Effect of Relative Humidity on Longevity, Ovarian Maturation, and Egg Production in the Olive Fruit Fly (Diptera: Tephritidae)

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ABSTRACT The effect of five constant relative humidity (RH) regimes (12, 33, 55, 75, and 94%) on ovarian maturation, adult longevity, and egg production of Bactrocera (Dacus) oleae (Gmelin) (Diptera: Tephritidae) was studied at 25 ± 1°C and a photoperiod of 16:8 (L:D) h under laboratory conditions. The experimental insects developed in the preimaginal stages at 16:8 (L:D) h and 25 ± 1°C, and the adults were maintained in plastic cages in sealed plastic containers with saturated water salt solutions in their base to control RH. In the experiments concerning longevity and egg production, the adults were maintained individually, whereas in those concerning ovarian maturation they were in groups of 10 females and 10 males. Ovarian maturation was much reduced and the number of mature oocytes in the ovaries was very low in 7-d-old females maintained at 12 and 33% RH. By contrast, a high percentage of females of that age maintained at 55, 75, and 94% RHs had mature oocytes in their ovaries. Longevity of adult females, egg production and egg hatch were low at 12% RH. They were significantly higher at 33% RH, and even more so at 55 and 75% RHs but declined at 94% RH. These results could be useful for the mass rearing of B. oleae and for better understanding its population dynamics under field conditions.

KEY WORDS Bactrocera (Dacus) oleae, relative humidity, ovarian maturation, longevity, egg production

The olive fruit fly, Bactrocera (Dacus) oleae (Gmelin) (Diptera: Tephritidae) is a major pest of olives in Mediterranean countries. It overwinters as a pupa in the soil or as an adult in or outside the olive grove. Preimaginal stages develop in the olive fruit from late summer to late autumn or even winter if temperature permits. In regions where olive fruit are not collected and remain on the trees until spring, one or two additional generations are reported to develop in spring (Sacantanis 1957, Arambourg and Pralavorio 1970, Sigwalt et al. 1977).

Several studies recording the condition of ovaries of B. oleae females at different localities in Greece and Italy show that there is a period of reproductive immaturity in late spring to mid- or late summer, which seems to be independent of the peculiarities of certain years (Baranov 1937, Ayoutantis et al. 1954, Stavrakis 1973, Stavrakis and Fytizas 1980, Delrio and Cavalloro 1977, McFadden et al. 1977, Neuenschwander and Michelakis 1979, Girolami et al. 1983, Raspi et al. 2002). Certain investigators have suggested high temperatures, low humidity, and the absence of suitable olive fruit for oviposition as possible factors causing ovarian immaturity during spring and summer (Ayoutantis et al. 1954, Orphanidis 1967, Mourikis and Fytizas 1970, Fletcher et al. 1978). In the laboratory, ovarian maturation did not occur in a high percentage of females whose preimaginal stages developed under a short day photoperiod of 12:12 (L:D) h and a moderately low temperature of 19 ± 1°C and which as adults were maintained under a long day photoperiod of 16:8 (L:D) h and a higher temperature of 26 ± 1°C (Tzanakakis and Koveos 1986, Koveos and Tzanakakis 1990). This experimentally induced ovarian immaturity of B. oleae females was averted when the adult flies had access to olive fruit (Koveos and Tzanakakis 1990). On the island of Corfu, ovarian maturation in late summer was favored by the availability of new olive fruit, but it was inhibited by high temperatures (Fletcher et al. 1978, and references therein). In the context of the current study, massive egg laying has been recorded in various localities of northern Greece after the first autumn rains (D.S.K., unpublished data). We suspected that relative humidity (RH), which is low during the summer days but increases after the first autumn rains, might play a role in ovarian maturation and egg production of B. oleae. The object of the present work was to detect such a role of RH under laboratory conditions.

Materials and Methods

Stock Colony. The laboratory colony was established in autumn 2005 from field-infested ‘Megaritiki’
Table 1. Saturated salt solutions used in our experiments with the corresponding RHs and vapor pressure deficits

| Solution     | RH (%) | Vapor pressure deficit (kPa) |
|--------------|--------|-----------------------------|
| LiCl          | 12     | 2.96                        |
| MgCl₂·6H₂O   | 33     | 2.25                        |
| Mg(NO₃)₂·6H₂O | 55     | 1.51                        |
| NaCl          | 75     | 0.84                        |
| KNO₃          | 94     | 0.17                        |
| H₂O           | 12     | 2.96                        |
| LiCl          | 33     | 2.25                        |
| MgCl₂·6H₂O   | 55     | 1.51                        |
| NaCl          | 75     | 0.84                        |
| KNO₃          | 94     | 0.17                        |

olive fruit collected in the province of Thessaloniki in northern Greece. Stock colony flies were kept in wooden cages (30 by 30 by 30 cm) with two sides of metal net and the front one of glass, in a climatic room at 16:8 (L:D) h and 25 ± 1°C. A liquid diet of commercial sucrose, yeast hydrolysate, and water was offered daily to the stock flies. The intensity of light at the level of the flies varied from 500 to 500 lux and the RH was ≤ 60%.

