DOI: 10.1111/j.1570-7458.2007.00573.x

# The α-amylase inhibitor acarbose does not affect the parasitoid *Venturia canescens* when incorporated into the diet of its host *Ephestia kuehniella*

Jana Kaufnerová[1,2], Zuzana Munzbergová[3,4], Vojtěch Jarošík[2,3] & Jan Hubert[1]*

[1]*Research Institute of Crop Production, Drnovská 507, 161 06 Prague 6–Ruzyně, Czech Republic,* [2]*Department of Ecology, Faculty of Science, Charles University, Viničná 7, 128 01 Prague 2, Czech Republic,* [3]*Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Průhonice, Czech Republic, and* [4]*Department of Botany, Faculty of Science, Charles University, Benátská 2, 128 01 Prague 2, Czech Republic*

Accepted: 23 January 2007

*Key words:* food, biological control, Hymenoptera, Ichneumonidae, Lepidoptera, Pyralidae

**Abstract**

Amylase inhibitors (AIs) are suitable candidates for protecting plants and their products from attacks by herbivorous and granivorous insects. However, detailed studies of the suppressive effects of AIs on target and non-target insects are necessary before their application in post-harvest protection. To address this issue, laboratory bioassays were used to test the effect of the non-proteinaceous inhibitor acarbose on a stored product pest, the flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), and its parasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). Two sublethal concentrations (0.001 and 0.0001%, wt/wt) of acarbose were incorporated into the diet of parasitized and unparasitized larvae of *E. kuehniella.* Development time and fresh body weight of the larvae, together with the size of the wasps, were compared for insects reared on acarbose-treated and control diets. On the diet containing 0.001% acarbose, the developmental time was longer and relative weight gains of the *E. kuehniella* larvae were lower, but the weight of the larvae prior to pupation was similar to that of the control. The acarbose did not have a suppressive effect on the parasitoid *V. canescens*; in fact the wasps that emerged from the hosts reared on a diet containing 0.0001% acarbose were on average larger and heavier than the controls. These results demonstrate that it might be possible to enhance the control of stored product pests by using both biological control and AIs.

## Introduction

Larvae of the flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), feed on flour, but also infest other cereal products (Sedlacek et al., 1995). The moth is a pest in mills and bakeries, where larvae cause damage both by feeding and contaminating stored food. In addition, their bodies cause problems during food processing (Sedlacek et al., 1995) and the species is also allergenic (Armentia et al., 2004).

As for other stored product moths, chemical control is widely used, mainly because it is very cost effective (Zettler & Arthur, 2000). However, *E. kuehniella* resistance to some chemical insecticides limits the application of chemical control (Cox et al., 1984; Price, 1985; Arthur, 1996), and there are also indications that it is developing a tolerance and/or resistance to bio-pesticides based on the toxins of *Bacillus thuringiensis* (Rahman et al., 2004). Consequently, there is a need to search for alternative means of controlling *E. kuehniella*.

Promising candidates are the inhibitors of insect amylases, naturally occurring in many plants as part of their defence (Carlini & Grossi de Sá, 2002; Franco et al., 2002). They are particularly abundant in cereals and legumes, and have a high insecticidal potential (see review by Carlini & Grossi de Sá, 2002). These inhibitors increase the post-harvest resistance of crops against beetle (Gatehouse et al., 1986; Chrispeels et al., 1998; Gatehouse & Gatehouse, 1998) and mite pests (Hubert et al., 2005).

Another way of controlling stored product pests are the applications of predators (Palyvos et al., 2006) or parasitoids. *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) is a parasitoid attacking larvae of *E. kuehniella* (Harvey & Vet, 1997), and the inundation of food processing

*Correspondence: Jan Hubert, Research Institute of Crop Production, Drnovská 507, Prague 6, Ruzyně, CZ 161 06, Czech Republic. E-mail: hubert@vurv.cz

 

premises with the wasp is an effective means of controlling moth infestations (Schöller et al., 1997; Prözell & Schöller, 2000). However, an adverse effect of amylase inhibitors (AIs) on this parasitoid would exclude its application in post-harvest protection. So far, this possible side-effect has not been studied.

The effect of the non-proteinaceous inhibitor acarbose (Gilles et al., 1996) at sublethal doses for *E. kuehniella* on the parasitoid *V. canescens* was studied. Several morphological features of the wasps, reared on acarbose-treated and control larvae, were measured and used as indicators of wasp fitness. In addition, parasitoids reared on acarbose-treated larvae were studied in order to determine whether exposure to the acarbose affected their host choice and preimaginal mortality.

## Materials and methods

### Experimental insects

The flour moth, *E. kuehniella*, was originally collected in Boršov near České Budějovice in South Bohemia, in September 2001. It was continuously reared on a mixture of wheat germ, glycerol, baker's yeast, and Pangamin (Rapeto a.s., Prague, Czech Republic) at the ratio of 10:10:2:1:0.5 (wt/wt/wt/wt/wt), in 1-l glass jars covered by muslin, in continuous darkness, at 27 ± 1 °C and 60% r.h. Eggs of the moth were collected by confining approximately 50 *E. kuehniella* adults in 500-ml plastic vials of which the bottoms were covered with 0.6-mm mesh, which were placed in larger collecting vials, into which the eggs fell through the mesh. Eggs were transferred at 24-h intervals to the rearing jars.

