Original Article

Side Effects of IGR Cyromazine on *Nasonia vitripennis* (Hymenoptera: Pteromalidae), a Parasitic Wasp of House Fly Pupae

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(Received 30 Nov 2008; accepted 12 May 2009)

Abstract

Background: Combination of cyromazine as an Insect Growth Regulator (IGR) and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) a parasitic wasp may be an effective tool for reducing the house-fly populations in poultry houses and livestock farms. This study was conducted to assess the side effects of the IGR cyromazine on the level of parasitism and numbers and the longevity of emerged *N. vitripennis* parasitoids from house fly pupae.
Methods: Cyromazine treated cloth target was used as the contaminating method of the parasitoids which was applied in this research study.
Results: The Weibull distribution showed that there was no significant difference among controls and cyromazine treated targets for longevity data. There was no significant effect of cyromazine on the level of parasitism of *N. vitripennis* using χ^2 test. One-way ANOVA showed that the actual numbers emerging were significantly higher in the control than in two cyromazine treatments; however, it is a useful phenomenon because of reducing the hyperparasitism.
Conclusion: There is a good consistency between using *N. vitripennis* and 1.1% or 0.9% cyromazine treated targets. Therefore cyromazine treated targets can be applied as a safe delivery vehicle for applying the cyromazine IGR in the poultry houses and livestock farms in an Integrated Pest Management (IPM) program.

Keywords: *Nasonia vitripennis*, Cyromazine, IGR, Weibull distribution,

Introduction

Commercial poultry houses and livestock farms are rapidly expanding worldwide to meet the needs of the increasing human population (Axtell 1999). Therefore, the increase in accumulating manure is unavoidable. This phenomenon provides breeding places for different groups of pests, with house-flies being the most abundant species in poultry facilities and livestock farms. As a result, *Musca domestica* is the primary object of most fly management and control programs (Wilhoit et al. 1991).

Chemical control methods, using different insecticides belonging to the chlorinated hydrocarbon, organophosphate, carbamate and pyrethroid groups, can provide a rapid and easy means of suppressing house-fly populations and have become popular since the 1950s. The use of those insecticides, however, has created several problems including resistance to insecticides, environmental pollution and the creation of new pests. As a consequence, extensive research has been carried out to find suitable alternatives, particularly ones, which can be incorporated into Integrated Pest Management (IPM) programs (Senior 1998, Vazirianzadeh 2003).

Combination of an insecticide and a parasitic wasp is a very serious issue in the IPM

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(Emden 1984, Zhao 2000, Wang 2008). They have also expressed that chemical control and biological control are two important strategies and key points for the success in an IPM program.

Combination of cyromazine as an Insect Growth Regulator (IGR) and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) a parasitic wasp may be an effective tool for reducing the house-fly populations in poultry houses and livestock farms. However, the effective exploitation of natural enemies in IPM fly management programs requires defining the compatibility of parasitoids with insecticides in use (Scott et al. 1991). In addition, the parasitoids are at the risk of direct and indirect contacts with the IGRs therefore the consistency between two agents is an essential figure. Consequently, it was also necessary to evaluate the potential detrimental effect of cyromazine on, or compatibility with, the important species of natural enemies, in this case one species of Pteromalid parasitoid. Those criteria have also been discussed by Ruberson et al. (1998), Haseeb et al. (2004), Desneux et al. (2007), Wang et al. (2008) and as direct effects (acute effects) and indirect effects (chronic effects) of insecticides on biological control agents, like mortality or parasitism rate, longevity, egg viability, consumption rate and behavior, respectively.

The objectives of the current study were to assess the side effects of the IGR on the level of parasitism, numbers of emerged parasitoids and the longevity of parasitoids after emergence.

Material and Methods

Cyromazine treated cloth target was used as the contaminating method of the parasitoids which was applied in this research study.

IGR

In this research one commercial formulation of cyromazine, Neporex® (2% w/w), was used. In this case 32% w/v sugar solution was used as a solvent.

Insects

Parasitoids *N. vitripennis* came from Wye College, UK, colonies. Originally *N. vitripennis* parasitoids were reared in *Sarcophaga* sp. (Diptera: Sarcophagidae) pupae. In the present study, the parasitoids were reared in the constant environmental chambers (25°C, 65% RH and 12 h L: 12 h D) of Cardiff School of Biosciences using house-fly pupae in 2003. Moreover, the rearing was carried out using 1000 cm^3 and 250 cm^3 glass jars with proportion of 1 parasitoid/5 house-fly pupae. They were fed with 5% honey solution. House-fly pupae were taken from the "Chicken house" a wild strain originated from a Carefilly poultry house of Wales, UK as well.