Control of RH. The experimental adult insects were subjected to 12, 33, 55, and 75% RH at 25 ± 1°C and a photoperiod of 16:8 (L:D) h. This photoperiod and temperature at a RH of 60% has long been known as favorable for reproduction of B. oleae (Tzanakakis 1989). To maintain the different RHs, sealed plastic containers (30 by 40 by 30 cm) with a plastic cup placed at their base and filled with an appropriate saturated water salt solution (5 cm in depth) were used (Wexler and Hasegawa 1954, Winston and Bates 1989). To maintain the different RHs, sealed plastic containers (30 by 30 by 30 cm) with two sides of wooden cages (30 by 30 by 30 cm) with two sides of plastic cups covered with a piece of wet cotton wool in a climatic room at 25 ± 1°C and a photoperiod of 16:8 (L:D) h. Pupation took place outside the olive fruit. The newly formed pupae were collected daily and the number of mature oocytes in their ovaries was determined under a binocular microscope, as described by Tzanakakis and Koveos (1986).

Flies Destined for Longevity and Egg Production Measurements. The preimaginal stages were grown as described above, and the adults kept in pairs in cylindrical plastic cages. In each plastic cage, one newly emerged adult female and one male were placed and provided with a liquid diet of yeast hydrolysate, commercial sucrose, and water. At the base of each cage, one olive fruit was added and renewed every second day. To get the least possible disturbance of the RH within the experimental units, the cages were inspected once every other day. On each inspection day, dead flies were removed and survival and the number of oviposition holes on each fruit was recorded. Also, a small piece (2 by 2 cm) of Whatman no. 1 paper soaked with 100 µl of tap water was offered to the flies. Subsequently, olive fruit on which eggs had been laid were maintained for 5 d at the same RH and temperature (25 ± 1°C) as the parental adults. Egg hatch was recorded by dissecting the fruit under a stereomicroscope.

Statistical Analysis. The effect of RH on the percentage of females with mature oocytes was analyzed using a chi-square test (SPSS Inc. 2005). Analysis of variance (ANOVA) was used to evaluate the effect of RH on the mean number of mature oocytes per female during the first week of adult life (first group of experimental treatments) as well as on female life span (second group of experimental treatments) and the total egg production as well as fertile oviposition (percentages of egg hatch of the total number of eggs laid per female) (SPSS Inc. 2005). Following a significant difference, means were compared by Student–Newman–Keuls test. Before data analysis, log transformation of total egg production or fertile egg production was used, and homogeneity of variances was subsequently tested by Levene’s test (SPSS Inc. 2005). When data failed to meet the requirements for parametric analysis, the nonparametric Kruskal–Wallis test was used, and means were subsequently separated by Mann–Whitney U test (SPSS Inc. 2005). The significance level for interpretation purposes was P = 0.05 for all tests. Normality tests (for skewness and kurtosis) were used before data analysis on all data sets.

Results

Ovarian Maturation of Females under Various RHs. RH affected the percentage of females with mature oocytes (χ² = 74.59, df = 4, P < 0.05) as well as the number of mature oocytes in the ovaries (F = 15.518; df = 3, 102; P < 0.05) (Table 2). At the lowest (12%) RH, the percentage of females with mature oocytes as well as the number of mature oocytes in the ovaries was very low. At 33% RH, the percentage of females with mature oocytes increased significantly, but the mean number of mature oocytes in the ovaries was again very low. At the higher RHs of 55 and 75%, the percentages of females with mature oocytes were

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| Solution     | RH (%) | Vapor pressure deficit (kPa) |
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| MgCl₂·6H₂O   | 33     | 2.25                        |
| Mg(NO₃)₂·6H₂O | 55     | 1.51                        |
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Longevity and Egg Production under Various RHs. Longevity of females was also affected by the RH ($\chi^2 = 51.308; \text{df} = 4; P < 0.001$). As shown in Table 3 and Fig. 1, at 12% RH mean longevity was ≈19 d, whereas at 33% RH it was almost double. An even higher longevity was observed at 55 and 75% RHs, whereas at 94% RH the mean longevity dropped to ≈31 d.

