Journal of Stored Products Research 47 (2011) 238–243

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Fumigant toxicity of allyl isothiocyanate to populations of the red flour beetle *Tribolium castaneum*

J.C. Santos [a], L.R.A. Faroni [b], A.H. Sousa [a], R.N.C. Guedes [a,*]

[a] Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil
[b] Departamento de Engenharia Agrícola, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil

ARTICLE INFO

Article history:
Accepted 21 March 2011

Keywords:
Allyl isocyanate
Biofumigant
Mustard oil
Respiration rate
Susceptibility

ABSTRACT

The phasing out of methyl bromide as a fumigant, the phosphine resistance problems in stored product insect-pests, and the ever-growing concerns with human health and environmental safety have been guiding the search of alternative fumigants. Allyl isothiocyanate (AITC) is the main component of mustard oil with reported pesticide activity and potential as a fumigant of stored foodstuffs. The fumigant toxicity of AITC was assessed in adults of 18 populations of the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebronidae). These populations were all susceptible to AITC with negligible variation among them. Two of these populations were further used to test the AITC susceptibility of eggs, larvae (early and late instars), and pupae of *T. castaneum*. All of the developmental stages of both populations were similarly susceptible to AITC. No cross-resistance between phosphine and AITC was observed. Despite the significant variation in body mass, respiration rate, and fitness among the populations of *T. castaneum*, they were not correlated with AITC susceptibility. Larvae and adult malformations were observed when larvae and pupae were exposed to AITC. These results show the potential of AICT as an alternative fumigant against stored product insects.

1. Introduction

Fumigants play a major role in stored product protection, but unlike residual pesticides where new compounds have become available and continue to do so, the development of new fumigants have not been forthcoming (Bell, 2000; Zettler and Arthur, 2000). Of the two fumigants in widespread use, methyl bromide is an ozone-depletor and is being phased out as agreed through the Montreal Protocol (Montreal Protocol on Substances that Deplete the Ozone Layer, 1994; Bell, 2000). The other fumigant, phosphine, has some issues with insecticide resistance, requires air-tight conditions necessary for its desired efficacy, and it is under regulatory review in several countries because of environmental and human safety concerns (Champ and Dyte, 1976; Garry et al., 1989; Environmental Protection Agency, 1993; Anonymous, 1997; Chaudhry, 2000; Zettler and Arthur, 2000). Regarding new fumigants, sulphuryl fluoride is used in few countries with only recent use extension to food commodities and food handling facilities in the USA, Canada and Europe (Bell, 2000; Prabhakaran, 2006; Emekci, 2010). Consequentlly, there is a high level of interest in the development of new alternative fumigants for stored product protection.

A recent focus of attention among alternative fumigants are the biofumigants, which reflects the growing attention received by biopesticides or biorational pesticides (Isman, 2006; Rajendran and Sriranjini, 2008; Rosell et al., 2008). Natural products, and particularly plant products, are potential new insecticides for crop protection as insecticides and chemical backbones for the synthesis of new insecticides (Kidd, 2000; Rajendran and Sriranjini, 2008). Among plants, cruciferous vegetables (including cabbage and mustard, among others) produce glucosinolates, which are secondary metabolites of plant defense involved in plant–plant, plant-microorganism, and plant–insect interactions (Chew, 1988; Mewis et al., 2002; Tsao et al., 2002; Agrawal and Kurashige, 2003; Müller, 2009). Glucosinolates are the storage form of isothiocyanates in the plants, which are released upon hydrolysis by thioglucosidase (=myrosinase) yielding an aglycone that, after undergoing non-enzymatic rearrangements, produces the said isothiocyanates (Chew, 1988; Tsao et al., 2002).

The use of green manure, crop residues and seed meals of Brassica plants (cruciferous vegetables) as biofumigants against soil pathogens and nematode suppression have been recognized (e.g., Zasada et al., 2009; Lu et al., 2010; Motisi et al., 2010). In addition, isothiocyanates generated by the glucosinolate-myrosinase system exhibits herbicide, bactericide and insecticide activity (Lin et al., 2000; Demirel et al., 2009; Hara et al., 2010). One of such isothiocynates, the allyl

* Corresponding author. Tel.: +55 (31) 3899 4008; fax: +55 (31) 3899 4012.
E-mail address: guedes@ufv.br (R.N.C. Guedes).

