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Determination of the lower thermal thresholds and day-degree requirements for eggs and larvae of Dacus oleae (Gmel.) (Diptera: Tephritidae) under field conditions in Crete, Greece

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A new method is described to determine the lower temperature threshold and the heat requirements of immature *Dacus oleae* (GMEL.) in day-degrees. It utilizes only observed developmental times in the field and field weather data.

From October to March five sets of sleeve cages installed on three olive varieties in July were sequentially infested each month with adult *D. oleae* for a period of 20 day-degrees (°d) above 10°C. All fruit were then sampled seven times or until the last larva had left the olive for pupation. From dissection data the time for 50% transformation from one stage to the next was calculated, and expressed in day-degrees above several chosen threshold temperatures. The threshold having the smallest variance between the data from the different months was defined as the lower temperature threshold. The temperatures used for these calculations were measured in the shady interior of the olive tree. Temperatures were also recorded from different parts of the canopy of the tree, outside and inside the sleeve cages, and on the surface as well as within the pulp of the olives.

Based on shade temperatures, which agreed with official weather data, eggs required 68°C above 6°C over the whole season. Development was hastened in the black varieties, which also showed higher surface temperatures on the olives directly exposed to the sun. Larval developmental times decreased linearly in all varieties from 146°d above 10°C in October to 93°d in April. The first instar consumed 30%, the second 25%, and the third 45% of this physiological time. The increase in the speed of development from fall to spring is attributed to the ripening of the olives, which thereby provided a better substrate for the larvae.

The pulp of the olives in the direct sunshine tended to be cooler than the surrounding air. But at minimum temperatures in the early morning and in shaded olives throughout the day, pulp temperatures usually were a fraction of a degree higher than in the air. Irrespective of the season they were about 1°C higher than the shady interior of the tree. It is estimated that the mean difference in emergence time of full grown larvae from exposed olives in the south as compared to shaded olives in the north of the same tree can reach two weeks in winter.

Predicting the development of insect populations is one of the most important aspects of pest management. For this purpose developmental time in different climatic conditions has to be known. The duration of a life stage is conveniently expressed on a physiological time scale utilizing two key parameters, namely the lower thermal threshold and the number of day-degrees (°d) above this threshold. To some extent both of these parameters are characteristic of a species. They are most often obtained from laboratory experiments under different, usually constant temperature conditions. At each temperature the speed of development is calculated (= 1/duration). Then the lower thermal threshold, which is the temperature at which development equals zero, is extrapolated. This extrapolation is most easily done linearly, thereby yielding a value which deviates little from experimental results with low temperature cabinets (for a discussion of this controversial method see HOWE, 1967). Formulae which take into account the sigmoid pattern of the speed of development curve, and which incorporate the flattening

and eventual decrease at high temperatures, have been utilized (DAVIDSON, 1944; STINNER *et al.,* 1974). But as long as extremely high and low temperatures are excluded the classical linear approach with temperature sums holds true.

Other difficulties which go beyond the mere fitting of a curve to the laboratory data touch on the question of the reality of the laboratory results when applied to the field situation. Cabinets with smoothly fluctuating temperatures simulating specific field situations are rare because of their high cost. The application of laboratory data to the field therefore generally involves the assumption that a certain constant temperature for rearing gives the same durations of the life stages as fluctuating temperatures around this mean. This assumption, though widely used in computer modeling, may be violated specially in the lower temperature range (MESSENGER & FLITTERS, 1959). Furthermore, physiological difficulties may arise in eggs and larvae at constant low temperatures near the threshold, thereby making it impossible to experiment under those conditions (HOWE, 1967).

Sometimes the lower thermal threshold and the day-degree requirement of a species cannot be reasonably obtained from a laboratory study because of lack of equipment, difficulties in rearing the insect, or because the mentioned drawbacks are considered too severe. It would therefore be desirable to have a procedure to determine the two key parameters under field conditions. This paper describes such a method by utilizing only the classical approach of temperature sums taken directly from thermograph records in the field. In essence the method is based on the concept that the lower thermal threshold and day-degree requirements can be calculated from field observations with the same accuracy as the insects' development can be predicted from the lower thermal threshold and the accumulated day-degrees. If in a field experiment the exact duration of a stage and the course of the temperature during this period are known, this period can be expressed in several ways in day-degrees by choosing various threshold temperatures. Among these values, one is then defined as the lower thermal threshold or developmental zero temperature.