Experiments

Two series of treatments were conducted. In the first, newly emerged parasitoids were exposed for 48 h in 1000 cm^3 glass jars to targets containing either 1.1 or 0.9 g/100 ml in 32% sugar solution giving 0.02 mg a.i./cm^2 and 0.016 mg a.i./cm^2 respectively and for the control, 32% sugar treated targets only. This ensured that the parasitoids were exposed to the IGR. Then five females of *N. vitripennis* parasitoids (two-day-old) plus two males of *N. vitripennis*, taken from the 1000 cm^3 glass jars, were placed in a 25 ml glass test tube with twenty-five one day-old house fly pupae (Morgan et al. 1989, Mann et al. 1990, Scott et al. 1991). Three replicates were carried out, each replicate containing ten test tubes. This means that each replicate contained fifty female adult parasitoids and 250 house-fly pupae. Some tiny holes were made around the middle of the test tubes for ventilation. Then 30 pieces (5×5 cm) of polyester cloths were dipped in the 1.1 g/100 ml and another 30 in 0.9 g/100 ml cyromazine solutions. Then the tops of test tubes were sealed with the treated clothes. After 48 h the pupae were replaced

with twenty-five fresh pupae, the numbers being kept constant during the experiments. Then, a sample of ten pupae was collected from each test tube, placed individually in plastic Petri dishes (Geden et al. 1992) and kept for 3 weeks in the constant environmental room (25° C, 65% RH and 12 h L: 12 h D). The level of parasitism and the numbers of emerged parasitoids were recorded. This procedure was carried out until all of parasitoids died. The cloths were treated with 1.1 g/100 ml and 0.9 g/ml of IGR in 32% w/v sugar solution every 24 h. Three replicates were done with 32% w/v sugar-only treated cloths as controls.

The second treatment was used to examine the longevity of parasitoids under the IGR and control regimes. Fifty newly-emerged *N. vitripennis* (per replicate) were placed individually in test tubes, to remove the effects of population density of population longevity and to determine accurately individual longevity. The top of the test tubes were covered by cloths treated with either 1.1 or 0.9 g/ml cyromazine in 32% sugar solution or 32% sugar solution alone (control). Then 5 house-fly pupae (one-day-old) were put in each test tube. The house-fly pupae were replaced with fresh ones every 48 h. The cloths were treated with same concentration each 24 h. The number of dead parasitoids was recorded every 48 h. Three replicates per treatment were conducted.

Data analysis

Chi-squared tests were used to test the effects of the IGR on the level of parasitism. Where replicates were found to be homogeneous (again using Chi-squared tests), the results of each treatment were summed.

To determine the effects of IGR on the numbers of emerged parasitoids, one-way ANOVA was used, after preliminary diagnostic checks for normality of residuals (Ryan-Joiner test) and homogeneity of variances (Bartlett's and F-tests). The Least Significant Difference (LSD) method was used to detect any differences between treatments and controls, with Bonferroni corrections where necessary.

To determine the longevity parameters of parasitoids, the Weibull distribution was used. It enables statistical comparison of the shape and scale of different survival curves, providing valuable information which is lost if longevity is summarized as a mean with standard deviation, or as a single LT_{50} value, as commonly done (Pinder III et al. 1978, Tingle and Copland 1989).

In addition, Minitab performs a series of Chi-squared tests and provides 95% confidence intervals for testing weather two or more samples have equal shape or scale and come from the same population. Also, testing weather the distribution parameters are consistent with specified values (Manual of Minitab 13.1, 2000, University of Wales computing service, 2001 and communication with office of Minitab, 2001). As a result by means of the above mentioned procedures the longevity of parameters, scale and shape, were determined. As well LT_{50}s were obtained from the table of percentiles and used to compare the results of treatments.

Results

The results in Table 1 show that there was no significant effect of cyromazine on the level of parasitism of *N. vitripennis* (Mean percentage parasitism, $\chi^2= 0.46$; $P= 0.794$; d.f= 2).

One-way ANOVA (Table 1) showed that, while there was no differences in the level of parasitism between the treatments and control (using Chi-squared tests), the actual numbers emerging were significantly higher in the control than in two cyromazine treatments ($P< 0.001$). However, there was no difference between the 1.1% and 0.9% cyromazine treatments.

The longevity results, as described by the Weibull distribution, are summarized in Table 2 (as an example of Weibull distribution).

All shape parameters of controls and IGR-treatments were significantly larger than 1 ($P< 0.001$), indicating that all of the longevity curves belonged to the Type I shape category. Weibull results, using Chi-square tests for comparisons, showed that there was no significant different among controls and cyromazine treated targets for longevity data, shape parameters ($P= 1$), scale parameters ($P= 1$) and overall shape and scale. Those results explain that cyromazine did not effect on the longevity of *N. vitripennis* population. Comparisons of LT_{50}s for each treatment were also consistent with these results, showing no significant differences due to overlapping confidence intervals.