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doi:10.1016/j.jspr.2011.03.004

isothiocyanate (AITC) comes mainly from broken seeds of black mustard (*Brassica nigra*) or brown Indian mustard (*Brassica juncea*), and it is commercially produced by the reaction of allyl chloride and potassium thiocyanate (sometimes referred as synthetic mustard oil in this case) (Romanowski and Klenk, 2005). AITC is used as insecticide, bactericide, and nematicide possessing four active EPA registrations in the USA (Kegley et al., 2010).

Although AITC is available for foliar treatment against some insect species mainly for turf and ornamental plants in the US (Kegley et al., 2010), its fumigant activity has also been reported against adults of stored product insects (Worfel et al., 1997; Tsao et al., 2002; Wu et al., 2009). Here we further assessed the fumigant activity of AITC against adults of 18 populations of *Tribolium castaneum* (Herbst), the red flour beetle, some of which are resistant to phosphine (Pimentel et al., 2007; Sousa et al., 2008). This research aimed to determine: (1) AITC insecticidal activity on 18 populations of *T. castaneum*; (2) potential cross-resistance between AITC and phosphine; (3) AITC effect on immature stages of two populations of *T. castaneum*; (4) if insect body mass, respiration rate and fitness affect AITC toxicity; and (5) if AITC leads to potential insect malformations, since spiracle and crochet malformation were induced by isothiocyanates in the tobacco hornworm (*Manduca sexta* (Lepidoptera: Sphingidae)) (Ujváry et al., 1989).

2. Material and methods

2.1. Insect populations and fumigant

Eighteen populations of *T. castaneum* were used. They are representative populations collected between 2004 and 2007 from different stored product facilities from five Brazilian States; in addition two laboratory strains were also assessed (Table 1). Six of these populations (Bom Despacho, Campos de Júlio, Rio Verde, Uberlândia, Unaí-1, and Viçosa-1) were resistant to phosphine with resistance ratios ranging from 37.0- to 186.2-fold (Pimentel et al., 2007; Sousa et al., 2008). These strains are periodically monitored. Field populations (>250 unsexed adults) were reared for at least two generations in standardized laboratory conditions before starting the bioassays. Insects were maintained in clear glass jars (1.5 L) at 26 ± 2 °C, 75 ± 5% r.h., and 24 h darkness. Ground maize kernels free of insecticide residues and with 13% moisture content (m.c.) were used as food substrate.

Table 1
Origin of the Brazilian populations of *Tribolium castaneum*.

Code no.	Site	State	Storage facility	Product	Month/Year
1	Água Boa	MT	Warehouse	Maize	August 2004
2	Aguanil	MG	Farm wood store	Maize (in the curb)	May 2005
3	Barra do Garças	MG	Rice Mill	Rice	March 2007
4	Bom Despacho	MG	Metallic bin	Maize	May 2005
5	Bragança Paulista	SP	Farm wood store	Maize	March 2005
6	Campos de Júlio	MG	Metallic Bin	Maize	June 2005
7	Frutal	MG	Metallic Bin	Sorghum	December 2005
8	Guaxupé	MG	Metallic Bin	Maize	August 2005
9	Nova Era	MG	Warehouse	Maize	May 2005
10	Piracicaba	SP	Laboratory	Maize	August 2004
11	Picos	PI	Metallic Bin	Maize	February 2007
12	Rio Verde	GO	Metallic Bin	Maize	July 2005
13	Sacramento	MG	Horizontal bulk store	Maize	May 2005
14	Uberlândia	MG	Metallic Bin	Maize	August 2004
15a	Unaí I	MG	Metallic Bin	Maize	August 2004
15b	Unaí II	MG	Metallic Bin	Maize	July 2004
16a	Viçosa I	MG	Laboratory	Maize	March 2004
16b	Viçosa II	MG	Animal food industry	Maize	September 2004

Commercial allyl isothiocyanate (AITC) was obtained from Petite Marie Química Fina (95% pure; São Paulo, SP, Brazil), where it is produced by the reaction of allyl chloride and potassium thiocyanate, as commonly employed in the food industry. An AITC-soybean oil stock solution (0.5% v/v) was prepared for the fumigation bioassays. Soybean oil was used as solvent to minimize AITC sorption which would take place if applied to grains using a more volatile solvent (Paes et al., 2011).