The parthenogenetic parasitoid *V. canescens* was collected at the same time and place as its host *E. kuehniella*, and was continuously reared on the last instar of *E. kuehniella* in 135 × 182 mm plastic boxes (Aliachem, Pardubice, Czech Republic) provided with larval food and kept at L16:D8, 25 ± 1 °C, and 65 ± 5% r.h. (Eliopoulos & Stathas, 2003). Parasitoid adults were fed a 1:1 (wt/wt) mixture of honey and water.

### Control and experimental diets

The control diet of *E. kuehniella* was a 1:1 mixture of oat flakes and wheat germ, which was ground to a powder and was sieved. Experimental diets consisted of the same mixture as the control diet, but the α-amylase inhibitor acarbose (Gilles et al., 1996) was added. Acarbose was chromatographically purified from Glucobay tablets (Bayer AG, Leverkusen, Germany), identified by mass spectrometry, and quantified by elementary analysis. Our preliminary study indicated a lethal dose from 0.01% of acarbose concentration. Therefore, to test sublethal doses, we chose acarbose concentrations of 0.001 and 0.0001% (wt/wt). The inhibitor was homogeneously incorporated into the diet in a suspension in distilled water, followed by lyophilization and remoisturization of the mixture (Kluh et al., 2005).

### *Ephestia kuehniella* bioassay

After 14 days from egg hatch on the control diet, the *E. kuehniella* larvae were weighed and were individually placed in 10-ml glass tubes containing 0.2 ± 0.01 g of either the control (no acarbose) or experimental (0.001 or 0.0001% of acarbose) diet. There were 12 replicates of each treatment. The openings of the tubes were covered with muslin and the tubes were kept in chambers in continuous darkness at 30 ± 1 °C and 60% r.h.

Larval mortality, time to pupation, relative growth rate (RGR), calculated as $W_{21}/(W_{14} \times 7)$, where $W_{14}$ is the weight of 14-day-old larvae (i.e., at the onset of the experiment) and $W_{21}$ is the weight of larvae 7 days later (Xie et al., 1996), and finally the weight just prior to pupation, defined as the weight of a larva just before it pupated, were monitored weekly. The larvae were weighed on a Mettler AE 240 microbalance (Mettler-Toledo, Columbus, OH, USA), to a precision of 10 ± 5 μg.

The Kaplan–Meier survival analysis was used to compare the proportion of larvae that pupated when fed control and acarbose-treated diets. The distribution of pupation rates was compared using Gehan–Wilcoxon multisample and two-sample parametric tests (Cox, 1972; Cox & Oakes, 1984). The RGR and weight before pupation had a normal error distribution and were analysed using analysis of variance (ANOVA); differences among means were compared using least square differences (LSD) tests and a 5% level of significance. All the statistical calculations were done in Statistix, version 7 (Analytical Software, Tallahassee, FL, USA).

### In vitro analysis of *Ephestia kuehniella* larvae

The experimental design differed from that used in the bioassay as the larvae were starved for 24 h after they were removed from the experimental or control diet, so that they excreted the food from their guts. After they were starved, the larvae were weighed and 10 were individually placed in 10-ml glass tubes and were fed on control or acarbose diets as in the previous experiment. The larvae were killed in the last larval instar and were weighed after 6 h drying at 120 °C in an incubator (Memmert UNB; Memmert GmbH, Schwabach, Germany). The percentage dry weights of the larvae fed control and acarbose diets were angular transformed and compared using an ANOVA test.

Individual larvae were homogenized separately (glass homogenizer; Kavalier, Sázava, Czech Republic) in 0.25 ml

of physiological solution using Ultra T8 homogenizer (IKA, Wilmington, NC, USA). The homogenates were centrifuged at 9981.62 ***g*** and 4 °C using Jouan MR23i centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 5 min and the supernatants were analysed to determine their protein and glycogen contents.

Protein content was measured after dilution of 50 µl of the supernatant with 0.95 ml of physiological solution (0.9% NaCl in distilled water). The solution was placed on a microtitration plate, 0.25 ml of Bradford reagent (catalogue no. B-6916, Sigma-Aldrich, St. Louis, MO, USA) was added, and after 15 min the colour was measured at 590 nm using the Emax, ELISA reader (Molecular Devices Corporation, Sunnyvale, CA, USA) in two pseudoreplicates per larva. The protein content was expressed as 'protein per fresh larval weight' (mg $g^{-1}$). As a standard we used the bovine serum albumin (BSA) protein standard solution 1 mg $ml^{-1}$ (P0914, Sigma-Aldrich).

The glycogen content was measured by hydrolysis of α-1,4 glycosidic bonds in glycogen to glucose by α-amylases. Ten microlitre of supernatant was placed on a microtitration plate, in eight pseudoreplicates per larva. Half of the pseudoreplicates were incubated with 0.25 ml of physiological solution. The other half were hydrolyzed to glucose by adding 0.25 ml of α-amylase from *Bacillus subtilis* (catalogue no. 900-90-2, ICN Biomedicals Inc., Irvine, CA, USA), diluted with Bristle–Robinson buffer: pH 6 (Ferencik & Skarka, 1981), in an amount of 722.5 BU per sample. The samples were incubated at 37 °C for 16 h in PST-60HL Thermo-Shaker (Biosan, Riga, Latvia), and the amount of glucose assayed using Glucose GOD 1500 tests (Lachema-Pliva, Brno, Czech Republic), in which the colour that developed after 30 min of exposure on a microtitration plate was measured at 490 nm using an ELISA reader (Emax, Molecular Devices). As a standard we used the Glucose standard solution 1 mg $ml^{-1}$ (G6918, Sigma-Aldrich).