Table 1. Effect of cyromazine on mean percentage parasitism of *N. vitripennis* (2-day olds) over 10-12 days, treated targets

Treatments	Mean number of emerged parasitoids	SE	Mean percentage parasitism	SE
32% sugar solution only treated targets	1724.00	23.47	53.47	0.27
0.9% cy in 32% sugar solution treated targets	874.30	10.92	52.47	0.35
1.1% cy in 32% sugar solution treated targets	874	8.03	52.33	0.41
Comparison of all treatments	One-way ANOVA*F (P, df error/df of replicate)		χ^2 (P, df)	
	986.04 (0.001, 6/2)		0.46 (0.79, 2)	

cy=cyromazine

*LSD results showed that there was a highly significant difference between the sugar-only treated targets and both concentrations of cyromazine. However, there was no significant difference between the two concentrations of cyromazine.

Table 2. Longevity of parasitoids (newly emerged females) as described by the Weibull distribution, using different concentrations of cyromazine treated targets

Treatments	Shape value	SE of Shape	Scale value	SE of scale	Mean value	SE of mean	LT_{50} value	SE of LT_{50}	Curve type	r*	df of r
N[1]/control	1.868	0.267	9.028	0.511	8.016	0.474	7.420	0.374	I	0.992	3
N/0.9% cy[2]	1.872	0.260	8.975	0.498	7.986	0.461	7.380	0.371	I	0.993	3
N/1.1% cy[3]	1.878	0.253	8.887	0.481	7.889	0.443	7.311	0.365	I	0.994	3

All scale, mean and LT_{50} values are in days, Size of population = 150

[1] *N. vitripennis,* [2] 0.9%cyromazine in 32% sugar solution, [3] 1.1%cyromazine in 32% sugar solution, r* correlation coefficient

Discussion

According to Scott and Rutz (1988), Mandeville et al. (1990), Rutz and Scott (1990), Scott et al. (1991), Floate (1998), Floate and Fox (1999) the use of different classes of insecticides, including IGRs, in animal houses has adverse effects on an ecologically and taxonomically diverse group of insects, including both predators and parasitic wasps. Therefore, to apply integrated pest management effectively in poultry houses and livestock farms, using a combination of IGRs and parasitic wasps, it is important to assess the compatibility of the control agents.

The results obtained in this study report the different effects of cyromazine on *N. vitripennis*. There were no harmful effects on the level of parasitism of *N. vitripennis*, using 1.1%

and 0.9% cyromazine treated targets, but those concentrations were highly significant in reducing the number of *N. vitripennis* emerging per pupa from 6.45±0.1 in control to 3.33±0.03 and 3.34±0.04 in 1.1% and 0.9% cyromazine, respectively. It is possible, however, that this effect could be construed as beneficial in reducing the rate of superparasitism in this species, although this reduction might affect the next generation of the parasitoid.

Both concentrations of cyromazine caused the same effects on the number of emerged parasitoid, using target application. This phenomenon explains that the parasitoids could parasitize the treated pupae, normally. Presumably, some of their eggs did not hatch or some of larvae died after emergence in the host puparia. Both effects have been reported as the properties of cyromazine. The same level of parasitism between two concentrations and controls explain that the rest of larvae of parasitoids carried out the successfully parasitism.

Results of the study of Wang et al. (2008) showed that using IGRs (Hexaflumuron, Chlorfluazuron, Buprofezin and Fuxian) performed very low contact and residual toxicity, however with exhibition chronic effects of oral toxicity on longevity, fecundity and offspring emergence of *Anagrus nilaparvatae* (Hymenoptera: Mymanidae), an egg parasitoid of the rice planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). In contrast to the IGRs using the convenience insecticides presented the highest contact and residual toxicity in the study of Wang et al. (2008).

The results of study of Srinivasan and Amalraj (2003) using a combination of parasitoid *Dirhinus himalayanus* (Hymenoptera: Chalcididae) and insect growth regulator, triflumuron against house fly, *Musca domestica* (Diptera: Muscidae) show that it is effective in reducing puparia and fly density. Therefore, for sustenance of an effective fly control program, combination a parasitoid and an IGR may be used.

The Weibull results here show that 1.1% and 0.9% cyromazine did not affect the longevity of *N. vitripennis* and there was no significant difference amongst the treatments and control.

Consequently, there should be a good consistency between using *N. vitripennis* and 1.1% or 0.9% cyromazine treated targets. Then it is suggested using cyromazine treated targets as a safe delivery vehicle for applying the cyromazine IGR in the poultry houses and livestock farms in an Integrated Pest Management (IPM) program.

Acknowledgements

Iran Ministry of Health who sponsored financially this study is appreciated. Thanks Mr. P. Macaughen of Computing Service, Cardiff University of Wales for providing Weibull distribution to interpret the longevity results.

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