; however control had decreased to 75% by day 10 (Table 5). Broadcast applications of 7 and 9 g ai Dimilin SC-48 ha^{-1} gave 21 and 37% control, respectively, 3 d after application, 75 and 93% at day 7, and 96 and 97% 10 d after treatment. Control was slower in the plots receiving 50% coverage of Dimilin but when the final counts were made at 10 d after treatment, they had reached 83% and 88%. At the termination of population assessment, it was noted that surviving immature and adult insects could not move and were often observed with morphological abnormalities.

2000 – Trinidad, Colorado, USA.—This study was established to compare broadcast aerial application of Dimilin 2l at 17.5 g ai ha^{-1} with 50% coverage. Application was made to swaths 21.3 m wide with spray volume of 9.36 l ha^{-1}, including 0.29 l of COC. Alternate swaths received no treatment. The broadcast block was 10.3 ha and the 50%-treated block was 3.4 ha. The untreated control block was 1.7 km from treated blocks. Vegetation consisted of a mixed grass population of mainly buffalo grass (*Buchloe dactyloides*), grama grass (*Bouteloua* spp.) and blue stem (*Andropogon glomeratus*). Grasshopper populations were assessed visually as described by DeBrey *et al.* (1993) at 7, 14, 21 and 28 d after treatment. In the 50% coverage block, populations were sampled in the middle of each of the treated and untreated swaths. The population at treatment was mainly 2nd to 3rd instar *Melanoplus* spp., and averaged 40 nymphs per m^2. Grasshopper population counts were repeated in the untreated and broadcast blocks in the season following the application (2001).

A significant reduction of the grasshopper population in the broadcast block was observed 7 d after treatment with maximum control by 14 d (Table 6). A reduction in population at 7 d after treatment was also observed in the sprayed strips of the 50%-coverage block and continued throughout the remaining sampling times. In the untreated strips of the 50%-coverage block, there was a significant decrease in grasshopper numbers 14 d after treatment. At this time, the mean population density in the untreated strips was not significantly different from that in the strips treated with

Table 5. 1999 – Almaty, Kazakstan. *L. migratoria* population counts and percent control taken at 3, 7 and 10 d after treatment (DAT). Dimilin SC-48 backpack-applied as broadcast treatment (100% coverage) at 7 and 9 g ai ha^{-1}, and at 18 and 30 g ai ha^{-1}, as 40-m strips separated by 40-m untreated strips (50% coverage). Beta-cypermethrin broadcast applied at 15 g ai ha^{-1}.

Treatment	Untreated	Dimilin 48-SC				Beta-cypermethrin
Rate (g ai ha^{-1})		7	9	18	30	15
% Coverage		100	100	50	50	100
DAT		Mean number per m^2				
Pretreatment	52	45.2	48	45.5	45.8	44.5
3	52.3	36.5	30.7	41.5	41	1.8
7	49	9	3.2	20.5	17.8	2.8
10	42	1.8	1.2	6.8	5.8	11
		% Control				
3		21b	37c	10d	11d	96a
7		73b	93a	54c	61bc	93a
10		95ab	96a	83bc	88abc	76c

Means for percent control for a sampling period followed by different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test. Analyses were done on transformed numbers for the 3 d after sample time; however, actual mean percentages are shown.

Table 6. Trinidad, Colorado, USA. *Melanoplus* spp. population counts at 7, 14, 21 and 28 d after treatment (DAT). Dimilin 2l applied by air as broadcast treatment (100% coverage) at 17.5 g ai ha^{-1} and at 17.5 g ai ha^{-1} in alternating treated and untreated strips 21.3 m wide (50% coverage). Counts assessed in the 100% treatment and in the middle of each of the treated (T) and untreated (UT) strips of the 50% coverage treatment.