The pseudoreplicated samples were averaged, and the glucose content separately estimated for samples incubated with physiological solution and those hydrolyzed. It was expressed as a difference between glucose contents in samples incubated with α-amylase minus those incubated with physiological solution, in terms of glucose per fresh larval weight (mg $g^{-1}$). The protein and glycogen contents and protein:glycogen ratio for larvae fed the experimental and control diets were statistically compared using ANOVA.

#### *Venturia canescens* bioassay

The *E. kuehniella* hosts of *V. canescens* were similarly reared on a control diet or diets with either 0.001 or 0.0001% acarbose. In each treatment, a large number of 3-week-old larvae were exposed in glass tubes to ≤24-h-old *V. canescens*. Each wasp was removed after laying an egg, which was indicated by the characteristic cocking of the ovipositor (Rogers, 1972). The tubes were covered with muslin and returned to the chambers (60% r.h., 30 ± 1 °C, continuous darkness), where they were kept until the parasitoid adults emerged.

For assessing the morphological features, newly emerged wasps were killed by freezing, weighed on a microbalance (10 ± 5 µg), placed in physiological solution, and their hind tibia, forewings, and ovaries removed. Ovaries of 10 wasps from each treatment were placed in a drop of saline on a microscope slide, individual ovarioles teased apart with micropins, and the mature eggs counted after cutting the lateral oviducts (Eliopoulos et al., 2003). Adult weights and numbers of eggs were statistically compared by ANOVA.

The length of hind tibia and the length and width of the forewings of 16 wasps (Figure 1) (Slobodchikoff, 1983) were measured using digital image analysis. The individual images of the tibia and forewings were recorded using an Olympus 5050Z camera attached to an Olympus SZX – MDU 12 stereomicroscope (Center Valley, PA, USA), saved in .JPG format with a colour depth of 16.7 million at a resolution of 2592 × 1944 pixels, and analysed using Sigma-Scan Pro 5 (SPSS Inc., Chicago, IL, USA). The length of each hind tibia was measured 10 times and averaged; wings were measured once. The differences in the size of the wings and length of the tibia were tested using a multivariate

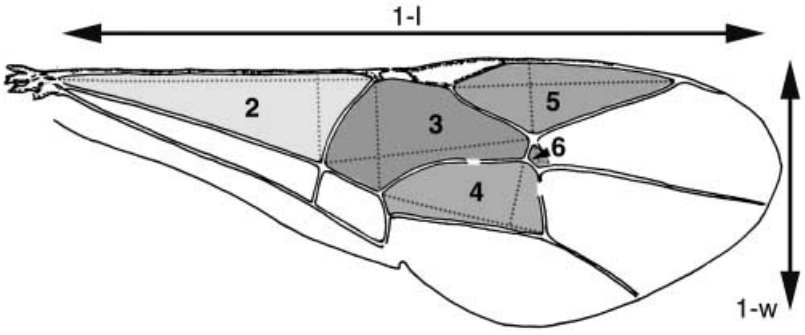


**Figure 1** The forewing of *Venturia canescens*. Indicated are the length (l) and width (w) of the whole wing (1) and of wing parts (2–6) that were measured using digital image analysis.

redundancy analysis (RDA) in CANOCO (Lepš & Šmilauer, 2003). The analysis was first done on relative values (data standardized by morphological parameters called species in Canoco terminology), as the different morphological parameters were measured on different scales. Then the same analysis was repeated on relative values after standardized by specimens (called samples in the Canoco terminology). In this way, the size differences between individuals were removed, and the results indicated differences in proportions, instead of absolute differences between the morphological parameters.

#### Arena experiment

The objective of this experiment was to compare the rate of parasitism of inhibitor-treated and control larvae. The arena consisted of a 135 × 182 mm plastic box (Aliachem) with 15 10-ml glass vials, each containing a 4-week-old larva of *E. kuehniella* and its diet. The control, 0.001, and 0.0001% acarbose diets were each replicated five times, giving a total of 15 vials. A ≤24-h-old wasp was released into the arena and monitored for 2 h. The sequence in which the wasp parasitized the larvae was recorded. At the end of the experiment, the parasitoid was removed, the larvae placed into the same conditions as the bioassay, and kept until the parasitoids emerged. This experiment was repeated 12 times.

The effect of arena and treatment on the probability of ovipositing into a vial was tested first. To do this, oviposition was used as the dependent variable, and arena and treatment as independent variables. Then the effect of sequence on the probability of ovipositing into a given vial was tested. Here the type of treatment was used as the dependent variable, and arena and sequence as independent variables. Only the first five oviposition events were considered, as a higher number of ovipositions were observed only in four arenas. Finally, logistic regression was used to study the number of parasitoids that emerged. The emergence of parasitoids was used as the dependent variable, and arena, sequence of parasitoid attack, and type of treatment as independent variables. The analyses were done using logistic regression in S-plus 7 (Insightful, Seattle, WA, USA).

