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Does the size of larval groups influence the effect of metabolic inhibitors on the development of *Phormia regina* (Diptera: Calliphoridae) larvae ?

PAUL W. C. GREEN[1, 2], MONIQUE S. J. SIMMONDS[1] and WALLY M. BLANEY[2]

[1]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, U.K.; e-mail: paul.green@rbkew.org.uk
[2]School of Biological and Chemical Sciences, Birkbeck College, Malet Street, London, WC1E 7HX, U.K.

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Abstract. A series of experiments were conducted to compare the susceptibility of *P. regina* larvae reared in isolation or in groups to the effects of diet-borne metabolic inhibitors: chlorogenic acid (CGA) and mimosine. Larvae were presented with diets containing 0.4 mM CGA or 0.4 mM CGA in combination with 22 mM lysine or methionine or with diets containing 1.5, 15 or 30 mM concentrations of mimosine. Methionine and CGA caused significantly reduced pupal weights when compared with larvae presented with lysine and CGA. All concentrations of mimosine resulted in 100% mortality with larvae unable to successfully complete pupation even at the lowest concentration. In general, larvae reared in groups were less susceptible to the toxic effects resulting from diet-borne metabolic inhibitors. The results are discussed in relation to the chemical factors that result from the feeding activity of saprophagous dipterans.

INTRODUCTION

Alteration to the nutrient levels in the diet of *Phormia regina* (Meigen) (Diptera: Calliphoridae) can affect the development of larvae (Hill et al., 1947; Brust & Fraenkel, 1955; McGinnis et al., 1956). If calliphorid larvae do not receive sufficient nutriment they produce smaller larvae, pupae and adults [for example, *Lucilia sericata* (L.), Daniels et al., 1991 and *L. caesar* (L.) Okorie and Okoke, 1990]. Metabolic inhibitors, such as CGA, can similarly retard the growth of insect larvae (Kimmins et al., 1995) by interfering with digestion of protein and absorption of the resulting amino-acids (Felton et al., 1992; Stevenson et al., 1993a, 1993b). Toxic and antinutritive effects of CGA can be reduced if sufficient quantity of specific amino-acids such as lysine, methionine and/or histidine are present in a substratum (Felton et al., 1992). These amino acids can react with and bind to CGA, reducing its toxicity by preventing further reactions with other dietary nutrients (Felton et al., 1992).

Protein metabolism can also be affected by chemicals that interfere with conversion of amino acids and peptides into body-mass. One such chemical is mimosine, a non-protein amino acid isolated from Legumes, such as *Leucaena* and *Mimosa*. Mimosine becomes incorporated into amino-acid metabolism and results in incorrectly formed molecules or toxic products (Wink, 1997).

Provided that there is sufficient space and nutriment available, calliphorid larvae from the genus *Lucilia* are known to obtain developmental benefits from the close proximity of conspecifics (Hanski, 1977). The feeding activity of groups of *Lucilia* larvae can aid digestion of a substratum as a result of alkaline and tryptic exudates from larvae (Hobson, 1932; Wigglesworth, 1965; Constable, 1994; Casu et al., 1996). By rearing *P. regina* larvae on 30 mL aliquots of a casein, agar and yeast (CAY) artificial diet at densities of 5, 10, 20 and 40 larvae, in a plastic Petri dish (9 cm diam.), an optimum group size for *P. regina* was found to be 10 larvae per Petri dish. Pupal weights declined and/or development-time increased when larvae were reared at lower or higher densities (Green, 1999). The aim of the experiments presented below was to observe if groups of 10 *P. regina* larvae benefit from the proximity of others and if they are able to either alleviate or circumvent the effects of metabolic inhibitors when compared with isolated larvae. Larvae reared upon diets containing metabolic inhibitors were compared with those reared upon diets without metabolic inhibitors.

MATERIALS AND METHODS

Insects

A colony of *Phormia regina* was maintained in the School of Biological and Chemical Sciences, Birkbeck College as described in Green et al. (submitted). Four-day-old larvae (10 to 12.9 mg) were used for the experiments to ensure that the weights of the starting populations were uniform.

Method

CGA (0.4 mM) (Sigma Chemical Co., UK) was incorporated into a casein (36 gl^{-1}) (Sigma Chemical Co., UK), agar (12 gl^{-1}) (Fisons, UK) and yeast (100 gl^{-1}) (Philip Harris Scientific, UK) (CAY) diet. Aliquots (30 mL) were poured into plastic Petri dishes (9 cm diam., Sterilin) and left to set and cool. This process was repeated until 8 different CAY diets containing a metabolic inhibitor and/or amino-acids had been prepared (Table 1). Groups (n = 3, per diet) of four-day-old larvae (n = 10) were dried in vermiculite and cleaned, using a soft-bristle paintbrush, before they were placed into a Petri dish containing a test diet. This was repeated for single larvae (n = 25, per diet). Fifty isolated larvae and 5 groups of larvae (n = 10) were presented with a CAY control diet (without metabolic inhibitors or amino-acids). All dishes were placed into a growth cabinet set to 23 ± 2°C, 12L : 12D h. Each dish was checked daily for

TABLE 1. Combinations of metabolic inhibitors and/or amino-acids added to CAY diets and presented to larvae of *P. regina*.