In this study such an approach was utilized for *Dacus oleae* (GMEL.), where laboratory rearing in the natural substrate poses problems. The female lays eggs underneath the skin of the olive fruit. The larvae hatch after a few days, and start tunneling in the pulp. Having completed their development they pupate either in the fruit on the tree (up to September), or they drop to the ground and pupate in the soil (from autumn to spring). The first infestation of the season in Crete occurs on irrigated table varieties in June and oil varieties in July and August. At this time all olives are still green and hard, and have a low oil content. In Crete the infestation then builds up through several successively overlapping generations from September until harvest, which in the lowlands starts in November and ends in March of the next year. In spring many olives are black, very soft, and high in oil content. Consequently climatic conditions and quality of the olives encountered by the larvae vary considerably throughout the season. Similarly, quality of olives is known to change with storage, thereby influencing the outcome of a laboratory experiment. Experiments with eggs and larvae in detached fruit have been done frequently for many purposes (SACANTANIS, 1953; MOORE, 1959; MANIKAS, 1974), but they do not allow a comparison of the speed of development on different types of olives at different times of the year. In the present study the effects of different storage times are excluded by working with olives on the tree.

Because the olives have to be dissected in order to measure the development of the eggs and larvae of *D. oleae,* each developing insect can be observed only

once. Such a destructive sampling procedure necessitates calculations like those developed for the determination of the median lethal dose in toxicology (FINNEY, 1971), and have been applied in studies on stored grain insects (HOWE, 1975).

MATERIALS AND METHODS

Sleeve cage experiment

The experiment was carried out near Souda, Crete, Greece, at sea level in 1976/77, on the three olive varieties Koroneiki, Tsounati, and Manaki. Koroneiki, an oil variety, has numerous small fruit which weigh about 0.7 g when mature. Harvest lasts from December up to February. On the experimental trees the first olives start turning black in December. Tsounati, the other main oil variety of the Chania district, carries fewer but bigger fruit of about 1.5 g each. Harvest starts at the end of December and lasts up to April. Most Tsounati olives in Souda do not change their colour beyond a pale green violet before they drop naturally. Manaki, which is cultivated in low numbers as a table and oil variety, has few, rather large fruit of about 2.8 g, which start turning black or at least mottled black in December.

The experimental plot consisted of nine trees (three per variety labled A, B, and C), approximately 4 m high and each having a good crop. At the end of June, before any natural infestation of *D. oleae* had occurred, five sleeve cages, numbered 1 to 5, were installed on the outer branches of each tree. These sleeves, measuring 75 x 120 cm and made from green plastic mesh of 1.7 mm, were left undisturbed in place until they were sequentially supplied as described below with ovipositing females to produce a controlled or artificial infestation of *D. oleae*. In late October, when the first cages were infested, all sleeves on the Koroneiki trees contained 600–700 olives apiece, but on the Tsounati or Manaki trees, the sleeves contained only 400–500 fruit.

The controlled infestation was initiated on October 25 by placing 45 young ovipositing females and 15 males in sleeve number 1 on each tree labeled A of each variety. All adults were reared from olives and were provided in the sleeve cages with water and a piece of wax paper covered with droplets of a mixture of protein hydrolysate, sugar, and egg yolk (ECONOMOPOULOS & TZANAKAKIS, 1967). After a period of time equivalent to approximately 20°d (above 10°C), the surviving adults were removed and together with sufficient new young adults to bring the total back to 45 females and 15 males they were transferred to sleeve number 1 on tree B. Again, after about 20°d had accumulated, the adults were removed from tree B to sleeve number 1 on tree C. This method of sequentially providing the sleeve cages on each tree with ovipositing females was repeated in November using cage number 2 and in December, February and in March with cages numbered 3, 4, and 5, respectively.