2.2. AITC toxicity bioassays

AITC concentration-mortality curves for *T. castaneum* were established using the AITC concentration range of 2.25–5.62 μL a.i./L in 0.56 μL a.i./L intervals. For the adult bioassays, AITC solutions in four dilutions were spread over filter paper (0.1 mL; Whatman no. 1; 2.25 cm^2; four replicates), which were placed over a Petri dish (5 cm diameter) at the bottom of a 0.8 L glass jar, and covered with organza to prevent direct insect contact with the oil solution containing AITC. Thirty non-sexed adults of *T. castaneum* (1–4 week old) were introduced and the jars were sealed with silicon (ORBIVED Neutro, Orbi Química Ltda, Leme, SP, Brazil) to prevent escape of fumigant. The exposure time was 24 h and the jars were maintained at the same environmental conditions as the insects were reared. Death was ascertained by lack of insect response after prodding with a fine hair brush. Soybean oil was used as a control. Four replicates (i.e., four jars with 30 insects each) were used for each AICT concentration and a minimum of four concentrations (in addition to the control) were used for each concentration-mortality bioassay.

For the immature bioassays, eggs (one to six days-old), early instar larvae (12 days-old), late instar larvae (18 days-old), and pupae (three to four days-old) from two populations of *T. castaneum* were used – Água Boa, which is susceptible to phosphine, and Bom Despacho, which is phosphine resistant (Pimentel et al., 2007). The immature stages were obtained from 500 non-sexed adults maintained in glass jars (3.25 L) containing 500 g dog food (Beneful®, Purina, São Paula, SP, Brazil) as food substrate for 24 h. This food source allows contrast with the insect eggs, which were sieved after six days (0.50 mm mesh), and inspected under stereomicroscope. The eggs were isolated and placed in Petri dishes (5 cm diameter) in groups of 30 providing them (if necessary) with ground maize kernel (30 g) until the desired developmental stage for the bioassays. The bioassays were carried out as described for adult insects, without food provision and for 24 h, after which the insects were again provided with ground maize kernel for seven days when mortality was assessed as above. The number of replicates and concentrations used were the same as for adults, as were the control treatments.

2.3. Respiration rate, body mass and instantaneous rate of population growth (r_i)

The respiration rate of adult insects was determined by their rate of CO_2 production, as measured with a TR3C carbon dioxide analyzer (Sable System International, Las Vegas, USA), using the methods described by Sousa et al. (2008), adapted from Guedes et al. (2006). Briefly, 20 non-sexed adult insects were used for each 25 mL chamber, and three of such chambers (i.e., replicates) were used for each *T. castaneum* population. The insects were acclimated for 12 h within the chambers and CO_2 production was assessed for 1 h using a current (600 mL min^{-1}) of CO_2-free air to purge the CO_2 produced in each chamber, which was measured in a infra-red CO_2 analyzer (μL h^{-1} $insect^{-1}$). A control chamber without insects was simultaneously used as a control. After this measurement, the insects were removed from the flasks and

Table 2
Relative fumigant toxicity of ally isothiocyanate (AITC) to adults of 18 Brazilian populations of *Tribolium castaneum*. The AITC concentration range used was 2.25–5.62 μL a.i./L (24 h exposure).