Treatment	Untreated	Dimilin 2l		
Rate (g ai ha^{-1})		17.5	17.5	17.5
% Coverage		100	50(T)	50(UT)
DAT	Mean number per m^2			
7	31.2a	10.9b	32.8a	16.4b
14	27.3a	0.4b	10.9b	7.3b
21	40.0a	0.0b	7.6b	7.3b
28	30.1a	2.8b	10.9b	2.8b

Mean counts within a sampling period with different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test.

Dimilin 2L. Grasshopper counts taken in 2001, the year after application, showed densities 55% less ($p = 0.06$) in the broadcast block of Dimilin 2L compared to the untreated block.

2000 – Edgemont, South Dakota, USA.—In 1999, aerial applications of Dimilin 2L were made to 259-ha blocks of rangeland. It was applied as a broadcast treatment (100% coverage) at 17.5 g ai ha^{-1} and at 50% coverage (13.1 g ai ha^{-1}, equivalent to 6.5 g ai per protected ha). Carbaryl (Sevin XLR PLUS) was included as the standard at 50% coverage (420 g ai ha^{-1}, 210 g ai per protected ha), and at 100% coverage (420 g ai ha^{-1}). These treatments gave 99, 98, 90 and 97% grasshopper control, respectively, at 28 d after application. Additional details on methods and results are addressed in Foster *et al.*, 2000. In 2000, the first three treatments and adjacent untreated control areas were monitored to see if any treatment exhibited residual effects in the subsequent season. Eighty sweeps with nets parallel to the surface at each of 12 sites in the middle of each treatment area were made; 80 sweeps in each of 20 untreated areas were also made. Each pass of the net touched the vegetation canopy. The grasshopper population represented a mix of instars at sampling. Species were not identified.

The year after treatment, grasshopper populations were reduced by greater than 90% with either treatment of Dimilin 2L, in contrast to the untreated control areas (Table 7). Populations were significantly lower than those found in the carbaryl block, which were reduced 60% compared to the untreated areas.

Table 7. 2000 – Edgemont, South Dakota, USA. Grasshopper population counts the year after treatment (1999). Dimilin 2l applied by air as broadcast treatment (100% coverage) at 17.5 g ai ha^{-1} and at 13.1 g ai ha^{-1} in alternating treated and untreated strips 30.4 m wide (50% coverage). Carbaryl applied at 420 g ai ha^{-1} in similar 50% coverage.

Treatment	Untreated	Dimilin 2l		carbaryl
Rate (g ai ha^{-1})		17.5	13.1	420
% Coverage		100	50	50
	Mean number per sweep			
	2.1a	0.1c	0.2c	0.8b

Mean counts followed by different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test. Analyses were done on transformed numbers. Actual means are shown.

2000 – Orenburg, Sol-Iletsky, Russia.—M.N. Kirilova of the Russian Plant Protection Institute reported results on pastureland in the Orenburg region of the Sol-Iletsky district. The location was treated in 2000 with 100% coverage of Dimilin OF-6 at 9, 12 and 15 g ai ha^{-1}. Another treatment had 20-m strips treated with Dimilin OF-6 at 36 g ai ha^{-1} separated by 40-m untreated strips, giving 33% coverage and a protected area rate of 12 g ai ha^{-1}. Deltamethrin (Decis EC) at 10 g ai ha^{-1} was included as the standard broadcast treatment. Total spray volume of 1 l per ha was applied with a motorized knapsack sprayer. These treatments and an untreated control were arranged in a complete randomized block design with two replicates. Blocks with 100% coverage were 10 ha; blocks with 33% coverage were 15 ha. Pastureland consisted mainly of wormwood (*Artemisia* spp.), feather grass (*Stipa* spp.) and miscellaneous vegetation; fifty to 60% was bare ground. Ninety-nine percent of the locust population at application consisted of 1st and 2nd instar Italian locust (*C. italicus*). Pretreatment densities and densities at 3, 6, 9, 12, and 15 d after treatment were determined by visual counts of live insects in areas approximately 0.25 m^2.

