

Effect of flour and pasta debris on larval development of *Rhyzopertha dominica*

Lidia LIMONTA, Daria Patrizia LOCATELLI

DeFENS - Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Italy

Abstract

The development of *Rhyzopertha dominica* (F.) larvae on different types of flour and on pasta debris was evaluated under laboratory condition. Tests were carried out on wheat flour, semolina, wheat bran, corn meal, corn starch, rice flour, buckwheat flour, chickpea flour, almond flour, chestnut flour, potato starch, and pasta debris. Two layers of flour or pasta debris, 3 and 6 mm high, were placed in Petri dishes and 20 first instar larvae were put into the middle of the substrate. Four replicates were carried out for each combination of substrate, particle size, and thickness of the layer. The emergence of adults was assessed daily. Chestnut flour and semolina permitted the development of larvae in a period of time similar to that observed in cereal kernels while chickpea flour and wheat flour were less appropriate food; in fact, only 25% of larvae underwent complete development into adults. In the case of corn meal, rice flour, and wheat bran, only 5% of adults emerged, while larvae were unable to develop on corn starch and potato starch. Although *R. dominica* is a well-known pest of pasta, pasta debris was less suitable for the development of larvae, as few adults emerged and a longer development time was observed. A three-millimeter layer of suitable food was sufficient for the development of larvae, although a higher number of adults was observed in the 6 mm layer.

Key words: lesser grain borer, larvae, food dust, thickness of food, suitable diet.

Introduction

From an economic point of view the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera Bostrichidae), is one of the most important beetles, infesting cereal kernels and pasta (Schwardt, 1933; Bashir, 2002; Edde and Phillips, 2006b). As a bostrichid, the original food of the insect should be wood and dried fruit, and thereafter the species adapted to feed on dry grains (Chittenden, 1911; Linsley, 1944; Potter, 1935). Larval development is through four larval instars (Potter, 1935; Howe, 1950; Thompson, 1966), and takes 46 days at 25 °C and 30 days at 28 °C and 70% RH (Howe, 1950). The species can develop within 18.2 and 39 °C, and 70% RH, on wheat kernels at 14% moisture content (Birch, 1945a; 1945b; 1953; Longstaff, 1999).

Fresh and dead twig, seeds, bulbs, and fruits belonging to different plant species have been reported as rearing media for *R. dominica* (Chittenden, 1911; Potter, 1935; Wright *et al.*, 1990; Morrison *et al.*, 2005; Jia *et al.*, 2008; Edde and Phillips, 2006a; 2006b; 2006c). In tests carried out with semolina, due to the small particle size, adults showed a poor mobility, turned over and died (Klys, 2006). First instar larvae could not develop in 0.5 mm of semolina, while, in 6 mm, the number of emerging adults was the same as that observed on kernels and the development time was shorter (Limonta *et al.*, 2011). The different behaviour can be explained as adults need a hard substrate to walk while larvae are able to move also on soft surface but we observed *R. dominica* adults walking and females laying eggs on thin layer of semolina, and adults emerged from semolina afterwards (unpublished results).

In this study, the development of *R. dominica* larvae on different types of flour and pasta debris was evaluated. Flours and debris can accumulate and become a breeding ground for pests in grain milling departments

and the food industry, due to improper cleaning of machines and facilities. The purpose of this study was to establish the layer of food that allows *R. dominica* development in order to plan cleaning operations.

Materials and methods

Insect rearing

Laboratory cultures of *R. dominica* kept on wheat seeds in a rearing room at 28 ± 1 °C, $70 \pm 5\%$ RH and a photoperiod of 16:8 (light:dark) since five years, were used for the experiments. Eggs were collected according to Elek (1994), incubated for 7-8 days, and assessed daily in order to obtain first instar larvae, 0-24 hours old.

Flour and pasta debris

Wheat flour, semolina, wheat bran, cornmeal, corn starch, rice flour, buckwheat flour, chickpea flour, almond flour, chestnut flour, and potato starch, used in tests, were purchased from the supermarket.

