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THE EFFECT OF CYROMAZINE ON DIFFERENT STAGES OF *LIRIOMYZA TRIFOLII* (BURGESS) (DIPTERA: AGROMYZIDAE)*

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ABSTRACT

Cyromazine in the form of 75% Trigard WP is currently recommended in Israel as an aqueous spray at weekly intervals against a serpentine leafminer, *Liriomyza trifolii* (Burgess) on gypsophila. The concentration of 15.6 ppm a.i. sprayed postinfestation on bean seedlings prevented further development of 1st and 2nd instar larvae of *L. trifolii*. Formation of viable pupae was prevented by still lower concentrations. With 3rd instar larvae concentrations of 15.6-31.2 ppm were required.

In preinfestation treatment experiments, sprayed uninfested bean seedlings were exposed to ovipositing females after various aging periods of the residues. Application of 62.5 ppm prevented infestation by neonates up to 20 days after treatment; 15.6 ppm prevented formation of pupae for 20 days and 1.95 ppm for 4 days.

KEY WORDS: Liriomyza trifolii, **control of immature stages, cyromazine (Trigard).**

INTRODUCTION

Liriomyza trifolii Burgess (Diptera: Agromyzidae), a serpentine leafminer of American origin, was introduced into Israel (Brosh & Hadar, 1980) in gerbera cuttings in the summer of 1978; at the end of that summer it was present in chrysanthemum and gypsophila and later on spread to other flower and vegetable crops (Katzir & Brosh, 1981). At present it is a major pest of gypsophila, gerbera and chrysanthemum, causing damage also to other flower and vegetable crops (Yathom et al., 1983).

Control measures include use of contact insecticides against the adult flies, and control of the larvae — which spend their entire life cycle feeding within mines in the leaves — by pesticides that penetrate into the mines.

Cyromazine 75% WP (Trigard wettable powder, Ciba-Geigy Ltd.), a systemic pesticide with IGR properties (Anonymous, 1983) is approved for use in Israel (Hadar, 1985). It acts as a stomach poison affecting development, retarding growth, and interfering with moulting and pupation processes.

Cyromazine at a rate of 0.5 lb a.i./100 gallons (227 g/440 liter = 516 ppm) of water provided 100% prevention of adult emergence by treatment of both newly

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hatched and late third-instar larvae of *L. trifolii* on chrysanthemum (Parrella et al., 1982). It also effectively controlled the pest on field-grown peppers (Chandler, 1985) and tomatoes (Schuster & Everett, 1983). Concentrations of 600, 300, 150, 120 and 60 ppm completely prevented adult emergence when sprayed on newly eclosed larvae in chrysanthemum; treatment of third-instar larvae with 120 and 60 ppm did not prevent emergence of a few adults (Parrella, 1983a). Cyromazine in the form of a 5% soluble concentrate was phytotoxic after repeated applications to chrysanthemum, but it was claimed that this was probably attributable to the carrier and not the active ingredient (Parrella, 1983b). This was later determined to be true as 75% WP is not phytotoxic to chrysanthemums. Cyromazine was distinct from other IGR's, *e.g.* the JHA fenoxycarb, by acting on larvae still in the mines (Parrella et al., 1983).

This study was undertaken to test two aspects of cyromazine activity: the effect of different concentrations on development of the leafminer when applied at each of the larval instars; and persistence of various concentrations of cyromazine and their efficacy in preventing a new infestation after various aging periods.

MATERIALS AND METHODS

Culture of *Liriomyza trifolii*

Young plants from a permanent culture of bean seedlings (*Phaseolus vulgaris*, Bulgarian variety) in pots (height 9 cm, diam 10 cm) were used both for rearing *L. trifolii* and for the laboratory tests.

A *L. trifolii* culture, originating from larvae found in *Chrysanthemum* leaves in the Jordan Valley, was maintained in the laboratory in a transparent perspex rearing cage (125x55x60 cm) kept at 27°C and a 16:8 LD photoperiod. Potted bean seedlings were exposed to an adult fly population in the rearing cage for 24 h. After excluding the flies the plants were kept under the same environmental conditions until the larvae were ready to pupate. The infested leaves were then cropped and kept on trays. Close to emergence, the trays were placed in the rearing cage.