Chemical	Diet								
	1	2	3	4	5	6	7	8	9
CAY control diet									
CGA (0.4 mM)		√	√	√					
lysine (22 mM)			√		√				
methionine (22 mM)				√		√			
mimosine (30 mM)							√		
mimosine (15 mM)								√	
mimosine (1.5 mM)									√

√ = chemical was present in a diet. For example, diet 4 contained CGA (0.4 mM) and methionine (22 mM).

pupae and dead larvae. Pupae were removed, cleaned and weighed and dead larvae were discarded. Diets were replenished every two days. Each experiment concluded when all larvae had pupated or died. Pupal weights were compared between pairs of diets, or between different densities of larvae using the Wilcoxon-Mann-Whitney test (WMW) (Siegel & Castellan Jr., 1988). A comparison was also made, using the WMW-test, between the times for which larvae survived ("longevity") when reared at densities of 1 or 10 larvae per Petri dish on CAY diets containing 30, 15 or 1.5 mM mimosine. Mortality was compared among diets using the Poisson analysis (Poisson) (Parker, 1988). After 2 days the pH of the CAY control diets was measured using pH test strips (0–14 range, Sigma Chemical Co., UK) at a point where the diet had been most liquefied by the feeding of larvae. Random samples (n = 5) were selected from the 25 dishes containing isolated larvae. Also the pH of the CAY diet in 5 Petri dishes without larvae was measured. The Student's t-test (Microsoft ® Excel 97) was used to compare the pH of diets in Petri dishes containing 0, 1, and 10 larvae.

RESULTS

Inclusion of methionine or lysine in a CAY diet did not affect the pupal weights (WMW, $P > 0.05$) or mortality (Poisson, $P > 0.05$) of *P. regina* larvae relative to a CAY diet control. A CAY diet containing lysine and 0.4 mM CGA caused more mortality of isolated larvae than of groups of larvae (Poisson, $P < 0.01$; Table 2). In all other instances mortality was not affected by group-size. Diets containing CGA and CGA in combination with methionine resulted in reduced pupal weights relative to those larvae presented with a CAY control diet (WMW, $P < 0.05$). However, larvae reared in isolation on diets containing 0.4 mM CGA and either lysine or methionine gave rise to less heavy pupae than those larvae reared in groups (WMW, $P < 0.001$; Table 2). Methionine and 0.4 mM CGA were more inhibitory to weight gain than lysine and 0.4 mM CGA, evidenced by significantly reduced pupal weights (WMW, $P < 0.001$; Table 2). All the larvae reared on diets containing mimosine died. There was, however, a difference in the onset of mortality. Most of the larvae exposed to 1.5 mM mimosine (60%) died at pupation with pupal cases incompletely tanned and prone to rupturing. In contrast, larvae exposed to 30 and 15 mM mimosine all died prior to the pre-pupal phase. Larvae reared in groups were able to survive for longer than isolated larvae when reared on a CAY diet containing 15 or 1.5 mM mimosine (WMW, $P < 0.05$; Table 3). Larvae reared in groups of 10 on an untreated CAY diet caused a significant change to the pH of the substratum, when compared with isolated larvae (t-test, $P < 0.05$; Table 4).

DISCUSSION

When reared upon casein, agar and yeast diets, groups of *P. regina* larvae gave rise to heavier pupae than those larvae reared in isolation. Furthermore, larvae reared in groups were able to reduce and in some instances delay the onset of the effects of CGA and mimosine, when compared with isolated larvae. Previous experiments have shown that larvae of *P. regina* reared on liver develop more quickly into heavier pupae when compared with those larvae reared in isolation (Green et al., submitted). Thus, individuals within groups of *P. regina* larvae are able to release more nutriment from diets than isolated larvae. Blowfly larvae produce tryptic and alkaline secretions and excretions (Hobson, 1932; Hanski, 1987) which pre-digest the substratum, with groups producing a greater volume of exudate than isolated larvae. The feeding activity of groups of larvae of *P. regina* was shown to have a greater effect on the chemical nature of the diet surfaces than the feeding of isolated larvae. Spe-

TABLE 2. Mean pupal weights (mg ± sem) and percentage mortality (± sem) when *P. regina* larvae were reared at different densities upon CAY diets that contained CGA and either lysine or methionine.