## Results

#### Suppressive effect of acarbose on *Ephestia kuehniella*

Larval mortality on control and acarbose diets was less than 15%. However, the length of larval development, expressed in terms of when the larvae pupated, was influenced by the inhibitor. The higher acarbose concentration, 0.001%, significantly prolonged larval development (Gehan–Wilcoxon test: $Z = 9.36$; $P>0.001$), but there were no differences between the 0.0001% acarbose and control diets (Figure 2A). Similarly, the time to when 50% of the larvae had pupated ($LP_{50}$) was 35 days when fed the control and 0.0001% AI diets and was 63 days on the 0.001% acarbose diet (Figure 2B).

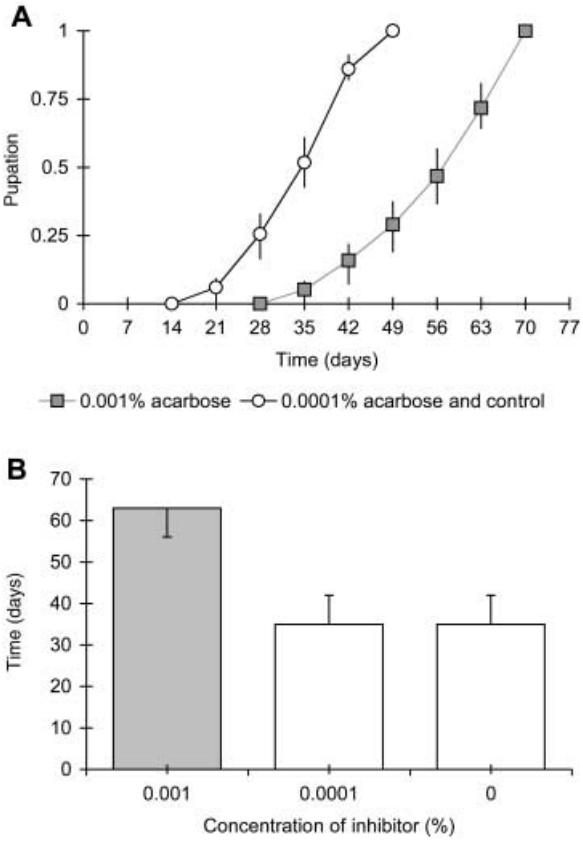


**Figure 2** (A) Comparison of the proportion of *Ephestia kuehniella* larvae that pupated and time to pupation (days) using the Kaplan–Meier analysis and (B) the time ($LP_{50}$) when 50% of the larvae pupated. Because there were no significant differences in the proportion of larvae that pupated when fed the 0.0001% acarbose and control diets, the curve in (A) is fitted for both treatments as a single fit; vertical lines are 95% confidence limits. Larvae that died before pupation were not analysed.

The acarbose also significantly influenced the increase in weight of the larvae, as indicated by the RGR. The lowest RGR was recorded on the acarbose concentration of 0.001%, whereas that on the 0.0001% acarbose diet did not differ from that on the control diet (Table 1). However, there were no differences in the final weight before pupation among the control and treated larvae. This means that the increase in weight of larvae on the 0.001% acarbose diet was slower, but they developed for longer and as a consequence the pupae were of similar weight to those reared on the control and 0.0001% acarbose diets.

**Table 1** Effect of the inhibitor acarbose on weight and nutrient contents of *Ephestia kuehniella* larvae, means and SDs (in parentheses). RGR is relative growth rate, and WLP is fresh mass of larvae before pupation. Dry mass (%), glucose, and proteins (mg $g^{-1}$) were measured in 21-day-old larvae; the glucose content from glycogen hydrolysis is an indirect assessment of glycogen. Mean values in a column followed by the same letter do not differ significantly (P<0.05) in least significant differences (LSD) tests

| | Growth parameters | | Nutrient contents | | | |
|---|---|---|---|---|---|---|
| Diet | RGR | WLP (g) | Dry mass (%) | Glucose from glycogen (mg $g^{-1}$) | Protein (mg $g^{-1}$) | Glycogen:protein |
| Control | 1.233a (0.002) | 0.0137 (0.0019) | 27.8a (3) | 3362b (447) | 84 (26) | 42.9a (12.1) |
| Acarbose 0.0001% | 1.091a (0.001) | 0.0137 (0.0013) | 31.1b (3.3) | 3896c (316) | 73 (11) | 53.8b (9.5) |
| Acarbose 0.001% | 0.402b (0.002) | 0.0114 (0.0032) | 29ab (3.5) | 2693a (271) | 71 (6) | 36.3a (6.8) |
| ANOVA | $F_{2,37} = 12.91$ P<0.001 | $F_{2,37} = 2.27$ P = 0.118 | $F_{2,59} = 4.48$[1] P = 0.016 | $F_{2,25} = 26.29$ P<0.001 | $F_{2,5} = 1.86$ P = 0.17 | $F_{2,25} = 8.51$ P = 0.001 |

[1]Analysis of data after angular transformation.

Although the weights of the larvae prior to pupation were similar in all treatments (Table 1), their nutrient contents differed. The larvae fed the lower acarbose concentration had the highest proportion of dry weight and glucose after glycogen hydrolysis, and glycogen:proteins ratio. Only the content of proteins per unit weight of larvae did not differ significantly among treatments.