Three days after application, approximately 60% of the locust nymphs in areas sprayed with Dimilin OF-6 exhibited symptoms of lethargy. A significant decrease in population numbers was observed at 6 to 9 d after application (Table 8). Over 95% control was observed 12 d after treatment. No differences were observed among rates, or between broadcast or barrier treatment applications for Dimilin OF-6, 9 d after application. Deltamethrin showed greater than 95% control 3 d after application; however, that decreased to approximately 65% after 12 d.

2001 – Delano, California, USA.—This trial was established on blocks of noncrop land within and adjacent to a large citrus planting. Dimilin 2L was applied by air at 17.5 g ai ha^{-1} to each of four blocks. One block (A) was 4 ha; the three others (B-D) were each 8.1 ha. The untreated block was at least 500 m from the other blocks. Application volume was 46.7 l ha^{-1}, which included 1.17 l ha^{-1} of COC. Forage of dry grass and weeds included soft chess (*Bromus hordeaceus*), annual ryegrass (*Lolium multiflorum*), California brome (*Bromus carinatus*), and wild oats (*Chasmanthium latifolium*). A mixed population of *M. devastator* and *Clinopleura melanopleura* (Scudder) [the latter species is a katydid rather than a grasshopper] was present and the population at application consisted of late instars and approximately 5% adults. Thirty-six estimates of population (DeBrey *et al.* 1993) were taken in areas of 0.093 m^2 the day prior to treatment and at 7, 14 and 21 d after treatment.

Densities of the insects in the treated blocks and in the untreated area ranged from 12 to 30 per m^2 prior to application. The density in the untreated area decreased 14 d after treatment (Table 9). Dimilin 2L activity was found in all treated blocks starting 7 d after treatment. Very few insects were found by 21 d after treatment where Dimilin 2L had been applied.

Table 8. 2000 – Orenburg, Sol-Iletsky, Russia. *C. italicus* population counts at 3, 6, 9, 12 and 15 d after treatment (DAT). Dimilin OF-6 knapsack-applied as broadcast treatments (100% coverage) at 9, 12 and 15 g ai ha^{-1} and at 36 g ai ha^{-1} in 20-m strips separated by 40-m untreated strips (33% coverage). Deltamethrin broadcast applied at 10 g ai ha^{-1}.

Treatment	Untreated	Dimilin OF-6				Deltamethrin
Rate (g ai ha^{-1})		9	12	15	36	10
% Coverage		100	100	100	33	100
DAT	Mean number per m^2					
Pretreatment	30.1	34.8	33.7	53.3	46.9	41.9
3	31.4	32.8	31.4	47.2	46.4	1.5
6	32.2	23.9	21.9	32.6	42	4.4
9	31.6	13.8	11	15	14.4	9.8
12	30	1.6	1.3	1.5	1.8	14.6
15	28.3	1.6	1	1.2	1.2	15.6
		% Control				
3		10bc	11bc	16b	5c	97a
6		36b	40b	43b	16c	90a
9		62c	69b	73ab	70b	78a
12		95a	96a	97a	96a	65b
15		95a	97a	98a	97a	60b

Means for percent control for a sampling period followed by different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test.

Table 9. 2001 – Delano, California, USA. Grasshopper (and katydid) population counts and percent control at 7, 14 and 21 d after treatment (DAT). Dimilin 2L applied by air as broadcast treatment to four distinct blocks (A to D) at 17.5 g ai ha^{-1}.

Treatment	Untreated	Dimilin 2L			
Rate (g ai ha^{-1})		17.5			
% Coverage		100			
DAT	Number per m^2				
Block		A	B	C	D
Pretreatment	30	12	20.4	16.8	27
7	31.8	1.2	3	0.6	10.8
14	18.6	0	3	0.6	7.8
21	7.8	0	0.6	0	4.2
		% Control			
7		90ab	85b	96a	62c
14		100a	76b	94a	51c
21		100a	89a	100a	31b

Means for percent control for a sampling period followed by different letters differ significantly ($p < 0.05$) according to Fisher's protected LSD test.

