Lower temperature thresholds for oviposition and egg hatching of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in a Mediterranean climate

Ó. Dembilio[1]*, G.V. Tapia[2], M.M. Téllez[2] and J.A. Jacas[3]

[1]Institut Valencià d'Investigacions Agràries (IVIA), Unitat Associada d'Entomologia Agrícola UJI–IVIA, Departament de Protecció Vegetal i Biotecnologia, Ctra Montcada-Nàquera km 4.5, E-46113-Montcada, Spain: [2]Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Unidad Control de Plagas, Centro La Mojonera, E-4745-Almería, Spain: [3]Universitat Jaume I (UJI), Unitat Associada d'Entomologia Agrícola UJI–IVIA, Departament de Ciències Agràries i del Medi Natural, Campus del Riu Sec, E-12071-Castelló de la Plana, Spain

Abstract

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is an economically important pest of palms worldwide. To better understand the phenology of *R. ferrugineus* populations in the Mediterranean basin, the objective of the present work has been to estimate the effect of temperatures within the range 10–25°C on the reproductive parameters of *R. ferrugineus* and to determine its lower temperature thresholds for oviposition and egg hatching. Our results confirm that oviposition in *R. ferrugineus* is strongly affected by temperature. Lower thresholds for oviposition and egg hatching (15.45° and 13.95°C, respectively) are below the mean monthly temperatures registered in winter in most of the northern shore of the Mediterranean basin. Under these circumstances, new palm infestations would be difficult during most of the winter. These results should be taken into account when planning some palm management practices, such as pruning or pesticide treatments, in areas under a Mediterranean climate.

Introduction

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is an economically important tissue-boring pest of palms worldwide (EPPO, 2008, 2009, 2010). The weevil was first described in India as a serious pest of coconut (Lefroy, 1906; Nirula, 1956) and date palms (Buxton, 1918). At the present time, this insect has been reported as a pest of 26 palm species belonging to 16 different genera (Malumphy & Moran, 2009). In the Mediterranean basin, where this species spread slowly during the mid-1990s and very quickly starting in 2004, *R. ferrugineus* has become the major pest of palms, especially *Phoenix canariensis* hort. ex. Chabaud, which is extremely sensitive to its attack (Dembilio *et al.*, 2009).

*Author for correspondence
Fax: +34 964728216
E-mail: dembilio@ivia.es

Adult *R. ferrugineus* are large rusty-brown weevils about 3 cm long with a characteristic long curved rostrum. They have strong wings, which enable them to undertake long flights. Female weevils lay their eggs singly at the base of the fronds in separate holes, which are often close to each other, made with their rostrum. The creamy-yellow eggs (approx. 2.5 mm long) are laid close to the surface of these wounds. Subsequently, females produce a secretion that seals the wound and protects the eggs.

Temperature is the main abiotic factor influencing the biology, ecology and population dynamics of poikilothermic organisms such as arthropods, and *R. ferrugineus* is not an exception. The thermal requirements of *R. ferrugineus* developing in live *P. canariensis* palms have been recently established by Dembilio & Jacas (2011). A thermal constant of 40.4 degree-days (DD) was obtained for the egg stage (lower temperature threshold, LTT, was 13.1°C). Larvae required 666.5 DD (LTT 15°C: Martín-Molina, 2004) to complete their development; this is 40 to 160 days depending on mean temperatures (summer-autumn or winter-spring generations, respectively). Pupal development required an additional 282.5 DD (LTT 13°C: Martín-Molina, 2004) and, therefore, could be completed in two weeks in summer but extended for several months during the rest of the year. The same authors concluded that under field conditions mean monthly temperatures below 10.3°C were lethal for neonate larvae and 4.5°C for older immature stages. Based on the strong relationship found between mean annual temperature (MAT) and the number of generations of *R. ferrugineus* estimated for different climatic stations in the Iberian Peninsula, these authors concluded that less than one generation per year could be expected in areas with MAT below 15°C and more than two where MAT is above 19°C. However, temperature could limit the reproduction of *R. ferrugineus*, not only by affecting preimaginal development, but also the oviposition behavior of adult females. Li *et al.* (2010) studied the effects of temperature on the fecundity of *R. ferrugineus* and concluded that the optimum temperature was 28°C (239.47 eggs per female) while upper and lower thresholds occurred somewhere within the ranges 36–40°C and 16–20°C, respectively. Mean temperatures in most of the northern shore of the Mediterranean basin (AEMET, 2011) fall within the range obtained by Li *et al.* (2010) for the lower oviposition threshold. Therefore, under these climatic conditions, the reproduction of *R. ferrugineus* could be impaired by temperature during the winter. To better understand the phenology of *R. ferrugineus* populations in the Mediterranean basin, the objective of the present work has been to estimate the effect of temperatures within the range 10–25°C on the reproductive parameters of *R. ferrugineus*.