Population	No. of insects	Slope (±SEM)	CL_{50} (95% FL) (μL L^{-1})	RT at CL_{50} (95% CI)	CL_{95} (95% FL) (μL L^{-1})	RT at CL_{95} (95% CI)	χ^2	*P*
Água Boa	480	23.38 (±2.00)	4.42 (4.35–4.49)	1.18 (1.14–1.22)	5.20 (5.07–5.38)	1.01 (0.96–1.07)	2.67	0.26
Aguanil	600	19.18 (±1.34)	4.01 (3.94–4.08)	1.07 (1.03–1.11)	4.89 (4.76–5.06)	0.95 (0.90–1.01)	3.81	0.28
Barra do Garças	540	19.24 (±1.33)	4.11 (4.04–4.19)	1.10 (1.06–1.13)	5.01 (4.87–5.19)	0.99 (0.93–1.04)	5.34	0.14
Bom Despacho	570	20.17 (±1.42)	4.14 (4.06–4.21)	1.10 (1.06–1.13)	4.99 (4.87–5.16)	0.98 (0.93–1.03)	5.07	0.16
Bragança Paulista	540	13.05 (±0.92)	4.28 (4.17–4.37)	1.14 (1.10–1.18)	5.72 (5.50–6.00)	1.12 (1.05–1.19)	5.07	0.16
Campos de Júlio	390	17.00 (±1.32)	4.43 (4.33–4.53)	1.18 (1.14–1.22)	5.53 (5.34–5.79)	1.08 (1.02–1.15)	5.74	0.12
Frutal	510	17.94 (±1.26)	4.32 (4.23–4.40)	1.15 (1.11–1.19)	5.33 (5.18–5.53)	1.04 (0.99–1.10)	2.98	0.39
Guaxupé	480	19.61 (±1.53)	4.01 (3.92–4.09)	1.07 (1.03–1.11)	4.86 (4.72–5.04)	0.95 (0.90–1.00)	5.54	0.13
Nova Era	480	20.30 (±1.60)	4.23 (4.15–4.30)	1.13 (1.09–1.17)	5.10 (4.96–5.28)	1.00 (0.94–1.05)	4.89	0.17
Piracicaba	600	10.51 (±0.83)	4.66 (4.55–4.79)	1.24 (1.20–1.29)	6.69 (6.32–7.21)	1.31 (1.21–1.42)	5.96	0.11
Picos	570	11.72 (±0.87)	4.15 (4.05–4.25)	1.11 (1.07–1.15)	5.73 (5.49–6.07)	1.12 (1.05–1.20)	0.89	0.82
Rio Verde	330	15.07 (±1.37)	4.45 (4.33–4.56)	1.18 (1.14–1.23)	5.72 (5.48–6.06)	1.12 (1.04–1.19)	3.70	0.29
Sacramento	600	10.35 (±0.85)	3.82 (3.70–3.93)	1.02 (0.98–1.06)	5.51 (5.27–5.86)	1.08 (1.01–1.15)	1.78	0.61
Uberlândia	570	6.59 (±0.71)	4.26 (4.09–4.42)	1.13 (1.08–1.17)	7.57 (6.84–8.80)	1.48 (1.30–1.69)	2.85	0.41
Unaí I	480	11.20 (±1.09)	4.17 (4.04–4.29)	1.11 (1.07–1.16)	5.84 (5.52–6.34)	1.14 (1.05–1.24)	2.98	0.39
Unaí II	600	12.21 (±0.96)	3.74 (3.63–3.83)	–	5.10 (4.91–5.35)	–	3.14	0.36
Viçosa I	570	16.87 (±1.15)	4.11 (4.03–4.19)	1.10 (1.06–1.13)	5.15 (5.00–5.34)	1.00 (0.95–1.06)	4.72	0.19
Viçosa II	600	11.88 (±0.85)	4.18 (4.08–4.28)	1.11 (1.07–1.16)	5.75 (5.52–6.06)	1.12 (1.05–1.20)	5.08	0.16

individually weighed on an analytical balance (Sartorius BP 210D, Göttingen, Germany). Respiration rate and body mass were presented separately, without normalizing the first by the second since this procedure may mask the effect of the individual (Haynes, 2001). These same determinations were also carried out for immature stages of two populations of *T. castaneum* (Água Boa and Bom Despacho) using the same procedures.

Instantaneous rate of population growth (r_i) was determined in Petri dishes (14 × 1 cm) containing 40 g ground maize kernels (13% m.c.) using ten replicates for each population. Twenty non-sexed adult insects (two to three weeks old) were released in each Petri dish and maintained at the same environmental conditions as the laboratory colonies for 60 days. After such period, the number of live insects was recorded and the r_i was estimated following Walthall and Stark (1997) where $r_i = \mathrm{Ln}(N_f/N_0)/\Delta t$ and N_f is the final number of live insects, N_0 is the initial number of (live) insects, and Δt is the duration of the experiment.