The number of eggs laid into the olives ($\chi^2 = 58.290; \text{df} = 4; P < 0.001$) and the percentages of egg hatch ($F = 701.221; \text{df} = 4, 148; P < 0.001$) were significantly affected by RH. As shown in Table 3, at the lowest RH of 12% the mean number of eggs laid per female was significantly lower than in the higher RHs tested and few of these eggs hatched. When the females were maintained at 33% RH, a more than two-fold increase in the number of eggs laid and more than a six-fold increase in the percentage of egg hatch were recorded. At 55% RH, both the number of eggs laid and the percentage of egg hatch increased significantly in relation to the respective values at the lower RHs. At 75% RH a further increase in the number of eggs laid and the percentage of egg hatch was observed. At the highest (94%) RH the number of eggs laid was significantly lower than the respective numbers observed at the lower RHs of 55 and 75%, but most of the eggs hatched. We therefore conclude that RHs in the range from 55 to 75% compared with lower and very high RHs favor an increased egg production and adult longevity.

Discussion

Our experimental data show that the increase of RH within certain limits has a marked positive effect on ovarian maturation, egg production, and longevity of *B. oleae* females. Field observations in various areas of Greece and Italy showed that during late spring and summer, ovarian maturation in females of *B. oleae* is suppressed. This reproductive immaturity of females was attributed to various factors such as photoperiod and temperature conditions prevailing during preimaginal and adult development (Tzanakakis and Koveos 1986, Koveos and Tzanakakis 1990, Raspi et al. 2002), the lack of suitable olive fruit in early summer and the hot and dry summer conditions (Fletcher et al. 1978, Fletcher and Kapatos 1983, Koveos and Tzanakakis 1990). According to Fletcher et al. (1978) and Fletcher and Kapatos (1983), ovarian maturation can be stimulated during summer in the presence of olive fruit, provided that temperature and RH are not too high and low, respectively. Our experiments were carried out at 25 ± 1°C and indicate that, when the females were maintained throughout their life at low RHs, the presence of olive fruit alone is not enough to favor ovarian maturation and egg laying. This may explain to some extent the observed low olive injury by the fly during the hot and dry summer period, although olive fruit suitable for oviposition is present. Yet, we may not exclude the possibility that RH may have an effect on bacteria present on the plant surface and through them on ovarian maturation and egg production of *B. oleae*. In other tephritid flies, low environmental moisture reduces the abundance of bacteria present on the leaf surface that are the main source of proteins for the flies and therefore indirectly affects fecundity and survival of females (Drew et al. 1983).

The results of the present work show that low RHs suppressed ovarian maturation and egg production and hatch, whereas relatively high RHs in the range from 55 to 75% favored them. In various areas of Greece during winter and spring, due to favorable temperatures and high RHs, females mature their oocytes and lay eggs, provided that olive fruit is available.

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**Table 2. Effect of relative humidity on ovarian maturation in 7-d-old *B. oleae* females maintained at 25 ± 1°C and a photoperiod of 16:8 (L:D) h**

| RH (%) | N<sup>a</sup> | % females with mature oocytes | Mean no. of mature oocytes per female (± SE) |
|--------|--------------|------------------------------|---------------------------------------------|
| 12     | 45           | 4.44<sup>b</sup>             | 4.5 ± 1.5<sup>a</sup>                        |
| 33     | 29           | 41.38b                       | 4.3 ± 1.3<sup>a</sup>                        |
| 55     | 66           | 60.61b                       | 21.5 ± 2.0<sup>b</sup>                       |
| 75     | 27           | 66.67b                       | 21.2 ± 3.1<sup>b</sup>                       |
| 94     | 39           | 92.31c                       | 15.4 ± 2.0<sup>b</sup>                       |

<sup>a</sup> Total number of females examined.

<sup>b</sup> Percentages within a column followed by the same letter are not significantly different at $P = 0.05$ by chi-square test.

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**Table 3. Effect of relative humidity on mean longevity and egg production of *B. oleae* females maintained throughout their adult life at 25 ± 1°C and a photoperiod of 16:8 (L:D) h with access to olive fruit**

| RH (%) | N<sup>a</sup> | Longevity (d) (± SE) | Mean no. of eggs per female (± SE) | % egg hatching (± SE) |
|--------|--------------|----------------------|----------------------------------|----------------------|
| 12     | 29           | 18.9 ± 1.8<sup>b</sup>| 109.4 ± 18.2<sup>a</sup>         | 12.3 ± 1.4<sup>a</sup>|
| 33     | 39           | 33.5 ± 2.7<sup>b</sup>| 214.9 ± 21.9<sup>b</sup>         | 77.2 ± 1.0<sup>b</sup>|
| 55     | 36           | 47.6 ± 2.8<sup>c</sup>| 443.7 ± 36.8<sup>c</sup>         | 81.9 ± 1.9<sup>c</sup>|
| 75     | 34           | 49.8 ± 3.3<sup>e</sup>| 590.8 ± 65.7<sup>c</sup>         | 85.9 ± 0.5<sup>d</sup>|
| 94     | 28           | 30.5 ± 3.9<sup>b</sup>| 233.5 ± 45.2<sup>a</sup>         | 86.8 ± 0.6<sup>d</sup>|

<sup>a</sup> Total number of females examined.

