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Sampling arthropod pests and natural enemies in stored barley

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Application of pest management programs for the food industry reduces pesticide applications, increases the effectiveness of pest suppression to acceptable levels and reduces the cost and risk of negative non-target effects. In these programs, efficient sampling is a decisive factor for undertaking management measures. In this study, a one year survey of arthropods attacking stored barley and their natural enemies was conducted in two experimental piles, in which no fumigation was applied. Sampling was performed on a monthly basis with a grain sampling trier, with pitfall traps and yellow sticky traps. The parasitoid *Anisopteromalus calandrae* was released twice in one of the piles with the aim of testing its establishment and control capacity on the infesting beetles.

Seven coleopteran and one psocid species formed the assemblage of pests that attacked the two barley piles, among which *Sitophilus granarius*, *Rhyzopertha dominica* and *Latheticus oryzae* were the most abundant species. Two parasitoids, *A. calandrae* and *Cephalonomia waterstoni*, and one predator, *Withius piger*, were the beneficial organisms found. Two releases of *A. calandrae* were successful in establishing the parasitoid, but they were not enough for controlling either *S. granarius* or *R. dominica*. Pitfall traps captured the same complex of species as the grain sample trier but the abundance of each species was not always the same in both devices. These traps were good for detecting the early presence of most species. Yellow sticky traps only captured some species from the pest complex and trapped high numbers of the parasitoid *A. calandrae*. They can be of help as indicators of the colonization of some specific pests, such as *R. dominica*, in the first weeks of the invasion process.

1. Introduction

There is much interest in alternatives to conventional insecticides for controlling stored-product insects because of insecticide loss due to regulatory action and insect resistance, and because of increasing consumer demand for products that are free of insects and insecticide residues (Phillips and Throne, 2010). The development of pest management programs for the food industry reduces pesticide applications, increases the effectiveness of pest suppression to acceptable levels and reduces the cost and risk of negative non-target effects.

In these programs, efficient sampling is a decisive factor for the timely and safe undertaking of management measures. In the case of stored grain, correct sampling indicates the early detection and degree of infestation, population density and the activity of different species in the product. At present, limited information is available on stored-grain pest composition and population dynamics, which limits the development of pest management tools and integrated pest management programs in these commodities. Among nonchemical strategies for stored product protection there is increasing interest in the use of natural enemies, such as parasitoids and predators, as a promising alternative for the future. However, again, limited information is available on this topic.

A diverse community of arthropods is associated with stored grains. Several surveys have been conducted in Mediterranean countries in stored-grain commodities to investigate the composition of these communities (Buchelos and Katopodis, 1995; Athanassiou and Buchelos, 2000, 2001; Athanassiou et al., 2011). Among the parasitoids identified, *Anisopteromalus calandrae* Howard (Hymenoptera: Pteromalidae) is one of the promising candidates for the biological control of a wide range of storage pests because of its high fecundity. It is a cosmopolitan, solitary,

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ectoparasitic parasitoid that attacks the larvae of numerous species of stored-product insects developing internally or concealed within the host substrate (Schöller et al., 2006; Chaisaeng et al., 2010).

In the present study, a one year survey of arthropods attacking stored barley and their natural enemies was conducted in two experimental piles, in which no fumigations of the grain were applied. Sampling was performed on a monthly basis with a grain sampling trier, with pitfall (probe) traps and yellow sticky traps. The parasitoid *A. calandrae* was released twice in one of the piles with the aim of testing its establishment and control capacity on the infesting beetles.

2. Materials and methods

Trials were carried out in a warehouse of IRTA located in Caldes de Montbui (Barcelona, Spain) which is surrounded by pastures and cereal fields. It is used to store cattle food during winter. Two piles of 3200 Kg (pile 1) and 1800 Kg (pile 2) (both 180 cm high) of barley from the previous year's harvest were located in two separate and isolated clean rooms (50 m apart one from the other) at the end of March 2010. The grain had a moisture content of 13% and had not been treated with any insecticide. *A. calandrae* were obtained from a stock culture maintained in the laboratory, which had originally been collected from grain silos near Barcelona, Spain. The parasitoid was reared at 28 ± 2 °C and 70 ± 10%RH on rice weevils (*Sitophilus granarius*) in brown rice. Two releases of *A. calandrae* (2400 and 1350 adults) were performed in pile 1 in two consecutive weeks (15th and 21st of April), while no releases of parasitoids were performed in pile 2. Parasitoids were released on the surface of the pile. Both piles were sampled on a monthly basis until January 2011 with a grain sampling trier, pitfall traps and yellow sticky traps.