The tests were carried out in a greenhouse at 27°C and under a 16:8 LD regime using plants which were infested as follows. Each treatment included three pots, each containing four bean seedlings; each seedling had two first true leaves. Thus there were altogether 12 seedlings with 24 first true leaves, which were exposed for 24 h to 50 ovipositing female flies. The insecticidal treatments were applied either postinfestation or preinfestation.

(i) Postinfestation treatment

To study the effect of different concentrations of cyromazine on the various larval instars, bean seedlings were infested as described above and kept until early larvae of the required instar appeared. The plants were then sprayed with aqueous dilutions of the cyromazine WP at concentrations ranging from 500 to 0.975 ppm a.i. The treated plants were observed for feeding damage, and the number of pupae developing on the leaves was recorded. The leaves were then cut off the plants and placed in petri dishes and, ultimately, adult emergence was recorded.

(ii) Preinfestation treatment

To test the persistence of cyromazine and its ability to prevent infestation, bean seedlings were sprayed as above with the same aqueous dilutions of cyromazine. Sets of treated plants were exposed to *L. trifolii* flies on the day of treatment or 1, 4, 7, 10 and 20 days later. The number of larvae that developed and pupated as well as the rate of adult emergence were recorded.

The efficacy of cyromazine on postinfestation or preinfestation application in controlling *L. trifolii* larvae infesting leaves was assessed in several ways.

1. The rate of feeding and the resultant damage were graded according to the size and number of mines and the portion of leaf area they occupied, as follows: a – leaves totally free of leafminer damage, b – some damage due to either neonate feeding mines or bigger, but separate and countable mines, c – the total leaf area, or a great part of it criss-crossed with mines of mature larvae.

2. The number of pupae formed was counted and served as an indication of the larvae that had successfully passed all previous molts.

3. The number of adults emerging from the pupae was recorded to assess pupal viability. In the control leaves the area available might have been too small to support all developing larvae, as intraspecific competition was shown to affect the ratio of emergent pupae to eclosed larvae (Parrella, 1983c).

RESULTS

(i) Postinfestation treatment

Leaf damage. Distribution of leaves according to the degree of feeding and damage by *L. trifolii* larvae is presented in Table 1. When leaves infested with first-instar larvae were sprayed, some damage was already present, but concentrations from 15.6 ppm and up prevented additional damage. At lower concentrations larval activity continued and a greater percentage of the leaves, although less than in the

TABLE 1. DISTRIBUTION OF LEAVES (%) ACCORDING TO DEGREE OF DAMAGE BY LEAFMINERS AFTER CYROMAZINE SPRAYS OF DIFFERENT CONCENTRATIONS ON BEAN LEAVES INFESTED WITH VARIOUS INSTARS OF *LIRIOMYZA TRIFOLII* (POSTINFESTATION TREATMENT). a – NO DAMAGE, b – SMALL LEAF AREA AFFECTED, c – STRONG FEEDING TO TOTAL LEAF AREA AFFECTED.

Larval instar treated:	1st			2nd			3rd		
Concentration (ppm)	Degree of damage								
	a	b	c	a	b	c	a	b	c
500	0	100	0	0	100	0	13	52	35
250	0	100	0	0	100	0	0	33	67
125	0	100	0	–	–	–	12	25	63
31.2	0	100	0	0	100	0	0	39	61
15.6	0	100	0	0	100	0	17	21	62
7.8	0	94	6	0	100	0	–	–	–
3.9	0	90	10	17	83	0	0	55	45
1.9	13	55	32	0	40	60	5	20	75
0.9	4	50	46	0	50	50	0	38	62
0 (Control)	13	13	74	4	42	54	0	20	80

– not examined

controls, showed higher feeding damage (grade b). When leaves with second-instar larvae were sprayed, results were similar.

Leaves with third-instar larvae already exhibited considerable damage (grades b and c) when sprayed.

Survival. The total number of pupae formed, their mean number per leaf, and their emergence rate are presented in Table 2. When leaves with first-instar larvae were sprayed, some viable pupae, with an emergence rate as high as in the controls, were formed only at the lowest concentration, 0.975 ppm.

When leaves with second-instar larvae were sprayed, pupae were found at 7.8 ppm, but they were deformed; only at the low concentrations of 1.9 and 0.975 ppm did flies emerge, although the emergence rate was lower than in the controls.