Five types of substrate, semolina, corn meal, rice flour, buckwheat flour, and chickpea flour, were sifted and two particle size fractions, 0.149-0.210 mm and 0.210-0.297 mm, were tested.

Pasta debris, collected from a pasta factory, was sieved and whole debris, >2.38 mm, 0.707-1.68 mm, and 0.4-0.5 mm particle size were assayed.

All the diets were kept at -18 °C for 20 days before the beginning of the tests.

Post-embryonic development

Twenty newly hatched larvae of *R. dominica* were placed in the middle of the substrate in Petri dishes (5 cm in diameter) containing 3 or 6 mm, of flour or pasta debris.

Four replicates were carried out, for each combination

of substrate, particle size, and thickness, and adult emergence was assessed daily until no insects emerged for 30 days.

Data analysis

The number of adults emerged and the development period on flour and on pasta debris were submitted to one-way analysis of variance (ANOVA), two-way ANOVA with diet and thickness as fixed factors while data on sieved flours were submitted to three-way ANOVA with diet, thickness, and particle size as fixed factors. The statistical differences among means were evaluated using the least significant difference (LSD) test at $\alpha = 0.05$ (SPSS22).

Results

Development of *R. dominica* on flour

In table 1, the mean number (\pm SE) of adults, starting from 20 first instar larvae, and the mean development of *R. dominica* on different types of flour, and two layer thicknesses, 3 mm and 6 mm, are reported. The highest number of adults in the 3 mm layer was observed on semolina, 8.2 ± 0.95 ($F = 31.591$; $df = 10, 33$; $P < 0.000$), and a significantly lower number was recorded on chestnut flour, 6.2 ± 0.48 . Chickpea flour and wheat flour were less suitable as rearing diet, as 4.2 ± 1.03 and 3.5 ± 0.50 adults developed respectively. Few adults emerged from wheat bran, rice flour, and corn meal, while, on corn starch, potato starch, buckwheat flour, and almond flour, larvae were unable to develop. The period from first instar larva to adult, in 3 mm, varied from 37.6 ± 1.86 to 29.2 ± 0.38 days ($F = 9.581$; $df = 6, 100$; $P < 0.000$). The longest developmental time was recorded on wheat bran, rice flour, chickpea flour and chestnut flour, and the shortest time was recorded on semolina and wheat flour.

In the case of buckwheat flour, adults were recorded only in the 6 mm layer. The highest number of adults (13.5 ± 3.00) was recorded in 6 mm of chestnut flour, significantly different from the number of adults observed in semolina (10.7 ± 1.60) ($F = 30.03$; $df = 10, 33$;

$P < 0.000$). Corn starch, potato starch, buckwheat flour, and almond flour, were unsuitable for the development of *R. dominica* larvae also in the case of 6 mm thickness. The shortest developmental period was recorded in 6 mm of semolina (27.5 ± 0.73 days) ($F = 18.111$; $df = 7, 196$; $P < 0.000$).

A two-factor ANOVA showed a significant ($F = 5.658$; $df = 10, 66$; $P < 0.000$) interaction between diet and the thickness of the layer, but diet had a stronger influence than thickness. LSD test confirmed that the number of adults of *R. dominica* was significantly higher on chestnut flour and semolina, followed by wheat flour and chickpea flour.

Development of *R. dominica* on different particle size

The number of adults and the developmental period of *R. dominica* recorded on semolina, corn meal, rice flour, buckwheat flour, and chickpea flour, sifted into two fractions, 0.210-0.297 mm and 0.149-0.210 mm, and two layers, 3 and 6 mm, are shown in table 2.