Material and methods

Experimental insects

Adult weevils collected in the province of Valencia in traps baited with ferrugineol (the male *R. ferrugineus* aggregation pheromone) and plant kairomones (ethyl acetate and pieces of palm fronds) were used to initiate the stock colonies. These colonies were established in 2007 and have been periodically supplemented with the introduction of additional wild specimens. Adult weevils were reared in a controlled environment cabinet at 25±1°C, 75±5% RH and a 16h light photoperiod in perspex cages (30×30×45 cm depth) with a density of 120–150 weevils per cage. These cages had a hole

(8 cm diameter) on the upper side covered by a mesh used for manipulation of the specimens and their bottom side consisted of a 2-mm metal mesh. Cages were set on top of a tray containing a folded piece of moistened filter paper containing thin apple slices used by female weevils as oviposition substrate and by both males and females as food. Apple slices were replaced three times per week (Dembilio *et al.*, 2009).

Experimental design

Newly emerged (<24-h old) male and female adult *R. ferrugineus* obtained from our laboratory colony were caged (one couple per cage) in plastic lunchboxes (15×10×10 cm) and provided folded pieces of moistened filter paper and thin apple slices as food and oviposition substrate. A total of 108 cages were prepared. Sixty cages were transferred to an environmental chamber at a constant temperature of 25°± 0.3°C. The remaining 48 cages were moved in groups of 12 to additional environmental chambers at a constant temperature of 10°, 15°, 20° or 23°±0.3°C. In all cases, the chambers were set at a relative humidity of 85–90% and a photoperiod of 16:8 (L:D). Cages were kept under these conditions for two weeks. On day 14, the 60 pairs at 25°C were split into five groups of 12. One of them was further kept at 25°C, whereas the remaining four were transferred to 10°, 15°, 20° or 23°C. From that day and for two more weeks, cages were supplied moistened filter paper and thin apple slices that were replaced daily. During this two-week period, daily oviposition was recorded. Eggs were further transferred into petri dishes (5 cm diameter) with moistened filter paper and kept under the same environmental conditions as their mothers (for those moved from 25°C to lower temperatures at day 14) or at 25°C (for those mothers exposed to the same temperature since emergence), and egg hatching was measured.

Statistical analyses

For those pairs where at least one egg was recorded, oviposition rate, fecundity and egg hatching during the two-week period considered were subjected to analysis of variance (one-way-ANOVA; $P < 0.05$). Egg hatching results were subjected to the angular transformation prior to the analysis to meet the requirements of ANOVA. Where appropriate, means were separated by Duncan's test ($P = 0.05$). Results obtained at constant temperature regimes (10–25°C) were further analyzed by linear regression analysis to estimate the lower thresholds for oviposition and egg hatching. The SPSS Statistics 18.0.0 software (IBM Corporation, Somers, NY, USA) was used.

Temperature

Mean, mean minimum and mean maximum monthly air temperatures from some climatic stations representative of the Mediterranean basin countries where *R. ferrugineus* is established were compared with oviposition and egg hatching thresholds to determine oviposition and egg hatching periods in the selected locations. The places chosen were Almeria, Melilla and Valencia (Spain) for the period 1971–2000 (AEMET, 2011) and Adana and Istanbul (Turkey), Algiers (Algeria), Alexandria (Egypt), Athens and Iraklion (Greece), Benghazi and Tripoli (Libya), Cagliari, Palermo and Rome (Italy), Marseille, (France), Split (Croatia), and Tel-Aviv (Israel) for the period 1931–2009 (World Climate, 2011).

