

## Evaluation of low humidity treatments to control *Acarus farris* (Acari: Acaridae) in Cabrales cheese

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**Abstract** Laboratory experiments were conducted to establish the best combination of relative humidity (r.h.) and time of exposure to be applied on Cabrales cheeses infested with *Acarus farris*. Laboratory assays revealed that less than 30 h were required to obtain 90% mortality of mites at 50% and 60% r.h. Males were more susceptible than females to low relative humidity, since their lethal period values (LP) were lower than those obtained for females at the same relative humidity. Moreover, the response within sexes to low moisture treatments changed as exposure time increased, since the LP50 obtained for each sex at 50 and 60% r.h. were statistically different whereas the LP90 showed no significant differences within sexes. Accordingly, two modifications of the traditional maturing process were established to assess the efficacy of low moisture treatments to control *A. farris* on infested cheeses. The first modification consisted of one single exposure of 48 h at 50% r.h. and the second one consisted of two exposures of 48 h at 50% r.h. separated by a time interval of 15 days. No significant differences in final population density were observed for both low moisture treatments compared to control cheeses. Therefore, low humidity treatments are not effective to control *A. farris* in Cabrales cheese, despite the good results obtained in laboratory assays.

**Keywords** *Acarus farris* · Mite control · Cabrales cheese · Relative humidity

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## Introduction

The use of low moisture treatments has been proposed as an environmentally safe method to control astigmatid mite pests on stored food, where these organisms constitute serious economic and health problems (Zdarkova 1991). Astigmatid mites are very susceptible to low values of relative humidity because their body fluids have a water activity greater than 0.99 (Arlian 1992), and, in addition, these mites present large surface/volume ratios and weakly sclerotized cuticles, which favour the loss of fluids through the body surface (Wharton et al. 1979; Evans 1992).

One of the most susceptible foodstuffs to mite infestations is cheese (Süss et al. 1978; Wilkin 1979). In Spain, mites usually infest Cabrales cheese, a typical regional blue cheese, which is manufactured in a traditional way. Prior to commercialization, Cabrales cheese is matured in natural caves for a variable period of time that can be up to 3 months. During the maturing period, mites can reach heavy infestations by feeding on cheese leading to weight reduction and high labour costs to clean the cheeses. The relative humidity in the maturing caves is greater than 90%, so that mite populations find proper conditions to develop and increase in number.

The main species observed infesting Cabrales cheese is *Acarus farris* (Oudemans), being able to produce severe losses when high population densities are reached (Sánchez-Ramos et al. 2007a). It has been reported that *A. farris* populations cannot develop at 70% r.h. (Sánchez-Ramos et al. 2007b). However, experiments in warehouse condition are needed to assess the effect of low moisture treatments to control this mite in foodstuffs with high water content, such as Cabrales cheese, since this feature could balance the low relative humidity conditions established to control the mites.

The aim of this study was to establish the feasibility of low moisture treatments to control *A. farris* infestations on Cabrales cheese. Prior to that, a laboratory evaluation was made to establish the best combination of relative humidity and time of exposure to be applied on infested cheeses.

## Material and methods

### Mites

A stock culture of *A. farris* was established from infested samples of Cabrales cheese obtained from the maturing cave of Corporación Agroalimentaria Peñasanta (CAPSA) in Carreña de Cabrales (Asturias, Spain). A colony reared in the laboratory for less than 2 years was used. The mites were maintained on Brewer's yeast and held in cylindrical plastic cages (12 cm diameter and 5.5 cm height). The cages were covered with round plastic plates that had a 5 cm diameter hole in the centre sealed with filter paper disks for ventilation. The rearing cages were kept in an environmental chamber (Sanyo MLR-350H, Sanyo, Japan) at  $25 \pm 0.5^\circ\text{C}$ , 90 r.h. and dark conditions, within a watered plastic tray to prevent escape. Adults were sexed by observing their secondary sexual characteristics (Hughes 1976).

Laboratory assays with mites were conducted using rearing cells (Barker 1967). These cells were concave slides (15–18 mm concavity diameter and 0.5–0.8 mm depth of well) covered with a cover glass (22 mm  $\times$  22 mm) initially sealed by contact with a small drop of water. After evaporation, the cover glass remained non-airtight fixed to the slide, thus allowing air and humidity exchange (Sánchez-Ramos and Castañera 2001). Previously, the

mites required at each assay were introduced inside each cell together with a Brewer's yeast flake (about 1.3 mg), with the help of a thin camel hair brush.

### Mite susceptibility to low relative humidity

Relative humidities of 50% and 60% were selected to evaluate their effect against adults of *A. farris*. As males and females are different in size, they were considered separately for the assays, since smaller individuals have greater surface/volume ratios and thus have a greater tendency to lose water through the body surface (Evans 1992; Wharton et al. 1979).

To test for low moisture effect, adult males and females <7 days were used. To assess the lethal period (LP), mortality values were registered at exposures of 0, 6, 9, 12, 15, 18, 21, 24, 27 and 30 h to selected humidities. For each combination of humidity, sex and time exposure, 5 slides with 10 individuals per slide were used. All experiments were made in an environmental chamber at  $15 \pm 0.5^\circ\text{C}$  and dark conditions.