Conditions	Mean pupal weight, mg ± sem[1]	Mean percentage mortality, ± sem[2]
10 larvae: CAY diet (control)	45 ± 1.8 a	12 ± 2.0 a
1 larva: CAY diet (control)	35 ± 0.6 b	20 a
10 larvae: CAY diet + 0.4 mM CGA	36 ± 1.3 b	60 ± 5.8 b
1 larva: CAY diet + 0.4 mM CGA	32 ± 1.2 c	30 ab
10 larvae: CAY diet + lysine and 0.4 mM CGA	42 ± 2.7 a	17 ± 3.3 a
1 larva: CAY diet + lysine and 0.4 mM CGA	26 ± 1.0 d	56 b
10 larvae: CAY diet + methionine and 0.4 mM CGA	28 ± 1.1 d	55 ± 3.3 b
1 larva: CAY diet + methionine 0.4 mM CGA	18 ± 0.7 e	60 b

[1]Larval weights and longevities of larvae were compared with the Wilcoxon-Mann-Whitney test.
[2]Mortalities were compared as Poisson counts.
Different letters in a column indicate significant differences, $P < 0.05$.

TABLE 3. Mean longevity (days ± sem) of *P. regina* larvae after exposure to CAY diets that contained either 1.5, 15 or 30 mM concentrations of mimosine.

Conditions	Mean longevity of larvae, days ± sem[1]
10 larvae: mimosine 30 mM	3 ± 0.1 a
1 larva: mimosine 30 mM	2 ± 0.2 b
10 larvae: mimosine 15 mM	4 ± 0.2 c
1 larva: mimosine 15 mM	3 ± 0.3 a
10 larvae: mimosine 1.5 mM	4 ± 0.6 c
1 larva: mimosine 1.5 mM	3 ± 0.2 a

[1]Larval weights and longevities of larvae were compared with the Wilcoxon-Mann-Whitney test.

cifically, the pH of a CAY diet was increased by the feeding of groups of larvae, becoming more alkaline after 2 days. Similarly, groups of *Lucilia cuprina* (Weidemann) larvae have been shown to alter the pH of an acidic diet (pH= 3.5 to 6.5) by 0.5 pH units after 3 days by their feeding activity and exudates (Guerrini et al., 1988).

At an alkaline pH CGA is converted to a reactive CGA-*o*-quinone which binds readily to protein and amino-acids (Hurrel & Finot, 1982). Therefore, toxic effects of CGA could have been exacerbated in the short term by the alkaline secretions and excretions from larvae.

However, increased alkalinity in the substratum due to the effects of grouped larvae could also enhance the alkylation of either lysine or methionine (Felton et al., 1992), removing more free CGA from the diet and reducing the toxic effects of CGA. Methionine may have been less reactive than lysine at the pH produced by the feeding of groups of *P. regina* larvae. Therefore, CGA and methionine may have not been as likely to form less toxic alkyl-amino-acids.

Mimosine resulted in 100% mortality of larvae, even when at a 1.5 mM concentration. The molar concentration of mimosine in CAY diets was greater than CGA so the difference in toxicity could have been due to difference in dosages. Larvae reared in groups were able to survive for a greater period of time on diets that contained mimosine, when compared with isolated larvae, although they also suffered 100% mortality. Thus, *P. regina* are highly susceptible to the toxic effects of mimosine at the concentrations tested.

Ishaaya et al. (1991) suggested that mimosine interferes with the digestive enzyme synthesis of larvae of *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) leading to reduced growth. The increased concentration of tryptic and alkaline exudates from groups of *P. regina* larvae may have released more nutriment from the diet and allowed groups of larvae to forestall the toxic effects of mimosine, particularly if digestive enzyme synthesis was affected. In conclusion, individuals within groups of *P. regina* larvae are less susceptible to metabolic inhibitors than isolated larvae. This would have important consequences for blowfly control regimes, if metabolic inhibitors were chosen as a control method, since the onset of mortality and growth-inhibition could be affected by the numbers of larvae within groups. Further work will investigate the susceptibility of grouped and isolated *P. regina* larvae to other plant-derived compounds.

Table 4. Effects of *P. regina* larvae on the mean pH (± sem) of CAY diets.

Numbers of larvae per Petri dish	Mean pH of larvae or diets, ± sem	
0 (control)	6a (diet)	
1	7.4 ± 0.20 b (larva)	
10	8.6 ± 0.20 c (larvae)	
		a < b, P < 0.01 b < c, P < 0.05

[#] the t-test was used to compare the pH data.
Different letters in a column indicate significant differences.

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