Table 1. Percentage ovipositing females, oviposition rate (eggs per female per day), fecundity (eggs per female) and egg hatching (%) of *R. ferrugineus* females obtained at different temperature regimes in the laboratory. Each value is the mean of 12 replicates (12 couples per temperature tested).

Temperature (°C)		Ovipositing females	Oviposition rate	Fecundity	Egg hatching
Day 0–14	Day 15–28				
25	25	100	2.38 ± 0.25 a	33.25 ± 3.46 a	75.5 ± 1.6 a
23	23	100	1.88 ± 0.09 b	26.25 ± 1.21 b	71.8 ± 5.0 a
20	20	100	0.88 ± 0.05 c	13.08 ± 0.80 c	65.8 ± 4.8 a
15	15	0	–	–	–
10	10	0	–	–	–
25	23	100	1.82 ± 0.17 b	25.50 ± 2.31 b	75.8 ± 1.8 a
25	20	83.3	0.55 ± 0.09 cd	7.67 ± 1.29 d	71.7 ± 4.1 a
25	15	66.7	0.37 ± 0.08 de	5.17 ± 1.06 de	60.5 ± 8.9 a
25	10	50.0	0.05 ± 0.02 e	0.75 ± 0.28 e	12.5 ± 9.0 b
ANOVA (F, P)[1]		–	45.73; <0.0001	48.08; <0.0001	14.20; <0.0001

[1] df were 6 and 83 in all cases.
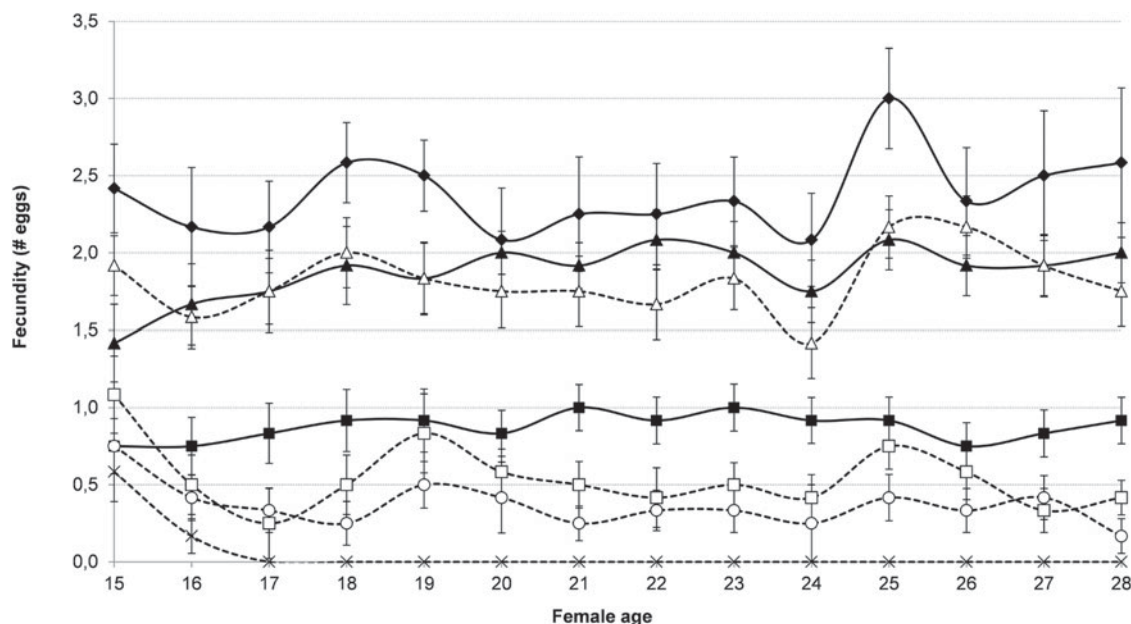Within a column, data followed by the same letter are not different ($P < 0.05$; Duncan's test).

Fig. 1. Daily oviposition rate of 15-day-old *R. ferrugineus* females at five different temperatures (10°, 15°, 20°, 23° and 25°C) when exposed either to the same temperature since emergence (continuous lines) or to 25°C from adult emergence to day 14 (dashed lines)*. (–♦–, 25–25°C; –▲–, 23–23°C; –■–, 20–20°C; - -△- -, 25–23°C; - -□- -, 25–20°C; - -○- -, 25–15°C; - -x- -, 25–10°C).