#### The indirect effects of acarbose on *Ventruria canescens*

The diet could affect the parasitoid *V. canescens* indirectly via its host larva. The inhibitor-treated hosts, however, did not adversely affect the wasp; those reared on larvae fed the lower concentration of acarbose were even affected positively. The positive effect of the 0.0001% AI diet is apparent in the significantly ($F_{2,58} = 9.94$; P<0.001) larger wasps that emerged in this treatment (Figure 3A). Morphological features also varied significantly between treatments (Tables 2 and 3). The absolute values (non-standardized) RDA analysis indicated that the main morphological difference between the treatments is that wasps emerging from hosts reared on the acarbose 0.0001% diet were much larger. The relative values (after standardization by specimens) showed the wasps that emerged from hosts reared on the 0.0001% acarbose diet had the largest forewing parameters 4-w, 4-l, and 3-l, the control individuals had relatively very long tibia, and those reared on hosts treated with 0.001% acarbose can be recognized mainly by their relatively short forewing parameters, 6-w and 6-l (Figure 4). The number of mature eggs in the parasitoids that emerged from hosts reared on the diet with 0.0001% of the inhibitor was higher than in those that emerged from control and 0.001% treated hosts (Figure 3B), but this difference was not statistically significant ($F_{2,22} = 1.31$, P = 0.29).

The treatments had no significant effect on the probability of oviposition (dev = 2.71, d.f. = 2,166, P = 0.26). There was, however, a significant effect on the sequence in which the

**Table 2** Comparison of morphological parameters; means and SDs (in parentheses) of hind tibial length and the length and width of six parts of the forewing (see Figure 1) of *Venturia canescens* adults, reared on control and acarbose-treated host larvae of *Ephestia kuehniella*. n = 20 wasps on control, 32 on 0.001, and 24 on 0.0001% of acarbose

| | | Length of wing part (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| Host diet | Length of tibia | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | 1.82 (0.10) | 4.08 (0.26) | 1.73 (0.11) | 1.12 (0.08) | 0.90 (0.07) | 1.17 (0.08) | 0.10 (0.01) |
| Acarbose 0.0001% | 1.97 (0.07) | 4.32 (0.14) | 1.85 (0.08) | 1.22 (0.05) | 0.98 (0.05) | 1.17 (0.15) | 0.12 (0.02) |
| Acarbose 0.001% | 1.82 (0.11) | 4.07 (0.20) | 1.74 (0.09) | 1.12 (0.06) | 0.90 (0.05) | 1.16 (0.07) | 0.09 (0.02) |
| | | Width of wing part (mm) | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | | 1.49 (0.10) | 0.50 (0.04) | 0.62 (0.04) | 0.38 (0.03) | 0.38 (0.04) | 0.10 (0.01) |
| Acarbose 0.0001% | | 1.55 (0.08) | 0.54 (0.02) | 0.66 (0.03) | 0.40 (0.03) | 0.40 (0.05) | 0.12 (0.02) |
| Acarbose 0.001% | | 1.49 (0.07) | 0.50 (0.03) | 0.63 (0.03) | 0.38 (0.02) | 0.39 (0.02) | 0.09 (0.02) |

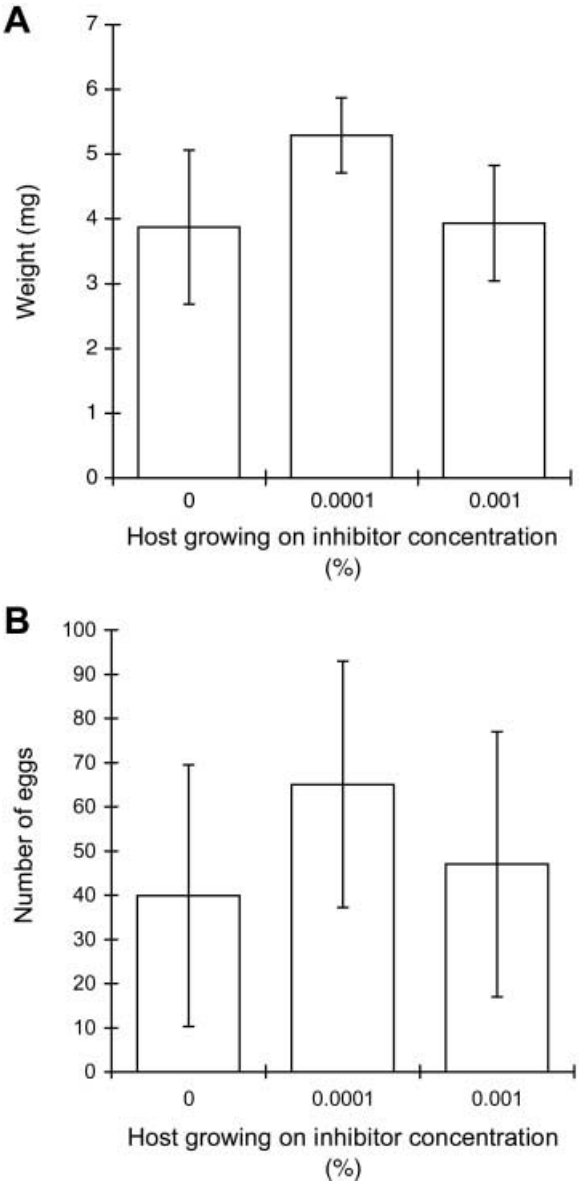


**Figure 3** The effect of acarbose-treated host (*Ephestia kuehniella*) larvae on the parasitoid *Venturia canescens*: (A) weight (mean ± SD) of wasps (mg) and (B) number (mean ± SD) of eggs in the parasitoid's ovaries.