In the particle size fraction 0.210-0.297 mm, 3 mm layer, the highest number of adults was observed in semolina, 6.5 ± 0.64 ($F = 44.426$; $df = 4, 15$; $P < 0.000$), and the shortest development period, 32.5 ± 1.72 days, in chick pea flour ($F = 5.864$; $df = 3, 55$; $P < 0.001$). In the 6 mm layer the highest number of adults was observed in semolina, 8.7 ± 0.75 , and in chickpea flour, 10.0 ± 1.41 , that was significantly different from the number of adults developed on other sifted flours ($F = 26.11$; $df = 4, 15$; $P < 0.001$). The longest development period was observed in chickpea flour, 43.5 ± 6.75 days, and it was significantly different from the development period on the other diet ($F = 21.507$; $df = 3, 98$; $P < 0.05$). The development period on buckwheat was not considered as there only one adult emerged (33.0 days).

In the particle size fraction 0.149-0.210 mm, 3 mm layer, again the highest number of adults was observed in semolina and chickpea flour, 6.7 ± 0.85 and 5.2 ± 0.63 respectively ($F = 24.069$; $df = 4, 15$; $P < 0.001$), while, in the 6 mm layer, the number of adults registered in semolina, 8.5 ± 0.64 , was significantly different from the number of adults in chickpea flour, 6.7 ± 0.63 ($F = 40.395$; $df = 4, 15$; $P < 0.001$). In 3 mm the longest

Table 1. Mean number (\pm SE) of adults and mean development time (\pm SE) (days), from newly hatched larva to adult, of *R. dominica*, reared on different substrates and two different layer thickness, 3 mm and 6 mm.

Substrate	3 mm		6 mm	
	Adults	Days	Adults	Days
Wheat bran	$2.0 \pm 0.41d$	$37.6 \pm 1.86a$	$2.7 \pm 0.25cd$	$37.3 \pm 0.99a$
Wheat flour	$3.5 \pm 0.50c$	$29.2 \pm 0.38c$	$6.2 \pm 0.48c$	$29.5 \pm 1.11bc$
Semolina	$8.2 \pm 0.95a$	$30.8 \pm 0.80bc$	$10.7 \pm 1.60b$	$27.5 \pm 0.73c$
Cornmeal	$1.2 \pm 0.25de$	$33.6 \pm 2.71abc$	$2.5 \pm 0.29d$	$39.5 \pm 0.70a$
Corn starch	$0.0 \pm 0.00e$	-	$0.0 \pm 0.00e$	-
Potato starch	$0.0 \pm 0.00e$	-	$0.0 \pm 0.00e$	-
Rice flour	$1.5 \pm 0.29d$	$37.3 \pm 0.80a$	$5.0 \pm 0.41c$	$31.7 \pm 0.87b$
Buckwheat flour	$0.0 \pm 0.00e$	-	$4.0 \pm 1.08cd$	$36.2 \pm 1.79a$
Chestnut flour	$6.2 \pm 0.48b$	$35.1 \pm 0.77a$	$13.5 \pm 1.50a$	$39.0 \pm 1.11a$
Chickpea flour	$4.2 \pm 1.03c$	$35.5 \pm 0.73a$	$5.0 \pm 0.71c$	$36.2 \pm 0.68a$
Almond flour	$0.0 \pm 0.00e$	-	$0.0 \pm 0.00e$	-

The means followed by different letters in the same column are significantly different (LSD, $P < 0.05$).

Table 2. Mean number (\pm SE) of adults of *R. dominica* that emerged from different diets and two different particle size fractions, 0.210-0.297 mm and 0.149-0.210 mm, with two different layer thicknesses, 3 mm and 6 mm, and mean development time (\pm SE) (days) from newly hatched larva to adult.