(*No oviposition was observed for females kept at 10° and 15°C for the entire 30-day period.)

Results

Within the temperature range considered (10–25°C), oviposition rate and fecundity increased with temperature (table 1). Highest fecundity and oviposition rate were obtained at 25°C, (33.25 eggs per female and 2.38 eggs per female and day, respectively), whereas no oviposition was observed for females kept below 15°C. Interestingly, females moved from 25° to 15°C at age 14 days could oviposit a mean of 5.17 eggs during the entire experimental period, and those moved from 25° to 10°C could lay a mean of 0.75 eggs during the first two days of exposure (fig. 1 and table 1). Compared to the fecundity obtained at 25°C, these values correspond to a reduction of 84 and 98%, respectively. These reductions are much higher than those observed at 23° and 20°C (24 and 77%, respectively).

Egg hatching was not significantly different between 20° and 25°C and was the same irrespective of whether the mothers had been exposed to the same temperature since emergence or moved from 25°C to a lower temperature at age 14 days (table 1). The only exception was observed for females moved from 25 to 10°C. In that case, an 83.5% reduction in egg hatching was calculated.

Based on the results obtained at constant temperature regimes (table 1), a threshold of 15.45°C was estimated for oviposition (table 2). Taking into account all results obtained

Table 2. Relationship between (*x*) temperature and (*y*) oviposition rate (eggs per female and day), fecundity (eggs per female) and egg hatching (%) of *R. ferrugineus* females obtained at different constant temperature regimes (15°, 20°, 23° and 25°C) in the laboratory (see table 1).

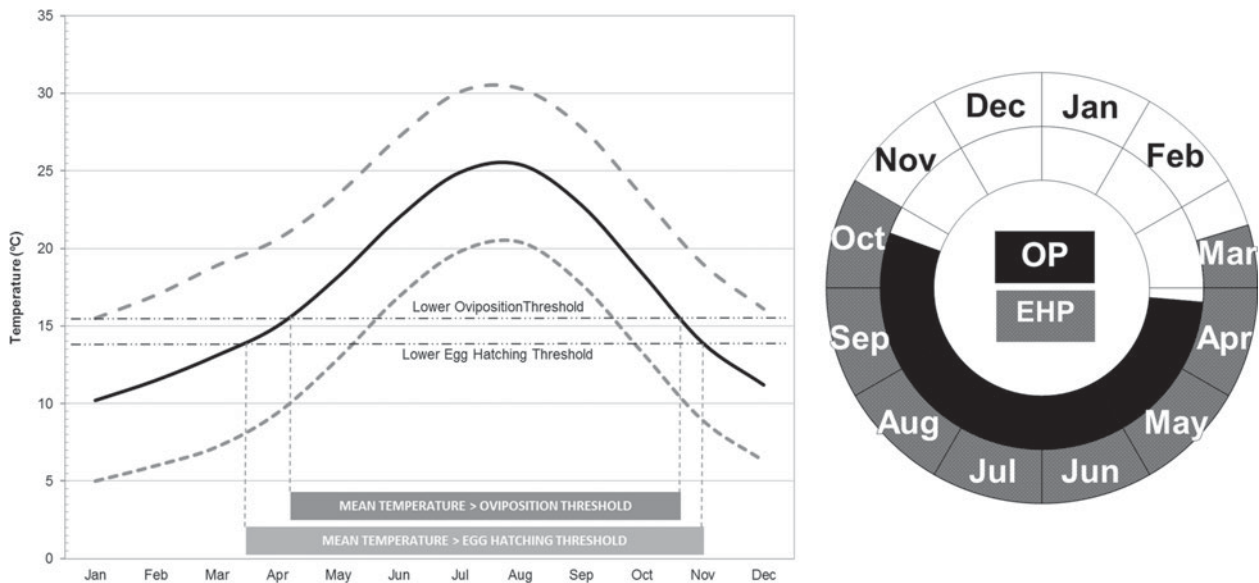| *y*	*r*	F, P	Regression	Lower threshold (°C)
Oviposition rate	0.819	177.426, <0.0001	$y = 0.240x - 3.709$	15.45
Fecundity	0.892	179.370, <0.0001	$y = 3.358x - 51.531$	15.35
Egg Hatching	0.869	141.388, <0.0001	$y = 7.535x - 105.136$	13.95

df were 1, 47 in all cases.