### Low relative humidity treatments on cheese

The cheeses used for the assays were provided by CAPSA. The maturing period employed by CAPSA is about 2 or 3 months and at day 30 from the entry in the cave, cheeses are wet and wrapped in paper to avoid heavy moisture loss. Therefore, the same procedure was also followed in the field experiments. The environmental conditions during the maturing are about  $15^\circ\text{C}$  and 90% r.h., which from now on will be stated as *standard conditions*.

To establish the initial and final mite density of treated and untreated cheeses, five samples of  $2.5 \times 2.5$  cm were taken out of each cheese (total area:  $31.25 \text{ cm}^2$ ). The samples were put into plastic vessels containing 20 ml of 96° ethanol and shaken to remove the mites. The ethanol with the mites was then transferred into a 9 cm diameter Petri dish where mite density was estimated by using a counting card disk placed under the Petri dish according to the method described by Solomon (1945). Only mobile stages were considered since the eggs remained stuck to the cheese samples. Mite density was expressed as number of mobile stages/ $\text{cm}^2$ . The initial mite density was established at the beginning of the treatments and the final population density was estimated after 80 days from the beginning of the maturing process. The mite density in the different treatments was compared with that obtained from control cheeses matured under standard conditions.

From the information previously obtained in the laboratory assays, two modifications of the traditional maturing process were used to assess the efficacy of low moisture treatments on infested cheeses. The first modification consisted of one single exposure of 48 h at 50% r.h. and the second one consisted of two exposures of 48 h at 50% r.h. separated by a time interval of 15 days. The first exposure at 50% r.h. was done in both cases before the wetting and wrapping of the cheeses. After 30 days in the cave, the cheeses were transferred to an environmental chamber under the selected relative humidity and at  $15 \pm 0.5^\circ\text{C}$  to simulate the temperature conditions of the maturing cave. After the treatments (and also between them in the second case) and to the end of the maturing process, the cheeses were kept in the chamber under standard conditions to avoid reinfestation from the mites in the cave. Four cheeses were utilized for each treatment and for the control.

## Data analysis

Lethal period values and 95% fiducial limits for the relative humidities tested in laboratory experiments were obtained by probit analysis using the computer program POLO-PC (LeOra Software 1987). Natural response and time exposures that produced mortality rates different from 0% and 100% were used for probit analysis. The differences in susceptibility between sexes at the relative humidities selected were analysed by the 95% confidence intervals of lethal period ratios (LPR) at the LP50 and LP90 (Robertson and Preisler 1992).

The comparison of initial and final mite density between treated and control cheeses was analysed using ANOVA, followed by Newman-Keuls multiple range test for separation of significantly different means. Data were previously transformed with  $\sqrt{(x)}$  transformation to meet the conditions of parametric statistics. The level of significance was  $P < 0.05$  in all cases.

## Results

### Mite susceptibility to low relative humidity conditions

Less than 30 h were necessary to obtain 90% mortality for males and females of *A. farris* at the relative humidities assayed. Males were more susceptible than females, since their LP50 and LP90 were lower than those obtained for females at the same relative humidity (Table 1). Nevertheless, the LP50 of males at 60% r.h. were not statistically different than the LP50 of females at 50% r.h. Also, the response to low moisture treatments changed as exposure time increased, since the LP50 obtained for each sex at 50% and 60% r.h. were statistically different whereas the LP90 showed no significant differences within sexes (Table 1). The probit regression lines are represented in Fig. 1.

### Low relative humidity treatments on cheese

For both types of treatment, initial population densities of treated and control cheeses were not statistically different (means  $\pm$  SE:  $51.8 \pm 18.2$  mobile mites/cm<sup>2</sup> for treatment 1;  $93.8 \pm 11.8$  mobile mites/cm<sup>2</sup> for treatment 2;  $91.2 \pm 20.2$  mobile mites/cm<sup>2</sup> for the control). In all cases, population density at the end of the maturing process was significantly greater than the initial one ( $410.4 \pm 135.5$  mobile mites/cm<sup>2</sup> for treatment 1;

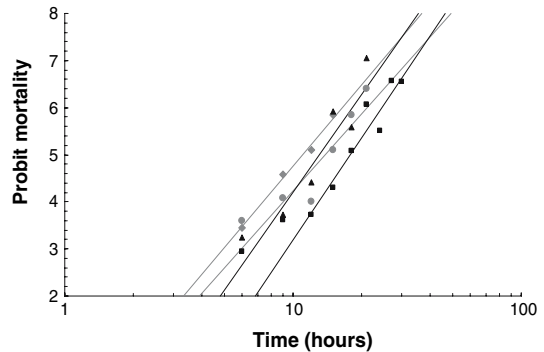
**Table 1** Lethal period values and probit slope of low moisture treatments at 15°C on males and females of *Acarus farris*