When leaves with third-instar larvae were sprayed, pupae were found at all concentrations, although only a few at the high concentrations, but from those formed between 500 to 125 ppm no flies emerged. At lower concentrations (3.9 ppm and less) the number of pupae and the emergence rate increased.

(ii) Preinfestation treatment

Damage. Distribution of leaves according to feeding damage after exposure of cyromazine treated plants to *L. trifolii* infestation is presented in Table 3. At 500 and 250 pm, many of the leaves showed no feeding damage (grade a) and the rest showed only initial neonate feeding (grade b), up to 20 days after treatment, *vs.* the majority of leaves heavily damaged (grade c) in the controls. No heavy feeding (grade c) occurred at concentrations of 62.5 and 15.6 ppm, which still reduced feeding and damage up to 20 days after application, though less than in the higher concentrations. At 1.95 ppm there were already leaves that showed heavy feeding damage, but their number was much smaller than in the controls. The 0.975 ppm spray proved to be inactive already after one day.

Survival. The mean number of pupae/leaf and the emergence rate, when treated plants were exposed after different aging periods of the residues to ovipositing females, are presented in Table 4. Concentrations of 500 to 15.6 ppm prevented formation of viable pupae up to 20 days after application; the few pupae found were dead. Formation of pupae was prevented by 1.95 ppm when infestation took place 4 days after spraying, but when leaves were infested 7 days after application pupae were formed but flies did not emerge. From the infestation on the 10th day after treatment a few flies emerged. 0.975 ppm did not prevent formation of pupae even when the plants were infested on the day of treatment.

DISCUSSION

The present recommendations for control of *L. trifolii* on flowers in greenhouses in Israel include sprays with cyromazine, at a rate of 250 g/ha of the 75% Trigard WP, once a week (Hadar, 1986; Sheinboim & Ohali, 1986). This quantity is applied in a spray volume of 300-500 1, that is, cyromazine concentrations of either 625 or 375 ppm a.i. The lower concentration lies between the two highest concentrations (500 and 250 ppm) used in the present study for both postinfestation and preinfestation treatments.

TABLE 2. EFFECT OF CYROMAZINE SPRAYED ON LEAVES INFESTED WITH 1ST-, 2ND- AND 3RD-INSTAR *LIRIOMYZA TRIFOLII* LARVAE (POSTINFESTATION TREATMENT).

Larval instar treated:	1st			2nd			3rd		
Concentration (ppm)	*Number of pupae* Total	Mean/ leaf (± SE)	% Emergence	*Number of pupae* Total	Mean/ leaf (± SE)	% Emergence	*Number of pupae* Total	Mean/ leaf (± SE)	% Emergence
500	0			0			10**		
250	0			0			6**		
125	0			0			9**		
31.2	0			0			1		
15.6	0			0			73	3.0 ±1.0a	14
7.8	0			25**	1.3 ±0.4a	0	–	–	–
3.9	0			1*		0	92	4.2 ±0.9ab	9
1.9	22*		0	34**	2.7 ±1.0ab	44	108	5.1 ±1.1ab	19
0.9	75**	2.5 ±0.6a	65	84**	3.8 ±0.8bc	35	132	6.3 ±1.1b	57
0 (Control)	159	6.9 ±0.6b	60	171	7.1 ±1.9c	68	117	5.8 ±0.2b	80

*Dead
**In part deformed and abnormal
– not examined
Within each column, values followed by the same letter do not differ significantly at P = 0.05 by Duncan's Multiple Range Test.

TABLE 3. DISTRIBUTION OF LEAVES (%) ACCORDING TO DEGREE OF DAMAGE CAUSED BY LEAFMINERS TO CYROMAZINE TREATED BEAN LEAVES EXPOSED TO *LIRIOMYZA TRIFOLII* INFESTATION (PREINFESTATION TREATMENT)
a – NO DAMAGE, b – SMALL LEAF AREA AFFECTED, c – STRONG FEEDING TO TOTAL LEAF AREA AFFECTED.