It should be noted that an accumulation of approximately 20°d required only 3 to 4 days if the weather was warm, but up to 11 days if it was cold and rainy. After the adults were removed from a given cage, the sleeve was momentarily removed, the branch shaken, and the sleeve then reinstalled along with a glass plate covered with poison bait to kill any flies which inadvertently were overlooked. In March when the olives in sleeves numbered 5 were infested, there were still 500–600 fruit in the cages on the Koroneiki trees, but only 110–280 on the

other two varieties. These differences were due to the natural fruit retention times of the three varieties. At the time of the last sample in April practically all Manaki and Tsounati olives had dropped naturally.

By using the method of producing a controlled infestation on three separate trees within each variety, staggered by about 20°d, it was possible to follow the development of the eggs and larvae under the natural climatic conditions of each month from October to March. No infestation was attempted during January, as the temperatures were too low. Sleeves were also established for July, August, and September. But despite a relatively cool and wet summer, which was favourable for the development of *D. oleae,* the number of eggs produced within the sleeves was too low for an analysis of the duration of the different stages.

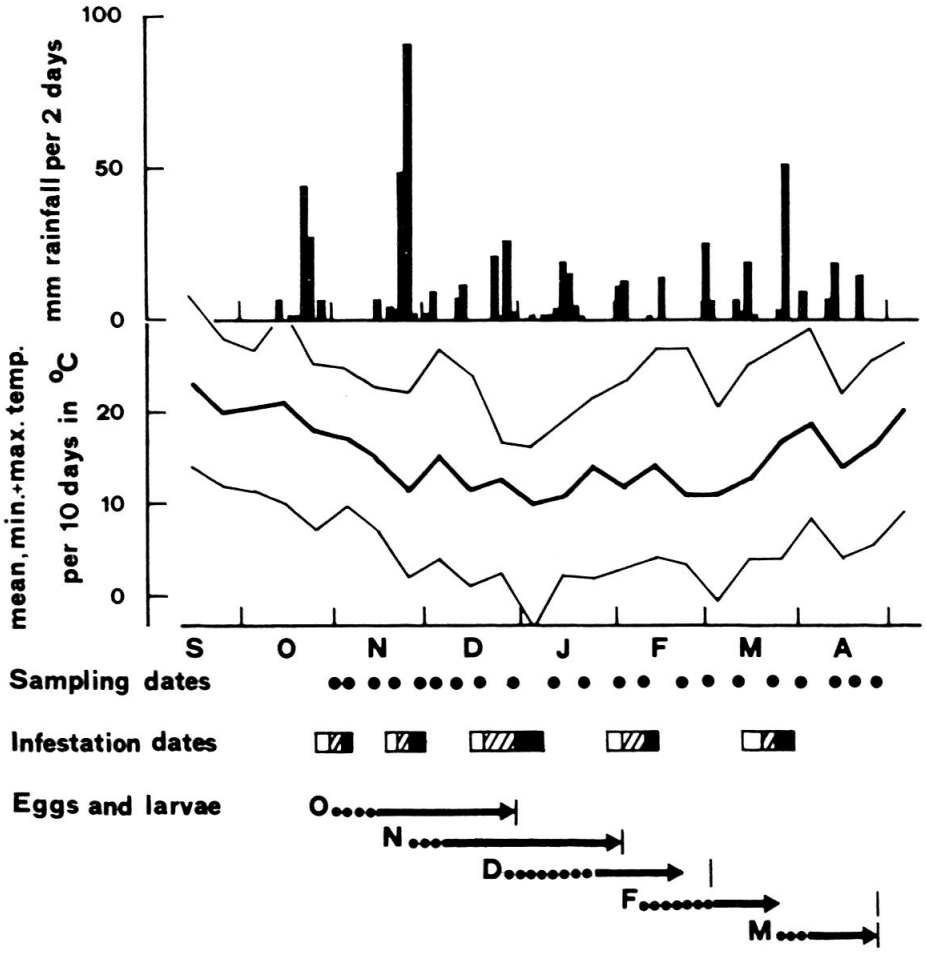

Fig. 1: Weather data, infestation dates, and duration of the immature stages of *D. oleae,* in Crete 1976/77. The symbol ▨ represents the dates when the sleeve cages contained ovipositing females on tree A, B, and C respectively. •••••• = eggs; ▬▬ = larvae; ➡ = last living larva; | = last sample from the sleeves infested in Oct., Nov., Dec., Feb. or March.