Discussion

Diflubenzuron was tested in trials conducted over 10 y at eight locations in four countries on several species of grasshoppers and locusts with varied population densities. From studies discussed and papers reviewed, it appears that all pest species of grasshoppers and locust are susceptible to diflubenzuron. It was applied in broadcast (100% coverage), barrier and alternating systems, by ground and air, using five different formulations, and at rates ranging from 60 to 6 g ai ha^{-1}. Of the variables involved in these test programs, rate and application system seemed to have the most effect on speed of control. At lower rates and with alternating treated and untreated strips, it took >10 d before maximum levels of control were seen (35-g rate at 25% coverage, Volgograd, Table 4; 50% coverage at Almaty, Table 5; 50% coverage at Colorado, Table 6; 33% coverage at Orenberg, Table 8). Speed of control was also markedly different between diflubenzuron and the pyrethroids. Diflubenzuron performance did not peak until 7 to 10 d after application, but thereafter was maintained. The pyrethroids gave quick knockdown but then control dropped off (Volgograd, Table 4; Almaty, Table 5; Orenberg, Table 8). The efficacy of treating alternate strips relies on grasshoppers moving from the untreated strips to foliage treated with diflubenzuron, which remains active for up to 28 d (Table 6; Royer *et al.* 2001). This allows flexibility in the type of application method chosen (broadcast, barrier, alternating strips or RAAT) and treatment cost.

Effects on terrestrial nontargets susceptible to diflubenzuron would be lessened overall by using barrier or alternating sprays (Tingle 1997a). Such applications were tested in the USA, Russia and Kazakhstan. Diflubenzuron use resulted in control when applied in alternating swaths at a protective rate of 8 to 9 g ai ha^{-1} (Tables 5, 6). Application of 25 to 33% coverage, with protected area rates from 9 to 20 g ai ha^{-1}, gave acceptable control (Tables 4, 8). A protected area rate of 6.5 g ai ha^{-1} has also been shown to give >95% control of grasshopper populations under a RAAT approach, using alternating treatment and nontreatment swaths (Foster *et al.* 2000, Lockwood *et al.* 2001) in the USA. Field research in the USA has shown that 25 to 33% coverage can give acceptable levels of control (Lockwood *et al.* 2001). This type of application greatly reduces costs, making it more affordable to the rancher (Lockwood & Schell 1997). Foster *et al.* (2000) calculated the economic advantage for diflubenzuron using the RAAT approach in grasshopper control in USA rangeland. The RAAT strategy depends on movement of grasshoppers from untreated to treated swaths and on conservation of predators. Choice of rate and type of application (broadcast, barrier, RAAT) must be balanced against vegetation type and vigor. Dense, tall vegetation requires higher rates and sparse, short vegetation can be treated with lower rates (Lockwood & Schell 1997).

Visible effects of diflubenzuron on grasshoppers and locusts were noticed as early as 3 d after treatment in one of two sites in Argentina (Table 3, Site 2), Kazakhstan (Table 5) and in Russia (Tables 4, 8), in the form of lethargy and some reduction (≤20%) of population. Lethargic movement, cessation of feeding and delay in molting may be the response to gastric juices leaking through the peritrophic membrane of the grasshopper's midgut. Others (Cooper *et al.* 1995, Suleimenova *et al.* 1998, Yskak & Komissarova 1999) have also observed these symptoms as early as 3 d after treatment. Thus, reduction of populations of these pests on rangeland/pasture can be expected to start after approximately 3 d. Maximum control of populations occurred by 14 d after application in all reported trials. The most efficacious use of diflubenzuron occurs when applications are made on middle instars, which are growing rapidly and molting. While treatment of adults is less effective during the year of the application, it can reduce fecundity and egg laying, which results in smaller populations the following year. Grasshopper populations were monitored the year after application to immature stages in Trinidad, Colorado and Edgemont, South Dakota (Table 7). In both trials, populations remained lower in the treated in contrast to the untreated areas. In South Dakota there were 8 to 9% more grasshoppers in the area treated with carbaryl the previous year, compared to areas treated with diflubenzuron. After overwintering, the carbaryl block had a 30% higher grasshopper population than areas treated with diflubenzuron. This suggests that the effect of diflubenzuron on the reproductive capacity of adults in the year of treatment (as described above) is an important contributory factor to reduced populations the following year (Table 7).