2.4. Statistical analysis

The concentration-mortality data were subjected to probit analysis (PROC PROBIT; SAS Institute, 2009) and the confidence intervals for the toxicity ratios (TRs) were estimated following Robertson and Preisler (1992). The lethal concentrations (LC) were considered significantly different ($P < 0.05$) if their confidence limits did not include the value 1. TRs for AITC were subject to correlation analysis against the phosphine resistance ratios earlier estimated (Pimentel et al., 2007) using the Correlation Procedure (PROC CORR) in SAS (SAS Institute, 2009). Not all of the populations screened here were used by Pimentel et al. (2007), therefore only those common to both studies were used in this correlation analysis.

The data of adult CO_2 production and body mass, as well as rate of population growth were subjected to analysis of variance and Scott-Knott groupment analysis test, if appropriate (Scott and Knott, 1974; PROC GLM; SAS Institute, 2009). These data was also subjected to correlation analysis against AITC toxicity rate (PROC CORR; SAS Institute, 2009).

The results of CO_2 production of the immature insects were subjected to a two-way analysis of covariance, with insect populations and developmental stages as independent variables and body mass as covariate, complemented by linear regression analysis when necessary (PROC GLM; SAS Institute, 2009).

3. Results

3.1. AITC fumigant toxicity

The probit model was suitable for the concentration-mortality data obtained with the populations of *T. castaneum* based on the low χ^2 and high *P*-values obtained for each concentration-mortality curve ($\chi^2 < 5.7$; $P > 0.05$) (Tables 2 and 3). The toxicity ratio of adults from the 18 populations of *T. castaneum* exhibited negligible variation among them ranging from 1.0 to 1.24-fold at LC_{50} and from 0.95 to 1.48-fold at LC_{95} (Table 2). Very few adult populations exhibited significantly reduced susceptibility and such significance was always marginal based on Robertson and Preisler (1992). In contrast, there was significant slope variation among strains, ranging from 10.3 to 23.4, indicating heterogeneity of response

Table 3
Relative fumigant toxicity of ally isothiocyanate (AITC) to the delevopmental stages of two populations of *Tribolium castaneum*.

Population	Developmental stage	No. of insects	Slope (±SEM)	CL_{50} (95% FL) (μL.L^{-1})	RT at CL_{50} (95% CI)	χ^2	P
Água Boa	Egg	600	5.26 (±0.67)	3.88 (3.68–4.14)	–	3.53	0.31
	Early instar	540	5.45 (±0.70)	4.54 (4.33–4.77)	1.17 (1.08–1.26)	4.96	0.17
	Late instar	450	15.87 (±1.14)	4.16 (4.06–4.26)	1.07 (1.00–1.14)	4.21	0.23
	Pupa	570	10.60 (±0.85)	4.30 (4.19–4.41)	1.10 (1.04–1.18)	3.73	0.29
	Adult	420	23.38 (±2.00)	4.42 (4.35–4.49)	1.13 (1.07–1.20)	2.67	0.26
Bom Despacho	Egg	510	5.18 (±0.69)	3.67 (3.49–3.88)	–	4.27	0.23
	Early instar	510	5.51 (±0.77)	4.63 (4.42–4.88)	1.25 (1.17–1.35)	4.96	0.17
	Late instar	450	15.85 (±1.37)	4.13 (4.03–4.23)	1.12 (1.06–1.19)	4.14	0.24
	Pupa	570	13.04 (±0.96)	4.44 (4.34–4.54)	1.20 (1.14–1.27)	4.46	0.21
	Adult	570	20.17 (±1.42)	4.14 (4.06–4.21)	1.12 (1.06–1.18)	5.07	0.16

within some populations (e.g., Bom Despacho and Água Boa). Although some of the insect populations screened here are resistant to phosphine (as determined by Pimentel et al. (2007)), such resistance was not correlated with AITC susceptibility ($n = 11$; $r = -0.05$; $P = 0.87$).