Concentration (ppm)	Infested on: 0 day after treatment			1st			4th			7th			10th			20th		
	Degree of damage																	
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
0	0	67	33	0	0	100	0	10	90	0	14	86	0	10	90	0	14	86
500	42	58	0	4	96	0	0	100	0	14	86	0	21	79	0	29	71	0
0	10	74	16	0	24	76	26	26	48	5	9	86	0	10	90	0	14	86
250	62	38	0	50	50	0	38	62	0	86	14	0	4	96	0	14	86	0
0	0	0	100	0	36	64	0	43	57	10	85	5	0	48	52	0	21	79
62.5	0	100	0	20	80	0	17	83	0	4	96	0	26	74	0	0	100	0
0	–	–	–	0	75	25	0	6	94	0	62	38	0	48	52	0	21	79
15.6	–	–	–	33	67	0	100	0	0	0	100	0	15	85	0	0	100	0
0	0	36	64	13	57	30	4	38	58	0	18	82	0	48	52			
1.95	0	90	10	0	92	8	17	70	13	0	87	13	48	43	9			
0	0	37	63	32	62	6												
0.975	27	68	5	5	40	55												

– not examined

TABLE 4. PERSISTENCE OF CYROMAZINE RESIDUES FROM DIFFERENT CONCENTRATIONS SPRAYED ON LEAVES BEFORE EXPOSURE TO INFESTATION BY *LIRIOMYZA TRIFOLII* (PREINFESTATION TREATMENT)

	Infested on:											
	0		1st		4th		7th		10th		20th	
	day after treatment											
Concentration (ppm)	Number of pupae/ leaf (±SE)	% Emergence	Number of pupae/ leaf (±SE)	% Emergence	Number of pupae/ leaf (±SE)	% Emergence	Number of pupae/ leaf (±SE)	% Emergence	Number of pupae/ leaf (±SE)	% Emergence	Number of pupae/ leaf (±SE)	% Emergence
0	4.9 ±1.1	72	16.8 ±0.4	36	4.1 ±0.8	32	8.8 ±1.0	56	10.6 ±1.2	63	7.8 ±1.3	43
500	0		0		0		0		0		0	
0	1.2 ±0.3	39	6.9 ±0.9	55	2.4 ±0.7	85	7.8 ±3.4	91	8.2 ±0.5	57	7.8 ±1.3	43
250	0		0		0		0		0		0	
0	11.4 ±1.2	63	5.0 ±0.1	73	3.9 ±0.8	66	1.6 ±0.3	76	4.8 ±0.7	–	3.3 ±0.8	–
62.5	9*		0		1*		0		0		0	
0	–	–	2.4 ±0.5	–	14.8 ±2.2	57	3.3 ±0.7	44	4.8 ±0.7	–	3.3 ±0.8	–
15.6	–	–	0		0		0		0		0	
0	–	–	3.5 ±0.7	74	4.1 ±0.9	34	5.4 ±1.2	86	4.4 ±0.6	65		
1.95	–	–	3**	–	0		3.2 ±0.3	0	2.8 ±0.9	17		
0	4.9 ±0.8	58	2.2 ±0.7	–								
0.975	0.8 ±0.1	47	4.1 ±0.6	–								

*Dead pupae (total number per experiment)
**Deformed and abnormal pupae (total number per experiment)
– not examined

When leaves infested with first- and second-instar larvae were treated, the concentrations between 500 and 3.9 ppm prevented further feeding damage, pupation, and consequently development of a new generation. In analogous postinfestation experiments with third-instar larvae, there was already conspicuous leaf damage due to the mines established before treatment. At the high concentrations only a few larvae pupated to deformed pupae, from which no flies emerged. Also at low concentrations many larvae pupated to pupae of normal aspect, but the emergence of adults was much lower than in the controls.

In the preinfestation experiments long persistence of cyromazine was found.

A repellent effect to adults was excluded, as only with 500 ppm on the day of application was some repellent effect displayed.At 250 ppm and down, no repellent effect was noted, even on the day of application. Concentrations down to 15.6 ppm prevented infestation due to penetration of neonate larvae, until 20 days after the treatment. Also at the lower concentration of 1.95 ppm, which did not prevent development of larvae, leaf damage and formation of pupae, the emergence rate was negligible or very low at the different aging periods.

The recommended cyromazine sprays are, indeed, efficient and prevent fresh infestation by the serpentine leafminer damage. It is important to remember that cyromazine does not kill the larvae directly, but is an IGR interfering with molting, and therefore its action does not express itself immediately after application on the leaf. Also, the interruption of feeding activity is not immediate.

In order to reduce damage it is advisable to use an adulticide in addition to the cyromazine treatment, thus decreasing oviposition and infestation pressure.

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