Fig. 2. Mean, mean minimum and mean maximum monthly temperatures at Valencia airport climatic station (Spain) for the period 1971–2000 (AEMET, 2011) and estimated lower thresholds for oviposition and egg hatching. The circular diagram represents oviposition and egg hatching periods (OP and EHP, respectively) at the same location (——, mean; — —, maximum; – – –, minimum).

(table 2), a slightly lower value, 13.95°C, was calculated for egg hatching. When these values were compared to mean monthly temperatures in Valencia (fig. 2) and the remaining selected locations (fig. 3), windows for oviposition and egg hatching could be established. In most of the northern shore of the basin, the oviposition period (OP) extended from early April to mid-October/early November and the egg hatching period (EHP) from mid-March to mid/late October. However, these periods were much longer in the southern shore and, although oviposition would stop during the coldest winter months, egg hatching would continue during the whole year in the southwestern shore of the basin.

Discussion

This study confirms that oviposition in *R. ferrugineus* is strongly affected by temperature and that lower temperature thresholds for oviposition and egg hatching (15.45° and 13.95°C, respectively) are very close to mean annual temperatures registered in most of the northern shore of the Mediterranean basin and clearly above mean monthly temperatures in winter in this area (fig. 2). The fact that females moved from 25° to 15°C at age 14 days could oviposit during the entire experimental period, whereas those kept at this temperature since emergence did not lay any egg may be

indicative of the existence of an imaginal diapause in *R. ferrugineus* that deserves further studies. Li *et al.*, (2010) obtained no oviposition for females exposed to a constant temperature of 16°C and established the lower temperature threshold for oviposition at 16.48°C. This temperature is almost 1°C higher than that found in our study. In their studies, Li *et al.* (2010) used a stock colony established with individuals collected in Wenchang (China). This city is located at a latitude of 19°32′N, which is almost half the mean latitude of the Mediterranean basin (around 40°N), and has a tropical monsoon climate with an mean annual temperature of 23.6°C. Local adaptation of the weevil to prevalent environmental conditions in Wenchang and Valencia (with a mean annual temperature of 17.2°C) could at least partly explain the differences observed. Furthermore, El-Mergawy *et al.* (2011) demonstrated, using RAPD-PCR, that populations found in Mediterranean countries (Egypt, Spain, France) have a different origin from those found in Asia (Arabic Peninsula, Iran, Pakistan). These differences could also account for the relatively higher cold hardiness exhibited by the insects used in our assays compared to those from China.