Diet	0.210-0.297 mm				0.149-0.210 mm			
	3 mm		6 mm		3 mm		6 mm	
	Adult	Days	Adult	Days	Adult	Days	Adult	Days
Semolina	6.5 \pm 0.64a	40.5 \pm 1.15a	8.7 \pm 0.75a	35.2 \pm 0.58b	6.7 \pm 0.85a	44.9 \pm 0.61a	8.5 \pm 0.64a	45.8 \pm 0.68a
Corn meal	1.7 \pm 0.25c	35.6 \pm 3.54ab	2.5 \pm 0.29bc	35.9 \pm 1.58b	3.2 \pm 0.25b	39.8 \pm 1.81b	3.2 \pm 0.25d	40.0 \pm 1.56b
Rice flour	1.7 \pm 0.25c	40.7 \pm 1.67a	4.2 \pm 0.75b	33.6 \pm 1.21b	3.0 \pm 0.41b	34.8 \pm 1.63c	4.7 \pm 0.48c	31.6 \pm 1.13c
Buckwheat flour	0.0 \pm 0.0d	-	0.2 \pm 0.25c	33.0*	0.0 \pm 0.0c	-	0.5 \pm 0.29e	31.0 \pm 0.00c
Chickpea flour	4.0 \pm 0.41b	32.5 \pm 1.72b	10.0 \pm 1.41a	43.5 \pm 6.75a	5.2 \pm 0.63a	43.5 \pm 1.06a	6.7 \pm 0.63b	42.8 \pm 0.88b

Means followed by different letters in the same column are significantly different (LSD, $P < 0.05$). *Value not considered.

Table 3. Mean number (\pm SE) of adults of *R. dominica* that emerged from whole and sifted pasta debris in two different layers, 3 mm and 6 mm, and mean development time (\pm SE) (in days) from newly hatched larva to adult.

Particle size of pasta	3 mm		6 mm	
	Adults	Days	Adults	Days
Whole debris	0.5 \pm 0.29b	53.5 \pm 9.5	0.7 \pm 0.48b	50.0 \pm 3.46ab
>2.38 mm	3.0 \pm 0.91a	58.6 \pm 3.14	3.0 \pm 0.91a	60.1 \pm 1.98a
0.707-1.68 mm	0.5 \pm 0.29b	53.0 \pm 2.00	0.7 \pm 0.25b	45.3 \pm 0.33b
0.4-0.5 mm	0.2 \pm 0.25b	71.0*	1.0 \pm 0.41b	53.5 \pm 6.70ab

Means followed by different letters in the same column are significantly different (LSD, $P < 0.05$). *Value not considered.

development period was observed in semolina and chickpea flour, 44.9 \pm 0.61 and 43.5 \pm 1.06 days respectively ($F = 13.676$; $df = 3, 69$; $P < 0.001$), while in 6 mm the longest development period was observed in semolina, 45.8 \pm 0.68 days, and it was significantly different from the development period on chickpea flour and corn meal ($F = 32.715$; $df = 4, 90$; $P < 0.001$).

The results of tests with sifted flours (table 2) were compared with the number of adults recorded with the same diet not sifted (table 1). A three-factor ANOVA showed a significant ($F = 3.165$; $df = 8, 90$; $P < 0.005$) interaction between diet, the thickness of the layer and particle size, but diet had a stronger influence and particle size was not significant.

Development of *R. dominica* on pasta debris

In tests with whole and sifted debris collected from a pasta factory (table 3), the highest number of adults of *R. dominica* was observed in the fraction greater than 2.38 mm, for both the considered layer thicknesses, 3 mm ($F = 6.333$; $df = 3, 12$; $P < 0.05$) and 6 mm ($F = 3.677$; $df = 3, 12$; $P < 0.05$). On whole debris and in the 0.707-1.68 mm and 0.4-0.5 mm fractions, the number of adults was significantly lower. The number of adults was influenced by particle size ($F = 8.348$; $df = 3, 24$; $P < 0.001$), while neither the thickness of the layer ($F = 2.087$; $df = 1, 24$; NS) nor the interaction of the two factors ($F = 0.174$; $df = 3, 24$; NS) was significant.

In 3 mm of pasta debris, development from newly hatched larva to adult was not significantly different ($F = 0.766$; $df = 3, 13$; NS). The development period on 0.4-0.5 mm size fraction, 3 mm layer, was not considered as there was only one adult emerged (71.0 days).