larvae were parasitized (dev = 8.7, d.f. = 1,36, P = 0.003). The fastest parasitism was observed in 0.001% acarbose treatment and the slowest in 0.0001% acarbose treatment (Table 4). The probability of adult emergence (Table 4), however, did not depend on treatment (dev = 0.3, d.f. = 2,41, P = 0.86). It also did not depend on the sequence in which the larvae were parasitized (dev = 2.74, d.f. = 1,54, P = 0.10).

## Discussion

The AIs block larval α-amylase (α-1-4-glucan-4-gluconohydrolases) and prevent the hydrolysis of α-1-4 linked sugar polymers, such as starch or glycogen, into oligosaccharides or glucose, and as a consequence the larvae starve (Pueyo et al., 1995). In this study, we tested the non-proteinaceous low molecular weight inhibitor acarbose, which is used commercially as an antidiabetic agent. The acarbose blocks both α-amylase and α-glucosidases (Asano, 2003), and thus suppresses the whole pathway of starch hydrolysis.

For the construction of transgenic plants, proteinaceous inhibitors, coded by a single gene, seem to be a more feasible method than the non-proteinaceous inhibitors studied here (Franco et al., 2002). Although recent advances in genomics and gene expression technology have made incorporation of non-proteinaceous inhibitors feasible in the foreseeable future (Kinney, 2006), there is a realistic alternative to the incorporation into transgenic plants. This alternative is the massive application of suppressive proteins in stored products such as mixing of protein enriched pea flour with wheat grain, a technique that has

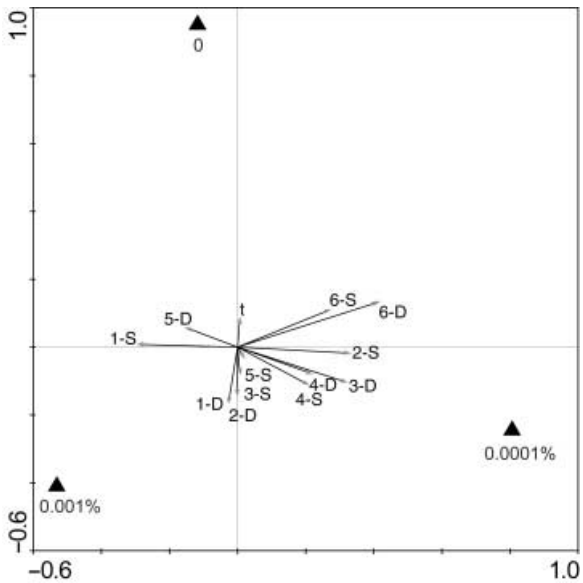


**Figure 4** The effect of inhibitor-treated hosts (*Ephestia kuehniella* larvae) on morphological parameters of adult *Venturia canescens*. Data are analysed using redundancy analysis on relative values (data were standardized by specimens). In this way the figure shows differences in shape after removing the differences in size. The single arrows indicate the different size parameters; the triangles indicate the different concentrations of acarbose (0, 0.001, and 0.0001%). The wing parameters 1–6 are shown in Figure 1.

**Table 3** Comparison of morphological parameters of *Venturia canescens* adults, reared on control and acarbose-treated host larvae of *Ephestia kuehniella*, using the redundancy analysis in the CANOCO program. Two types of results are shown. Results based on absolute values (non-standardized data) show differences mainly in absolute size; relative values (standardized data by morphological parameters and by specimens) show differences in proportions of the different morphological parameters, i.e., in shapes

| Statistics | Absolute values | Relative values |
|---|---|---|
| Percentage of variance explained | 18.9 | 5.7 |
| F | 8.515 | 2.226 |
| P | 0.002 | 0.002 |

already shown to have a high suppressive potential against stored product beetles (Hou & Fields, 2003).

In our experiments, two sublethal concentrations (0.001 and 0.0001%) of the non-proteinaceous AI differentially affected developmental time, larval weight, and nutritional status of *E. kuehniella* larvae. The increase in time to pupation and the slower increase in larval weight at the higher acarbose concentration indicate that sublethal doses of this acarbose negatively affect the development of larvae of *E. kuehniella*. It is known that the adverse effect of high levels of protease inhibitors consists of a complex process that includes a feedback mechanism that results in a chronic hyperproduction of proteases, disorganization of the digestive procedure, loss of appetite, and starvation (Broadway & Duffey, 1986; Cloutier et al., 2000). However, in spite of the observed adverse effect in our experiment, the larvae finally accumulated enough nutrients for successful pupation, and were of similar weight prior to pupation as the control and the lower acarbose concentration-treated larvae. The low concentration of acarbose did not have any negative effect on the time to pupation or the RGR compared to control larvae. That the larvae fed the lower AI concentration had the highest nutritional status might be caused by the fact that the lower concentration of acarbose may stimulate α-amylase production, which may cause a higher starch hydrolysis and glycogen accumulation compared to control larvae.