The olives in all nine sleeves infested during the same month were sampled on seven separate dates (fig. 1) spread over the insect's egg and larval development times. Sampling was discontinued when the last larvae had pupated. Each sample consisted of 50–100 olives and this provided 20–50 living immature stages for the calculations. Dissections were done under the microscope and the number and condition of each instar (L_1, L_2, L_3) as well as the number of larval exit holes was recorded.

For the calculations of day-degrees temperature was recorded with a hygrothermograph (Siap, Bologna, with chart number 7068) installed in the centre of a Koroneiki tree. It was compared with the readings of the official weather station 2 km away at the Institute of Subtropical Plants and Olive Tree, Chania, where rainfall was also recorded. A summary of infestation dates, sampling dates, and immature developmental times in relation to climatological data is shown in fig. 1. Day-degrees above different thresholds were calculated for each day of the season by directly counting the squares below the temperature curve. For each group of three sleeves which were infested at the same time, e.g. on tree B of each variety, the number of day-degrees above the different thresholds was then tabulated for each sampling date. As a starting point the midpoint of the interval, during which females were ovipositing, was chosen.

Mean developmental times, and lower thermal thresholds

The mean physiological time when 50% of the population had transformed to the next stage was estimated using the method of Spearman-Kärber (FINNEY, 1971). The procedure is exemplified for one sleeve and one chosen threshold in tab. 1.

Tab. 1: An example showing the calculations for the mean duration of the egg development in one sleeve cage, Crete 1976. Oct. sleeve on Tsounati tree B, for a threshold of 6°C.

Date of sample	Cumulative day-degrees above 6°C since day 0	Number of living immatures in the sample				Calculations		
		eggs	larvae	total	%larvae	Interval in °d	Transformation to larvae during this interval	°d
	°d	N	N	N	p%	$½(x_{i+1}+x_i)$ = A	$p_{i+1}-p_i$ = B	A + B
31 Oct.[1]	0	0	0	0	0			
2 Nov.[2]	25.8	105	9	114	7.9	12.9	0.079	1.019
5 "	63.5	33	81	114	71.1	44.7	0.632	28.250
13 "	156.8	4	102	106	96.2	110.2	0.251	27.660
20 "	233.5	0	54	54	100	195.2	0.038	7.418
						mean developmental time Σ(A x B)		64.3

[1]defined as day zero. Females were left in the sleeve from 29 October to 2 November
[2]first sample

For the analysis, which was performed for each stage and each chosen threshold separately, each sleeve contributed one value, namely the calculated mean developmental time. Since one repetition was lost alltogether in November, the analysis of variance was carried out only for four months, giving the following distribution of the 35 degrees of freedom: 3 for months, 2 for varieties, 6 for interactions, 2 in blocks, and 22 in the error term. For each chosen threshold the regression between the mean developmental times found in each month (y) and the month (x) was then calculated, whereby the months were merely numerated from one to five (November included) for simplicity reasons. In a more accurate evaluation concerning the developmental times for larvae in day-degrees above 10°C, the mean number of day-degrees for each single sleeve was utilized as dependent variable (y) and the calendar day, when this sleeve was infested as independent variable (x), with October 1 defined as day 1.