The grain sampling trier (Consultores cerealistas S.A., Castelldefels, Barcelona, Spain) was an aluminum tube of 1.50 m long, with 8 elongated holes (10 cm long each) separated 2.5 cm between holes. Only grain located at depth levels of 0–10 cm, 25–35 cm, 50–60 cm and 90–100 cm was collected. Three samples of these depths per sampling date were taken in each pile. Grain was sieved out (sieve of 2 mm opening) and all adults present were counted and identified. Three pitfall traps (AgriSense, Suterra) per pile were buried just below the grain surface and insects captured were counted and identified. Two yellow sticky traps (20 × 20 cm, Sanidad Agrícola Econex S.L., Santomera, Murcia, Spain) per pile were hung above each pile (Pi) or in a wall 5 m apart from the pile (Wa in pile 1) or in a door 5 m apart (Do in pile 2). Temperatures of the grain bulk were measured with one set of 5 probes (DaqPRO Data Logger) in each pile that registered data at depths of 0, 20, 40, 60 and 80 cm at hourly intervals.

At the end of January 2011, the quality of the grain was assessed in 5 ml of each of the three samples extracted with the grain sampling trier and from the four depths. The number of intact and damaged grains and the proportion of flour were evaluated.

2.1. Data analysis

The Simpson's index of diversity was used to determine species diversity within each pile for captures with the grain sampling trier and with pitfall traps (Southwood and Henderson, 2000). This index is calculated as:

$$1 - D = 1\sum (p_i)^2$$

where, $1 - D$ is the index of diversity and p_i is the proportion of individuals of an i species relative to the total insects captured with each sampling method. To test for relationships among captures of the different species present in the two piles with the three sampling methods and with grain temperature Spearman's coefficient of rank correlation was calculated and its significance was tested in JMP (SAS Institute Inc., 2009).

3. Results

There was an important diversity of species present in these two piles. Among the species attacking the grain, there were 7 species of Coleoptera belonging to 5 different families: *S. granarius* (L.) (Curculionidae), *Rhyzopertha dominica* (F.) (Bostrichidae), *Latheticus oryzae* Waterhouse, *Tribolium castaneum* (Herbst), *Tribolium confusum* Jacquelin du Val (Tenebrionidae), *Cryptolestes ferrugineus* (Stephens) (Laemophloeidae) and *Oryzaephilus surinamensis* (L.) (Silvanidae). Also, there was Psocoptera attacking the grain, *Liposcelis* spp. (Liposcelidae). Among the beneficial organisms, we found two Hymenopteran parasitoids, *A. calandrae* (Howard) (Pteromalidae) and *Cephalonomia waterstoni* (Gahan) (Bethylidae), and one predator *Withius piger* (Simon) (Withiidae) belonging to the order Pseudoscorpionida.

The Simpson's index of species diversity was high in the two piles: 0.72 for pile 1 and 0.67 for pile 2 when sampling with the grain sampling trier, and 0.68 for pile 1 and 0.64 for pile 2 when sampling with pitfall traps.

3.1. Grain sampling trier

Both piles supported a similar and extremely high number of beetles at the end of the experiment: at the last sampling date total cumulative coleopterans were 8022 in pile 1 and 8704 in pile 2 per kg of grain in each sampling date. The beetle's populations were low during spring (on the 30^{th} of march just two adults of *R. dominica* was found per 0.5 Kg of grain in pile 1 and none in pile 2) and started to increase in summer, and reached high densities in winter. However, the most abundant species varied in the two piles: in pile 1, *R. dominica, S. granarius* (primary pests feeding on whole grains) and *L. oryzae* (secondary pest feeding on already damaged grains) predominate, while only *R. dominica* and *S. granarius* predominate in pile 2 (Fig. 1, A&B). Other species of coleopterans, such as *C. ferrugineus, O. surinamensis, T. confusum, T. castaneum,* and the psocid *Liposcelis* sp., were present in lower numbers (Fig. 1, C&D).

A peak abundance of *A. calandrae* was found in pile 1 in mid-August, after four months had passed from the two releases of the parasitoid (Fig 1E). Taking into account the environmental conditions, this peak could correspond to the 4^{th}-5th generation developed in the grain pile. *C. watersoni* was also found in this pile, although in very low numbers. *A. calandrae* also appeared in pile 2 although it was not released. Its abundance was lower, while in that pile there was also presence of *C. watersoni* and abundance of the predator *W. piger* during autumn and winter (Fig 1F).

The two most abundant species, *S. granarius* and *R. dominica*, were able to penetrate up to 90 cm deep into the piles. However, most of the population, almost 80%, was located between 0 and 60 cm deep. This was also true for the less abundant species found: *L. oryzae*, *T. castaneum* and *T. confusum, C. ferrugineus, O. surinamensis* and the psocid *Liposcelis* sp. were also located at all heights sampled in the two piles. Also, the three natural enemies were present up to the deepest layer sampled, at 90 cm deep. Distribution of the species at the different depths sampled was similar during the experimental period (Fig. 2).

Abundance of individuals captured with the grain sampling trier was positive correlated among all beetle species except for *O. surinamensis.* Captures of psocids were negative correlated with captures of all beetle species except for *O. surinamensis.* Abundance of the parasitoid *A. calandrae* was positive correlated with