Reduced locust and grasshopper numbers the year after application of diflubenzuron were also noted in a trial in Kazakhstan (Kambulin 2000). Rangeland treatment with diflubenzuron on a grasshopper population consisting of approximately 70% adults resulted in 35% control of that population between 7 and 28 d after treatment in 1999 (Lockwood *et al.* 2001). Four weeks after initial monitoring of egg hatch in 2000, a 75% reduction in population density compared to untreated was observed in the diflubenzuron areas. This implies that adults consuming diflubenzuron residues do not produce normal levels of viable eggs, or that they do not copulate successfully. Laboratory studies have identified such responses in adult grasshoppers exposed to diflubenzuron (Mariy *et al.* 1981, Lim & Lee 1982, Kepner 1989, Bouaichi & Chihrane 2001). Suleimenova *et al.* (1998) also noted that adult locusts lost their ability to lay eggs when feeding on diflubenzuron-treated foliage in the field.

Dimilin 25W had no effect on populations from orders of Homoptera, Hymenoptera, Coleoptera, Hemiptera, Lepidoptera, or Neuroptera (Table 2). Numbers of Diptera were statistically greater in contrast to the untreated control at several sampling points for both rates of Dimilin 25W and carbaryl. Numbers of Araneae were statistically less in the lower-rate treatment of Dimilin 25W through 14 d after application. The significance of this reduction is questionable because no effect was determined with the higher rate of Dimilin 25W (Table 2). The spider population dropped 95% from the 14-d to the 21-d sampling date in the untreated control, and the statistical increase in population with the high rate of diflubenzuron at 21 d may not be meaningful, due to the crashing population. These findings of no or low effects on nontarget arthropods are supported by other research. For example Catangui *et al.* (1993b) found no significant population reduction with Dimilin 2F (8.4, 16.8 and 33.6 g ai ha^{-1}) or Dimilin 25W (16.8 g ai ha^{-1}) for Araneae from 14 to 77 d after treatment in rangeland in Amidon County, North Dakota, USA in 1993. Hymenoptera (12 families), Coleoptera (6 families), Neuroptera (2 families) and Diptera were also unaffected. Silks of spider webs have been shown to be sites of agrochemical spray deposition (Samu *et al.* 1992). Consumption of webs by spiders to recycle silks occurs and if residues of diflubenzuron are present ,eggs laid subsequently could be affected. No effects of Dimilin 2F and Dimilin 25W at 17.5 g ai ha^{-1} were found on Araneae, Coleoptera (9 families), Diptera (2 families) and Neuroptera (3 families) from

7 to 76 d after treatment or 1 y after treatment in a rangeland trial near Ludlow, South Dakota, USA in 1993 (Catangui *et al*. 1995, 2000). Hymenoptera (16 families) were also not affected except with Dimilin 2F at one of the 10 sampling points (49 to 56 d after treatment) where a 44% reduction in ants was determined. Ant densities returned to untreated levels in subsequent samplings.

Conclusions

Diflubenzuron can be used to control locusts and grasshoppers with low impact on nontarget arthropods and other fauna. This IGR is particularly suited to locust and grasshopper control because all stages of these insects, from eggs to adults, are sensitive to direct or indirect effects, either during molting or during the various aspects of reproduction. From papers reviewed and other studies discussed in this paper it seems that all pest species of grasshoppers and locust are susceptible to diflubenzuron. It can be effectively applied with a variety of equipment in broadcast, barrier or RAAT treatment systems. These options allow great economic flexibility but must be carefully chosen to suit the levels of infestation and density of vegetation.

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