Microclimatic measurements

The calculated day-degree estimates relied on temperatures taken in the shady centre of the tree. In order to record the real microclimatic conditions encountered by eggs and larvae, temperatures were measured in different parts of the canopy of a medium sized Tsounati tree, with the outer branches drooping to the ground. A portable, battery operated temperature recorder (Yellow Springs Instrument Company, Yellow Springs, Ohio 45387 USA) was utilized to measure temperatures within the canopy as well as on the surface and in the interior of the olives. Air temperatures were recorded with microsondes YSI 401, and temperature on the surface and in the interior of the olives with YSI 427. For the measurement of the surface temperature the sonde was pressed on the illuminated side of an olive on the tree by means of a wooden clothespin. After the reading the olive was picked, cut open, and the pulp temperature was recorded within about 10 seconds. Because of evaporation the temperature tended to drop negligibly in comparison to the surface temperature (by about 0.5°C per minute). Continuous measurements of the pulp temperatures on the tree proved to be unsatisfactory because small olives were mutilated too severely by introducing the relatively thick sonde, and bigger olives dropped too easily.

The following measurements were taken at about monthly intervals: In two extreme positions of the canopy, i.e. on the north and south sides, temperatures were recorded at man's height outside and inside a sleeve, in the air near an olive, on the surface and within the pulp of the same olive. In addition the temperature in the interior of the canopy, which corresponded to the hygrothermograph measurements, was taken as reference for each of the ten repetitions. Such a complete set of 130 measurements was recorded within about 75 minutes once around sunrise at daily minimum temperature, and once after noon at daily maximum temperature. At the same time the wind speed was recorded. The accuracy of the sondes was checked each day when measurements were made. It corresponded to the one specified by the manufacturer, who allows a difference of 0.1°C between two sondes. The oil and water contents of 60 olives were also determined.

Statistical procedures

Following the analysis of variance a few planned comparisons are made by means of the Dunn test (KIRK, 1968), whereas post hoc comparisons of numerous means are subjected to a Scheffé test (SNEDECOR & COCHRAN, 1967). Means followed by different letters are significantly different, and the critical interval $t_{Dunn} \cdot SE$ resp. $S \cdot SE$ is given. All tests are performed at the 5% probability level. Significant F and t values are indicated by an asterisk.

RESULTS

Egg and larval development in the field

The maximum durations for completion of the development of eggs and larvae at different periods of the year are represented in fig. 1. The sampling dates

Tab. 2: Minimum and maximum observed developmental times in days for eggs and larvae of *D. oleae*, Crete 1976/77.

Sleeves infested in	Mean number of living progeny per sleeve[1] N	Eggs maximum duration in days	Larvae minimum duration in days	Larvae maximum duration in days
October	312.2 ± 171.0	13.5	20.5	37.5
November	68.2 35.8[2]	15.0	39.5	62.0
December	166.6 137.2	24.0	35.0	63.0
February	138.3 85.6	28.0	22.0	49.0
March	256.4 102.5	23.0	12.0	38.5

[1] eggs, larvae, and exit holes. Mean ± SE, N = 9
[2] two repetitions only, third repetition failed because of bad weather

for the last eggs and last larvae from each mothly group of sleeves are shown together with temperature and rainfall data. Furthermore the observed extreme duration for the egg and larval development are presented in tab. 2. The midpoint of the interval when females were ovipositing is thereby taken as starting point. As in the method by Spearman-Kärber it is assumed that the first larvae all hatched together in the middle of the interval between the last recording of eggs only and the first sample with larvae. Since the minimum number of days for egg development is limited by the first sampling date, it cannot be estimated reasonably.

The mean durations for development (rather than the maxima and minima) more accurately summarize the development under the conditions within each sleeve. In order to facilitate comparisons these durations are expressed on a physiological time scale. For each monthly set of sleeves the number of day-degrees needed for 50% of all individuals to complete their egg and larval development is shown in tab. 3 for six different thresholds between 2° and 12°C. Every column describes the same experimental results equally well. In theory it is assumed that the day-degree requirement remains constant under different conditions. Therefore among the six chosen thresholds used in the computations in tab. 3 the one which yields the same number of day-degrees in all sets of sleeves from October through March is the lower thermal threshold. For the eggs the least variation (as measured by s_{yx}) occurs when 6°C is chosen as basis for the calculations. The mean heat sum needed for the egg development was 68.0°d ± 3.2°d SE above 6°C (calculated for N = 42, which includes 2 repetitions from the November set of sleeves). For eggs the day-degree requirement is the same throughout the year.