The concentration-mortality curves for the immature stages from the two most heterogeneous populations, Água Boa and Bom Despacho (which are susceptible and resistant to phosphine respectively), were also very similar among them, and even among developmental stages with marginally significant differences in toxicity rate based on their 95% confidence interval (CI) (Table 3). There was little variation between populations (which was high for adults), but large variation among stages with eggs exhibiting the most homogeneous response and adults the most heterogeneous response.

3.2. Respiration rate, body mass and instantaneous rate of population growth (r_i)

The adult populations of *T. castaneum* differed significantly in respiration rate ($F_{17,36} = 6.31$, $P < 0.001$), body mass ($F_{17,36} = 4.74$, $P < 0.001$), and also population growth rate ($F_{17,162} = 8.59$, $P < 0.001$) (Table 4). However, none of these traits correlates significantly with susceptibility to AITC (TR at LC_{50}) (respiration rate: $n = 18$, $r = 0.11$, $P = 0.67$; body mass: $n = 18$, $r = 0.02$, $P = 0.95$; and population growth rate: $n = 18$, $r = 0.28$, $P = 0.26$).

Respiration rate was influenced by the insect developmental stages ($F_{1,22} = 5.21$, $P = 0.03$) and its body mass ($F_{1,22} = 22.86$, $P < 0.001$), but not by populations ($F_{1,22} = 0.01$, $P = 0.97$) and there was no significant interaction between these sources of variance of respiration rate ($F_{1,22} < 2.91$, $P = 0.10$) based on the analysis of covariance. Respiration rate increased with body mass and also greatly differ with the developmental stage with only the pupa stage as outlayer (Fig. 1). Again none of these traits was significantly correlated with susceptibility of the different developmental stages to AITC (TR at LC_{50}) (respiration rate: $n = 10$, $r = 0.09$, $P = 0.81$; body mass: $n = 10$, $r = 0.36$, $P = 0.310$).

3.3. Insect malformations

Morphological abnormalities were observed in larva, pupa and adults of *T. castaneum* exposed to concentrations of AITC around the LC_{50} (4.50 μL L^{-1}). These abnormalities ranged from differences in sclerotization up to drastic abdominal malformations with a broader range of variation observed in adults generated from exposed pupa. Typical larva and adult malformations are exhibited in Fig. 2.

Table 4
Insect respiration rate (μL $CO_2.h^{-1}.insect^{-1}$), body mass (mg) and instantaneous rate of population growth (r_i) (±SEM) of (adult) insects from 18 populations of *Tribolium castaneum*. Means followed by the same letter within a column are not significantly different by Scott-Knott groupment analysis test ($P < 0.05$).

Population	Respiration rate (μL $CO_2.h^{-1}.inseto^{-1}$)	Body mass (mg)	Instantaneous rate of population growth (r_i)
Água Boa	1.198 ± 0.086 b	1.923 ± 0.002 a	0.025 ± 0.003 a
Aguanil	0.930 ± 0.075 c	1.873 ± 0.031 b	0.011 ± 0.002 d
Barra do Garças	0.710 ± 0.039 c	1.795 ± 0.020 b	0.009 ± 0.005 d
Bom Despacho	1.267 ± 0.092 b	1.835 ± 0.036 b	0.014 ± 0.003 c
Bragança Paulista	0.793 ± 0.306 c	1.950 ± 0.053 a	0.012 ± 0.009 c
Campos de Júlio	1.214 ± 0.006 b	1.981 ± 0.017 a	0.024 ± 0.006 a
Frutal	0.724 ± 0.118 c	1.861 ± 0.071 b	0.014 ± 0.005 c
Guaxupé	0.716 ± 0.009 c	1.713 ± 0.102 b	0.028 ± 0.002 a
Nova Era	1.185 ± 0.490 b	2.048 ± 0.041 a	0.021 ± 0.003 b
Piracicaba	0.671 ± 0.061 c	1.738 ± 0.025 b	0.013 ± 0.005 c
Picos	0.865 ± 0.120 c	2.101 ± 0.222 a	0.006 ± 0.005 d
Rio Verde	1.024 ± 0.142 c	1.870 ± 0.033 b	0.020 ± 0.004 b
Sacramento	0.859 ± 0.081 c	1.693 ± 0.007 b	0.016 ± 0.005 c
Uberlândia	0.654 ± 0.120 c	1.970 ± 0.053 a	0.019 ± 0.003 b
Unaí I	1.739 ± 0.387 a	1.996 ± 0.052 a	0.013 ± 0.004 c
Unaí II	0.724 ± 0.043 c	1.816 ± 0.005 b	0.009 ± 0.009 d
Viçosa I	0.717 ± 0.057 c	1.818 ± 0.035 b	0.019 ± 0.005 b
Viçosa II	1.787 ± 0.011 a	1.988 ± 0.048 a	0.023 ± 0.003 a