<sup>b</sup> Means within this column followed by the same lowercase letter are not significantly different (nonparametric Kruskal–Wallis and pairwise comparison with Mann–Whitney U test; $P > 0.05$).

<sup>c</sup> Means within this column followed by the same lowercase letter are not significantly different (ANOVA, Student–Newman–Keuls test, $P > 0.05$).
on the trees. During late spring and early summer, in females that emerged earlier in the season a period of reproductive immaturity occurs due to an increase in daylength and temperature, despite the presence of suitable olive fruit (Tzanakakis and Koveos 1986). In olive groves in northern Greece, we observed that serious olive fruit injury by the fly occurs from early September to late November, probably because of the relatively low field temperatures and the first autumn rains, which may increase air humidity and fruit growth. Variation in fruit growth and abundance, caused among other reasons by variation in rainfall, may affect the level of egg production and the larval population and hence the population dynamics of B. oleae and other tephritid flies (Meats 1989, and references therein).

The unfavorable effects of the low 12 and 33% RHs on ovarian maturation and egg production of B. oleae found in our experiments may be related to the increased rate of water loss through the cuticle and spiracles. In addition, at 94% RH, longevity, egg production, and egg hatch were significantly lower than at 55 and 75% RH.

By contrast with our results, in an Italian strain of B. oleae adult survival and fecundity did not differ significantly in the range of RHs from 40 to 100% (Tzanakakis 1989, and references therein). We have not observed how often the flies imbibed and regurgitated fruit juice at the various RHs, which may have affected hydration and/or nutrition and therefore, survival and egg production. Yet, under low RHs the adult diet may have been dehydrated to such extent that the flies

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**Fig. 1.** Age-specific survival and egg production of B. oleae females maintained at different RHs at 25 ± 1°C. Open circles, survival percentage; continuous line, number of eggs laid; and dotted line, number of eggs hatched (fertile oviposition).
could not take much advantage of it. Females of \textit{B. oleae} may perform better and produce more eggs when feeding on a liquid or a dry diet depending on whether the populations originate from humid or dry areas (Tzanakakis et al. 1967, Tsitsipis 1989). Furthermore, the high number of eggs that were laid on a single olive fruit may have caused considerable oviposition deterrence. It is to be expected that the number of eggs laid would be higher if the flies had daily access to plenty of fruit.

In our experiments, under low RH the percentages of hatched larvae in olive fruit were very low, which may be due to egg mortality or reduced mating because of dehydration. Tsitsipis and Abatzis (1980) found that eggs of \textit{B. oleae} reared on artificial diet suffered high mortality under low RH. Under drought conditions, 89% of the eggs of another tephritid fly, \textit{Dacus tryoni} (Froggatt), laid into fruit died either before hatch or as larvae (Meats 1989, and references therein). In addition, pupae of \textit{B. oleae} suffered high mortality, and the percentage of adult emergence was very low when maintained at 30% or lower RHs (Tzanakakis 1989, and references therein).

In conclusion, our results show that low RHs suppress, whereas high RHs favor ovarian maturation, egg production, and longevity of \textit{B. oleae} females. These effects of RH together with the effects of photoperiod, temperature, and availability of suitable olive fruit may explain, in some extent the observed reproductive immaturity and low fruit injury by \textit{B. oleae} during summer in the field. We should mention, however, that in the field during summer, low humidity would only be experienced for part of each day because during the night humidity is considerably higher. Daily formation of dew also could be a source of water for the flies during the dry summer period (Meats 1989, and references therein). In addition, there are sporadic early-to-midsummer summer rains in various areas of Greece; yet, reproductive maturation of the flies does not occur right after these rains. Rains certainly contribute to fruit growth, and the presence of suitable fruit could affect ovarian maturation and egg production of \textit{B. oleae} (Koveos and Tzanakakis 1990). Therefore, further experiments under field conditions are needed to elucidate the effect of RH in combination with fruit availability and temperature on survival and reproduction of \textit{B. oleae}.

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