Egg hatching values in the range 15°–25°C (table 1) are similar to those reported in other studies (Kaakeh, 2005; Li *et al.*, 2010; Dembilio & Jacas, 2011). Furthermore, the lower temperature threshold (LTT), 13.95°C, is very close to 13.1°C,
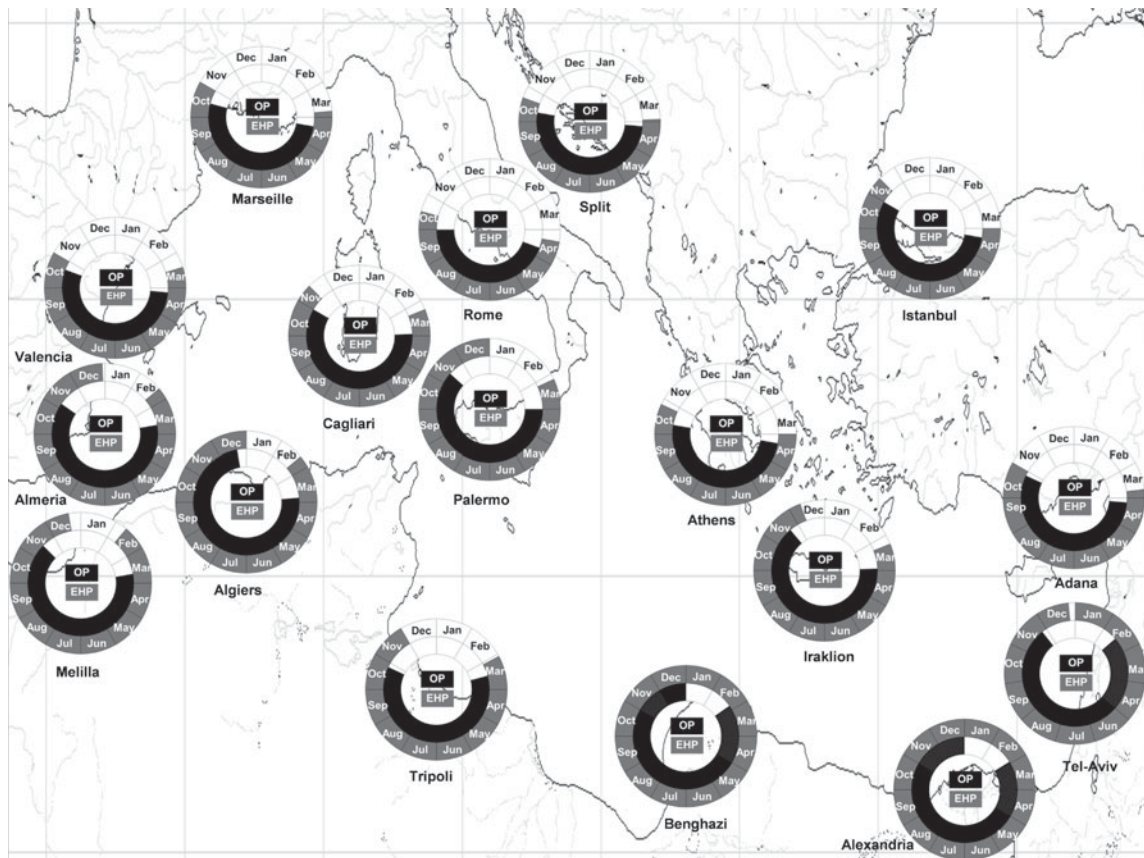
Fig. 3. Estimated oviposition and egg hatching periods (OP and EHP, respectively) based on mean monthly temperatures at some selected climatic stations in the Mediterranean basin.

which was the LTT obtained in a previous study by Dembilio & Jacas (2011). However, this value is again much lower than that reported by Li *et al*. (2010), who set this threshold at 18.28°C. The same reasons as before could explain the differences found, which are much higher in this case than when comparing oviposition thresholds.

Comparison of the temperature thresholds obtained in this study with mean temperatures in the Mediterranean basin clearly shows that oviposition can be seriously impaired between early November and mid-March in most countries within this area. Furthermore, because the window for oviposition is shorter than that for successful egg hatching (fig. 2), the occurrence of new infestations under these environmental conditions is quite unlikely from November until March, especially in the northern shore of the basin. A difference of 2° to 6°C between the outer atmospheric temperature and that inside infested palms has been reported in Egypt in winter and summer, respectively (Salama *et al*., 2009). Therefore, higher temperatures inside infested palms could not so severely limit mating, oviposition and egg hatching in already infested palms. However, even under these conditions, because immature mortality at this time is highest and the development rate of *R. ferrugineus* declines with decreasing autumn-winter temperatures (Dembilio & Jacas, 2011), the final effect of winter temperatures on the population dynamics of *R. ferrugineus* could be a synchronization of the whole population, that would peak in spring, as it is observed in

Valencia (Dembilio & Jacas, 2011) as well as in other areas in the Mediterranean basin (Abbas, 2010). Our results could also explain the decreased infestations occurring after the winter months in the Middle East on date palm (Abraham *et al*., 1998).

The results obtained should help in refining existing IPM strategies against this pest in different regions under a Mediterranean climate. Because wounds, such as those produced when pruning, emit volatiles that attract adult *R. ferrugineus*, pruning can increase the likelihood of a new infestation. Based on our results, pruning, as well as any other management practice producing wounds, should be best performed in winter, when oviposition is greatly reduced and both egg and immature mortality are highest (Dembilio & Jacas, 2011). However, even during this season, all wounds should be immediately protected with a tree wound seal preferably in combination with an appropriate insecticide. Similarly, the frequency of preventative treatments against the weevil (either chemical or biological) could decrease during the winter, thus reducing the non-target effects of these biocides on the environment and, especially, on humans.

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