**Table 4** The number of successful and failed attempts made by the parasitoid *Venturia canescens* to parasitize *Ephestia kuehniella* larvae reared on control and acarbose-treated diets

| | Host diet | | |
|---|---|---|---|
| Parasitism | 0.001% acarbose | 0.0001% acarbose | Control |
| Number of attempts | 18 | 15 | 23 |
| Successes | 6 | 8 | 6 |
| Failures | 12 | 7 | 17 |

The acarbose treatment had no adverse effect on the non-target beneficial *V. canescens*, in terms of its morphological features or prey choice. This is probably because acarbose was not transferred from the diet of host larvae to the larval parasitoid, and/or acarbose was in such a low concentration in the experimental diets that it was virtually absent in larval tissues consumed by the parasitoid during its development. The same results are probable if low concentrations of proteinaceous AIs are used.

A small host larva usually prolongs the development and decreases the size of the parasitoid (Harvey & Vet, 1997). In this study, small host size, due to the prolongation of host development on the 0.001% acarbose-treated diet, only marginally influenced the morphological parameters of the wasps. They did not differ in weight when reared on hosts fed the 0.001% acarbose and control diets. In addition, there were no differences in the success:failure ratio of parasitatism of acarose-treated and control hosts that would indicate a differential parasitoid mortality attributable to the acarbose in host's diet.

The morphological features of adult *V. canescens* indicate a positive effect of developing inside 0.0001% acarbose-treated hosts. The larger size and greater fresh weight of adults can be attributed to better nutrition, as *E. kuehniella* larvae reared on the acarbose 0.0001% diet contained the highest content of nutrients. Those larvae fed the 0.0001% acarbose diet had a higher percentage dry weight and glycogen content than the control and 0.001% acarbose-fed larvae. The higher nutrient contents of these larvae probably accounts for the greater weight and size of the wasps (cf. Harvey & Vet, 1997), which is consistent with a positive correlation between host and wasp mass (Roberts et al., 2004). Wasp size is also correlated with reproductive potential (Eliopoulos et al., 2003). However, there were no differences in the number of mature eggs in the ovaries of the parasitoids emerging from treated and control hosts.

These results indicate that AIs could be used for post-harvest protection. This is important, because there are increasing numbers of known AIs. These AIs are either being incorporated into crops (proteinaceous AI; Carlini & Grossi-de-Sá, 2002), or being massively applied to stored crops (Hou & Fields, 2003). It was already shown that low doses of acarbose fed to the granivorous mite *Acarus siro* do not adversely affect its predatory mite *Cheyletus malaccensis* (Hubert et al., 2007). The present study is the first to report that parasitoids are also unaffected by low doses of AIs. Thus, it might be possible to use stored crops with

enhanced resistance to *E. kuehniella* plus biological control using *V. canescens*. This combination could have a synergistic effect on post-harvest protection.

## Acknowledgements

Our special thanks are to Prof. A. F. G. Dixon for improving our English. We thank Jana Hýblová for incorporating the acarbose into the experimental diets, Václav Stejskal and Jan Lukáš for critical comments, and Marta Nesvorná and Šárka Tučková for technical help. This work was supported by grants GACR 522/04/1286 and COST-1P04OC842.20. It was also partly supported by MSM 0021620828, LC06073 and AV0Z6005908.

## References

Armentia AL, Martinez M, Barber C, Vega DJM & Callejo A (2004) Occupational asthma due to grain pests *Eurygaster* and *Ephestia*. Journal of Asthma 41: 99–107.

Arthur FH (1996) Grain protectants: current status and prospects for the future. Journal of Stored Product Research 32: 293–302.

Asano N (2003) Glycosidase inhibitors: update and perspectives on practical use. Gylcobiology 13: 93R–104R.

Broadway RM & Duffey SS (1986) The effect of dietary-protein on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. Journal of Insect Physiololgy 32: 827–833.

Carlini CR, Grossi-de-Sá MF (2002) Plant toxic with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 40: 1515–1539.

Chrispeels MJ, Grossi-de-Sá MF & Higgins TJV (1998) Genetic engineering with α-amylase inhibitors makes seeds resistant to bruchids. Seed Science Research 8: 257–263.

Cloutier C, Jean C, Fournier M, Yelle S & Michaud D (2000) Adult Colorado potato beetles, *Leptinotarsa decemlineata* compensate for nutritional stress on oryzacystatin I-transgenic potato plants by hypertrophic behavior and over-production of insensitive proteases. Archives of Insect Biochemistry and Physiology 44: 69–81.

Cox DR (1972) Regression models and life tables (with discussion). Journal of the Royal Statistical Society, Series B 34: 187–220.

Cox DR & Oakes D (1984) Analysis of Survival Data. Chapman & Hall, London, UK.

Cox PD, Bell CH, Pearson J & Beirne MA (1984) The effect of diapause on the tolerance of larvae of *Ephestia kuehniella* to methyl bromide and phosphine. Journal of Stored Products Research 20: 215–219.

Eliopoulos PA, Harvey JA, Anthanassiou CG & Stathas GJ (2003) Effect of biotic and abiotic factors on reproductive parameters of the synovigenic endoparasitoid *Venturia canescens*. Physiological Entomology 28: 268–275.