In 6 mm, the shortest period of time was observed in the 0.707-1.68 mm size fraction (45.3 \pm 0.33 days) and the

longest one in the debris with particle size lower than 2.38 mm (60.1 \pm 1.98 days), ($F = 3.556$; $df = 3, 18$; $P < 0.05$).

Discussion

In this study, the development of *R. dominica* was observed on different types of flour. Chestnut flour and semolina permitted the development of larvae in a period of time similar to that observed in cereal kernels (Howe, 1950). Chickpea flour and wheat flour were a less appropriate food, and in fact, only 25% of larvae completed development to adult. In the case of corn meal, rice flour, and wheat bran, only 5% of adults emerged.

Larvae were unable to develop on corn starch and potato starch while reproduction on small pieces of dried potatoes was observed (Edde and Phillips, 2006b), this behavior can be attributed to the different texture of the media.

Almond flour is not a breeding source for *R. dominica*, as larvae on this media did not develop into adults. This result agrees with those found by Kapoor (1964), who observed that oil seeds and spices are not a suitable food and development is slowed. The behaviour on seeds is different according to the plant species; for example, *R. dominica* can develop and reproduce on walnut seeds and acorn (Wright *et al.*, 1990; Jia *et al.*, 2008), while few adults survive on peanuts and do not reproduce (Edde and Phillips, 2006b).

Observations carried out on the sieved fractions, i.e. 0.210-0.297 mm and 0.149-0.210 mm, and two layer thicknesses, 3 and 6 mm, of semolina, corn meal, rice flour, buckwheat flour, and chickpea flour, demonstrated that development was influenced by the diet and the thickness of the diet, while particle size had no ef-

fect. This is in contrast to what was observed in a previous study, where the particle size of soft wheat flour influenced both the percentage of adults emerging and the mean development time of *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae), as flour with a smaller particle size had low rates of air diffusion (due to restricted interstitial space), which reduced the amount of available oxygen (Locatelli *et al.*, 2008).

Although *R. dominica* is a well-known pest of pasta, debris was less suitable for the development of larvae, as few adults emerged and a longer development time was observed. The best development was observed in the fraction of debris with a size larger than 2.38 mm, i.e. the size fraction with the largest particles, more similar to grain size.

Three-millimeter layer of suitable food was sufficient for the development of larvae, even if a higher number of adults was observed in 6 mm. In a previous study, it was observed that 0.5 mm of semolina was inadequate, as first instar larvae could not develop or fled, as larvae thrive inside a food layer (Limonta *et al.*, 2011). In fact, the body length of first instar larvae is 0.78 mm (Potter, 1935), and that of fourth instar larvae is 3.2 mm and the head is approximately 0.41 mm in diameter (Howe, 1950); therefore a 0.5 mm layer is insufficient for larvae to nest.

The development from newly hatched larva to adult on the different types of substrate varied from a minimum of 27 days on semolina to a maximum of 39 days on corn meal. The time slightly increased with decreasing particle size and almost doubled on pasta debris in one case, to 71 days.

The wide range of flour types that allow for the development of *R. dominica* has increased the number of food industries that can be colonized by this species, such that not only pasta factories are a suitable environment, but also other types of food processing plants. Moreover, this species can thrive on a small amount of food dust, increasing the importance of sanitation. The design of food processing machinery must consider the important issue accessibility to facilitate the cleaning of dust and debris.

References

BASHIR T., 2002.- Reproduction of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different host-grains.- *Pakistan Journal of Biological Sciences*, 5: 91-93.

BIRCH L. C., 1945a.- The mortality of the immature stages of *Calandra oryzae* L. (small strain) and *Rhyzopertha dominica* Fab. in wheat of different moisture contents.- *Australian Journal of Experimental Biology and Medical Science*, 23: 141-145.

BIRCH L. C., 1945b.- The influence of temperature on the development of the different stages of *Calandra oryzae* L. and *Rhyzopertha dominica* Fab. (Coleoptera).- *Australian Journal of Experimental Biology and Medical Science*, 23: 29-35.