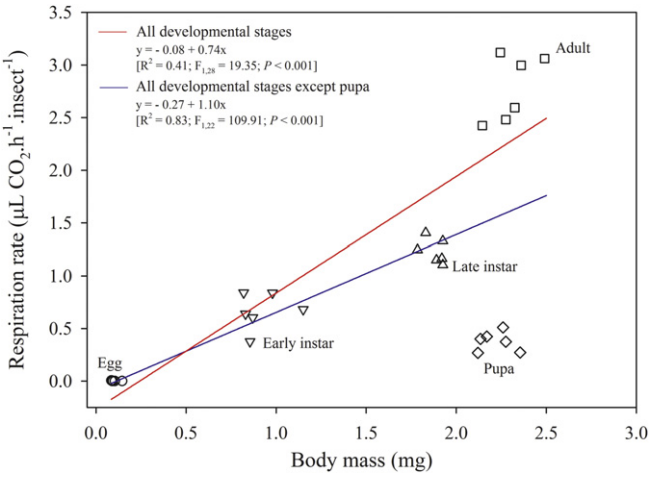

Fig. 1. Relationship between respiration rate (μL $CO_2.h^{-1}.insect^{-1}$) and body mass (mg) of individual insects from different developmental stages of the red flour beetle *Tribolium castaneum*. Each symbol represents the average determination of the 30 individuals encompassing each replicate (for each developmental phase from two insect populations). Two estimated regressions are exhibited, one including the pupal stage, and the other excluding it.

4. Discussion

The high toxicity of AITC to adults of *T. castaneum* were similar to other studies with the maize weevil *Sitophilus zeamais*, the lesser grain borer *Rhyzopertha dominica*, the book louse *Liposcelis entomophila*, the confused flour beetle *Tribolium confusun* and one other study with *T. castaneum* (Worfel et al., 1997; Demirel et al., 2009; Wu et al., 2009). All 18 populations of *T. castaneum* tested in our study, some of which are resistant to phosphine (Pimentel et al., 2007), were susceptible to AITC. In fact, AITC susceptibility was uniform among adults and immatures of *T. castaneum*, and our results were the first to be published for the immature stages. Furthermore, there was no correlation between phosphine resistance and susceptibility to AITC in *T. castaneum* indicating absence of cross-resistance between these compounds. The low variability of AITC susceptibility among the insect populations studied suggests that the defense mechanisms used for phosphine resistance are ineffective for AITC, as also observed for ozone (Sousa et al., 2008). However some populations of *T. castaneum* exhibited high heterogeneity of response to AITC (e.g., Água Boa e Bom Despacho, which are about twice as heterogeneous as other populations such as Piracicaba based on the slopes of the concentration-response curves), indicating their potential for future selection for reduced susceptibility (or resistance) to AITC.

Reduced respiration rate have been linked to phosphine resistance as a potential mechanism for reducing penetration in the insect body (Cotton, 1932; Chaudhry, 1997; Pimentel et al., 2007). Significant variation in respiration rate was also observed among adults and immatures of *T. castaneum*, which was similar to published results for ozone but not phosphine (Pimentel et al., 2007; Sousa et al., 2008). This variation was uncorrelated to AITC susceptibility, indicating that respiration rate does not seem to play any role in reducing AITC toxicity. Nonetheless this relationship may still exist, although undetected in our study due to the lack of interpopulational variation in AITC susceptibility among the insect

Fig. 2. Photos of normal (A, C) and malformed (B, D) larvae (A, B) and adults (C, D) of *Tribolium castaneum* when exposed to allyl isothiocyanate (AITC) at 4.50 μL L^{-1}. The malformed larva was exposed to AITC for 24 h during the early larval stage (12 days-old), while the malformed adult was exposed to AITC for 24 h during the pupa stage.

populations studied. Body mass and population growth rate, which were related to fitness cost associated with insecticide resistance and its mitigation (Guedes et al., 2006; Oliveira et al., 2007; Corrêa et al., 2011), also varied among populations of *T. castaneum* and did not correlate with AITC susceptibility. Again, the lack of interpopulation variation in TR make their (potential) correlation with AITC susceptibility very difficult to detect.