Eliopoulos PA & Stathas GJ (2003) Temperature-dependent development of the koinobiont endoparasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae): effect of host instar. Environmental Entomology 32: 1049–1055.

Ferencik M & Skarka B (1981) Biochemické a laboratórne metódy (The Methods in Biochemistry and Laboratory). ALFA, Bratislava, Slovakia (in Slovak languague).

Franco OL, Rigdem DJ, Melo FR, Grossi-de-Sá FM (2002) Plant α-amylase inhibitors and their interaction with insect α-amylases. European Journal of Biochemistry 269: 397–412.

Gatehouse AMR, Fenton KA, Jepson I & Pavey DJ (1986) The effects of α-amylase in vitro and effects on development in vivo. Journal of Science and Food Agriculture 37: 727–734.

Gatehouse AMR & Gatehouse JA (1998) Identifying proteins with insecticidal activity: use of encoding genes to produce insect-resistant transgenic crops. Pesticide Science 52: 165–175.

Gilles C, Astier JP, Marchis-Mouren G, Cambillau C & Payan F (1996) Crystal structure of pig pancreatic α-amylase isoenzyme II, in complex with the carbohydrate inhibitor acarbose. Europeaen Journal of Biochemistry 238: 561–569.

Harvey JA & Vet LEM (1997) *Venturia canescens* parasitizing *Galleria mellonella* and *Anagasta kuehniella*. Differing suitability of two hosts with highly variable growth potential. Entomologia Experimentalis et Applicata 84: 93–100.

Hou X & Fields PG (2003) Granary trial of protein-enriched pea flour for the control of three stored-product insects in barley. Journal of Economical Entomology 96: 1005–1015.

Hubert J, Dolečková-Marešová L, Hýblová J, Kudliková I, Stejskal V & Mareš M (2005) In vitro and in vivo inhibition of α-amylases of stored-product mite *Acarus siro*. Experimental and Applied Acarology 35: 281–291.

Hubert J, Hýblová J, Munzbergová Z, Pekár S, Kudlíková I et al. (2007) Combined effect of an antifeedant α-amylase inhibitor and a predator *Cheyletus malaccensis* in controlling the stored-product mite pest *Acarus siro*. Physiological Entomology 32: 41–49.

Kinney AJ (2006) Metabolic engineering in plants for human health and nutrition. Current Opinion in Biotechnology 17: 130–138.

Kluh I, Horn M, Hyblova J, Hubert J, Doleckova-Maresova L et al. (2005) Inhibitory specificity and insecticidal selectivity of α-amylase inhibitor from *Phaseolus vulgaris*. Phytochemistry 66: 31–39.

Lepš J & Šmilauer P (2003) Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press, Cambridge, UK.

Palyvos NE, Athanassiou CG & Kavallieratos NG (2006) Acaricidal effect of a diatomaceous earth formulation against *Tyrophagus putrescentiae* (Astigmata: Acaridae) and its predator *Cheyletus malaccensis* (Prostigmata: Cheyletidae) in four grain commodities. Journal of Economical Entomology 99: 229–236.

Price NR (1985) The mode of action of fumigants. Journal of Stored Products Research 21: 157–164.

Prözell S & Schöller M (2000) Commercial application of parasitoids and predators of stored-product pest insects. IOBC WPRS Bulletin 23 (10): 165–168.

Pueyo JJ, Morgan TD, Ameenuddin N, Liang C, Reeck GR et al. (1995) Effects of bean and wheat α-amylase inhibitors on α-amylase activity and growth of stored product insect pests. Entomologia Experimentalis et Applicata 75: 237–244.

Rahman MM, Roberts HL, Sarjan M, Asgari S & Schmidt O (2004) Induction and transmission of *Bacillus thuringiensis*

tolerance in the flour moth *Ephestia kuehniella*. Proceedings of the National Academy of Sciences 101: 2696–2699.

Roberts HLS, Trüe O & Schmidt O (2004) The development of the endoparasitoid wasp *Venturia canescens* in superparasitised *Ephestia kuehniella*. Journal of Insect Physiology 50: 839–846.

Rogers DJ (1972) The ichneumon wasp *Venturia canescens*: oviposition and the avoidance of superparasitism. Entomologia Experimentalis et Applicata 15: 190–194.

Schöller M, Prözell S, AL-Kirshi AG & Reichmuth CH (1997) Towards biological control as a major component of integrated pest management in stored product protection. Journal of Stored Product Research 33: 81–97.

Sedlacek JD, Weston PA & Barney RJ (1995) Lepidoptera and Psocoptera. Integrated Management of Insects in Stored Products (ed. by B Subramanyam & DW Hagstrum), pp. 41–70. Marcel Dekker, Inc., New York, NY, USA.

Slobodchikoff CN (1983) Why asexual reproduction? Variation in populations of the parthenogenetic wasp, *Venturia canescens* (Hymenoptera: Ichneumonidae). Annals of Entomological Society of America 76: 23–29.

Xie YS, Bodnaryk RP & Fields PG (1996) A rapid and simple flour-disk bioassay for testing substances active against stored-product insects. Canadian Entomologist 128: 865–875.

Zettler JL & Arthur FH (2000) Chemical control of stored product insects with fumigants and residual treatments. Crop Protection 19: 577–582.