BIRCH L. C., 1953.- Experimental background to the study of the distribution and abundance of insects-I: the influence of temperature, moisture and food on the innate capacity for increase of three grain-beetles.- *Ecology*, 34: 698-711.

CHITTENDEN F. H., 1911.- The lesser grain borer and the larger grain borer.- *Bulletin of the United States Bureau of Entomology*, 96: 29-47.

EDDE P. A., PHILLIPS T. W., 2006a.- Field responses of non-target species to semiochemicals of stored-product Bostrichidae.- *Annals of the Entomological Society of America*, 99: 175-183.

EDDE P. A., PHILLIPS T. W., 2006b.- Potential host affinities for the lesser grain borer, *Rhyzopertha dominica*: behavioral responses to host odors and pheromones and reproductive ability on non-grain hosts.- *Entomologia Experimentalis et Applicata*, 119: 255-263.

EDDE P. A., PHILLIPS T. W., 2006c.- Longevity and pheromone output in stored-product Bostrichidae.- *Bulletin of Entomological Research*, 96: 547-554.

ELEK J. A., 1994.- Methods for collecting eggs and monitoring egg-hatch and immature development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae).- *Journal of Stored Products Research*, 30: 261-265.

HOWE R. W., 1950.- The development of *Rhyzopertha dominica* (F.) (Col., Bostrichidae) under constant conditions.- *Entomologist's Monthly Magazine*, 86: 1-5.

JIA F., TOEWS M. D., CAMPBELL J. F., RAMASWAMY S. B., 2008.- Survival and reproduction of lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on flora associated with native habitats in Kansas.- *Journal of Stored Products Research*, 44: 366-372.

KAPOOR S., 1964.- Nutritional studies on *Rhyzopertha dominica* F. (Bostrichidae: Coleoptera). I. Effects of various natural foods on larval development.- *Indian Journal of Entomology*, 26 (3): 289-295.

KLYS M., 2006.- Nutritional preferences of the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae) under conditions of free choice of food.- *Journal of Plant Protection Research*, 46: 359-368.

LIMONTA L., MOROSINI M. C., LOCATELLI D. P., 2011.- Development of *Rhyzopertha dominica* (F.) (Coleoptera Bostrichidae) on durum wheat kernels and semolina.- *Journal of Entomological and Acarological Research*, 43: 33-38.

LINSLEY G. E., 1944.- Natural sources, habitat, and reservoirs of insects associated with stored food products.- *Hilgardia*, 16: 187-225.

LOCATELLI D. P., LIMONTA L., STAMPINI M., 2008.- Effect of particle size of soft wheat flour on the development of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae).- *Journal of Stored Products Research*, 44: 269-272.

LONGSTAFF B. B., 1999.- An experimental and modeling study of the demographic performance of *Rhyzopertha dominica* (F.). I. development rate.- *Journal of Stored Products Research*, 35: 89-98.

MORRISON A. A., MULDER P. G., SAMBARAJU K., 2005.- Host suitability of pecans for five species of storage pests.- *Proceedings of the Oklahoma Pecan Growers' Association*, 75: 55-58.

POTTER C., 1935.- The biology and distribution of *Rhyzopertha dominica* (Fab.).- *Transactions of the Royal Entomological Society of London*, 83: 449-482.

SCHWARDT H. H., 1933.- Life history of the lesser grain borer.- *Journal of the Kansas Entomological Society*, 2: 61-66.

THOMPSON V., 1966.- Biology of the lesser grain borer, *Rhyzopertha dominica* (F.).- *Bulletin of Grain Technology*, 4: 163-168.

WRIGHT V. F., FLEMING E. E., POST D., 1990.- Survival of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) on fruits and seeds collected from woodrat nests in Kansas.- *Journal of the Kansas Entomological Society*, 63: 344-347.

Authors' addresses: Lidia LIMONTA (corresponding author, lidia.limonta@unimi.it), Daria Patrizia LOCATELLI, Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy.

Received December 22, 2015. Accepted March 3, 2016.