Little is known about the mode of action underlying the insecticidal activity of glucosinolate breakdown products such as AITC. However, isothiocyanate compounds are generally regarded (1) as inactivators of the thiol group of essential enzymes (Hassall, 1990), and/or (2) as alkylating agents of nucleophilic groups of biopolymers such as DNA leading to cytotoxic properties that can affect the formation of the spiracular epidermis and crochet on the tobacco hornworm (*Manduca sexta*) prolegs, as observed with bis-isothicyanates (but not mono-isothiocynates such as AITC) (Ujváry et al., 1989; Weber et al., 1992). In addition, bis-isothiocyanates exhibits juvenile hormone-like effects in caterpillars of the tobacco hornworm *M. sexta*, unlike mono-isothiocyanates such as AITC, which did not affect growth and development of this species (Ujváry et al., 1989). We did observe morphological malformation, particularly in adults when the immature stages (larvae and pupae) were exposed to AITC, suggesting that mono-isothiocyanates may also affect insect growth and development. Ujváry et al. (1989) may not have detected such effect because they have used topical applications and not inhalation as the exposure method, which is critical for evaluating fumigants such as AITC.

Glucosinolates are also reported to affect insect respiration, but the results are controversial. While these compounds from rapeseed led to inhibition of respiratory gas exchange reducing oxygen uptake in mitochondria and increasing the respiratory quotient (RQ = CO_2 eliminate/O_2 consumed) without impairing food assimilation and growth of the yellow mealworm *Tenebrio molitor* (Pracros et al., 1992, 1997), distinct results were reported by Tsao et al. (2002) in cockroaches. AITC increased respiration rate (measured as CO_2 emission) in the American and German cockroaches (*Periplaneta americana* and *Blatella germanica* respectively) exposed to this fumigant leading Tsao et al. (2002) to speculate on its possible (mode of) action as an uncoupler of the mitochondrial oxidative phosphorylation.

Our results with populations of *T. castaneum* did not show any correlation between respiration rate and AITC toxicity ratios (at LC_{50}), and therefore does not provide support for Tsao's hypothesis of AITC acting as an unclouper of the oxidative phosphorylation (Tsao et al., 2002). However, Tsaos' work was based on cockroaches, which may exhibit distinct physiological response to AITC from *T.* castaneum. In addition, the use of soybean oil as solvent may have limited AITC evaporation reducing its potential effect on the respiration rate of *T. castaneum* and its fumigant efficacy against this species. Despite this, Pracros et al. (1992, 1997) also did not obtain any support for Tsao's hypothesis. In truth, our study provides evidence of AITC affecting insect growth and development based on the morphological malformations observed, but we need to grant that inhibition of gas exchange may also occur and may favor such malformations, what deserves future attention.

In conclusion, AITC exhibits homogeneous fumigant activity among different populations of *T. castaneum*. Such activity is not restricted to adults, but also to this species immature stages. No resistance to AITC was observed among the insect populations studied and there was no cross-resistance between AITC susceptibility and phosphine resistance suggesting the possibility of AITC use even against phosphine resistant insects as a management tactic. Respiration rate, body mass and population growth rate are not related to AITC susceptibility and adult malformation was observed when larvae and pupae were exposed to AITC. These results are suggestive of the potential of AICT as an alternative fumigant against stored product insects, but further studies to determine residue levels in kernel fractions with exposure time, temperature and cooking conditions are necessary, as are AITC stability under storage conditions among other studies.

Acknowledgments

Financial support provided by CAPES, CNPq and FAPEMIG were greatly appreciated, as was the technical assistance provided by O.D. Dhingra and A.M. Souza. The comments and suggestions provided by Dr. F. H. Arthur and the anonymous reviewers were greatly appreciated.

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