| % r.h. | Sex     | <i>n</i> | Slope $\pm$ SE | $\chi^2$ | d.f. | LP50 <sup>a</sup> (95% CI) | LP90 <sup>a</sup> (95% CI) |
|--------|---------|----------|----------------|----------|------|----------------------------|----------------------------|
| 50     | Males   | 250      | $5.7 \pm 0.8$  | 0.8      | 2    | 11.0 (10.2–12.0) a         | 18.4 (16.0–22.9) a         |
| 60     | Males   | 350      | $6.9 \pm 0.7$  | 20.6     | 4    | 13.1 (10.0–16.3) b         | 20.0 (16.1–39.9) a         |
| 50     | Females | 350      | $5.4 \pm 0.6$  | 20.4     | 4    | 13.8 (10.4–18.9) b         | 23.7 (17.8–72.7) b         |
| 60     | Females | 500      | $7.2 \pm 0.9$  | 16.0     | 7    | 17.9 (15.1–19.8) c         | 26.9 (23.9–33.9) b         |

*n* represents the number of individuals employed for probit analysis

<sup>a</sup> Values followed by different letters within each column are significantly different ( $P < 0.05$ ) according to the 95% confidence intervals of lethal period ratios (LPR). Lethal periods expressed in hours

**Fig. 1** Probit regression lines of *Acarus farris* adults exposed to low moisture treatments; (◆), males at 50% r.h.; (●), females at 50% r.h.; (▲), males at 60% r.h.; (■), females at 60% r.h



$445.0 \pm 80.2$  mobile mites/cm<sup>2</sup> for treatment 2;  $592.8 \pm 185.0$  mobile mites/cm<sup>2</sup> for the control). Moreover, no significant differences in final population density were observed for both types of low moisture treatments compared to the control cheeses.

## Discussion

The laboratory evaluation of the effect of low relative humidity on *A. farris* showed differences in susceptibility between males and females, which agree with the initial hypothesis of greater effect on males because of their smaller size. This has also been observed in *Tyrophagus putrescentiae* (Schränk) males and females treated by inhalation with different monoterpenes that produced a desiccation effect on the mites (Sánchez-Ramos and Castañera 2001). On the other hand, prolonged exposures to the two low moisture environments selected tend to reduce the differential effect observed at the LP50, since the LP90 obtained within sexes for each relative humidity were not statistically different. This could be explained by the different rate of water lost, as observed in *Dermatophagoides farinae* Hughes during prolonged exposures to relative humidities under its critical equilibrium humidity (Arlian and Wharton 1974; Wharton and Furumizo 1977). According to these authors, in the first 14 h of exposure, the water is lost rapidly, but then only slow diffusion through the cuticle continues. A similar mechanism could be acting in *A. farris*, so that, in the first hours of the exposure, the water would flow faster at the lowest humidity selected (50% HR), but, as time goes by, this flow would become similar resulting in similar LP90 values.

We have found that 30 h were enough to kill more than 90% of the experimental population of *A. farris* at the relative humidities selected. The rearing cells employed in the assays allowed a total exposure of mites to the moisture conditions, which might not be similar to the situation encountered in cheese. Because of that, we decided to increase the time of the treatments to 48 h for the experiments with cheeses. Longer exposures would not be advisable due to the risk of affecting the organoleptic properties of Cabrales cheese by desiccation.

The greater effect observed for males compared to females due to their smaller size suggests that immature stages would be even more susceptible to our experimental conditions as they are smaller than the males. The exception would be the eggs and the quiescent phases between stages. The egg shell represents a barrier that protects the embryo from external stresses and environmental hazards (Witalinski 1993) and therefore would act to prevent desiccation. The quiescent phases have two cuticular layers, since the

new cuticle of the body originates under the old one (Evans 1992), which would give greater protection under low moisture conditions than the normal cuticle. Accordingly, a two-exposure treatment was selected to kill those mites that were in egg or quiescent stages at the moment of the first exposure to low relative humidity.

Nevertheless, despite the great effect observed in laboratory assays, the low moisture treatments did not significantly reduce the mite density when applied to Cabrales cheeses. This could be related to the structure and high water content of the cheeses that probably allow mites to survive by changing their behavioural and feeding strategies. Mites under hydric stress search for shelter, which can then act as a barrier against water transpiration to the environment (Cutcher 1973). Also, mites can ingest the free water content in the food (Cutcher 1973; Arlian 1992). As a consequence, foodstuffs that provide high water content and numerous shelters to mite populations, such as Cabrales cheese, would be more difficult to protect by reducing the relative humidity. Similar results have been observed in Iberian hams exposed to relative humidity values below 60% (García 2004). These treatments reduced mite population on the ham surface but forced the mites to seek refuge in cavities inside the hams where they produced even greater damage.

We can conclude that low humidity treatments are not effective to control *A. farris* in Cabrales cheese, despite the good results obtained in laboratory assays. The viability of low relative humidity treatments to control mite pests of stored food depends not only on the susceptibility of mites to desiccation, but also on the characteristics of the products that might allow the survival and development of mite populations exposed to low moisture environments.

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