Since the Spearman-Kärber method for calculating the day-degree value when 50% of the eggs had hatched involves the use of the 0% and 100% values, a more accurate method was tried for the 6°C threshold. In a proper probit analysis (FINNEY, 1971) involving one cycle, all 36 percentage values, but not including any 0%'s and 100%'s, were utilized irrespective of month and variety. By this method the calculated mean was 69.4°d, which differs only little from the 68.0°d found with the Spearman-Kärber technique.

Tab. 3: Calculated developmental times in day-degrees above different chosen thresholds. Monthly means based on 9 sleeves, Crete 1976/77.

Sleeve thesholds	for eggs 2°C	4°C	6°C	8°C	10°C	12°C	for larvae 2°C	4°C	6°C	8°C	10°C	12°C
October[1]	94.4	82.7	71.0	59.6	46.4	36.1	337.1	288.0	239.0	189.9	142.3	100.0
November	90.4	74.9	59.3	44.5	30.6	19.0	457.3	370.9	285.2	202.3	127.8	72.1
December	107.3	84.8	62.7	41.4	22.7	10.6	424.0	343.4	263.5	186.6	119.6	71.8
February	116.7	96.9	77.1	58.4	42.2	29.2	282.5	230.6	181.1	136.2	96.1	63.8
March	97.1	81.8	67.0	53.5	40.4	29.1	242.5	206.6	171.3	137.2	104.1	74.3
ANOVA $\pm$ SE ($= \sqrt{2MSE/3}$)	26.5	21.3	16.5	12.1	8.8	6.3	42.0	33.7	25.9	18.8	12.7	8.9
F for months	0.89	0.64	0.83	2.82	8.47*	18.10*	21.02*	19.77*	17.87*	15.01*	15.46*	18.76*
varieties	4.50*	4.63*	4.72*	4.78*	4.21*	3.37	0.63	0.67	0.74	0.81	1.00	0.87
interactions	0.80	0.78	0.74	0.64	0.58	0.51	1.48	1.54	1.63	1.70	1.95	1.69
Regression[2]												
s_{yx}	10.90	8.47	7.86	9.45	11.12	11.54	81.67	59.65	38.00	18.18	8.03	11.60
slope b	3.17	2.02	0.98	0.17	-0.04	-0.38	-36.40	-30.31	-23.95	-17.15	-10.81	-5.97
t	<1	<1	<1	<1	<1	<1	1.41	1.61	1.99	2.98	4.26*	1.63

[1] not included in ANOVA because one repetition failed
[2] months (x = 1, 2, 3, 4, 5) versus °d (y = monthly means)

There is a significant difference in the duration of the egg development between varieties from December to March, i.e. when the olives reached maturity (see ANOVA tab. 3). Whereas in the blackish varieties Manaki and Koroneiki the mean duration was 57.4°d (a) and 61.8°d (a) respectively, in the green Tsounati fruit 87.6°d (b) were needed. (The critical interval $t_{Dunn} \cdot SE$ for C=3 is 24.7°d.)

If the same procedure is applied to the larval development (tab. 3) it becomes evident that the day-degree values obtained in spring in all calculations are smaller than the values from the autumn sleeves. This is also reflected in the significant F value for months in the ANOVA, or the high values for b in the regression analysis. Instead of choosing the set of day-degree values which deviate least from a common mean, the standard deviation from the regression (s_{yx}) was adopted as criterion for finding the lower thermal threshold. s_{yx} was at a minimum at 10°C, and therefore this temperature was defined as the lower thermal threshold for the larval development.

The regression of monthly means on months is significant (tab. 3). So is the regression concerning the mean durations (y) of all 42 single sleeves (incl. November) over the calendar day when the sleeves were started (x, with x=1 on Oct. 1): $y = 146.46 - 0.3027\ x$ ($t = 5.71^*$, $r^2 = 0.449$, SE of regression = 2.7°d). This means that it takes larvae significantly less day-degrees to develop in spring than during fall.

For clarity the calculated day-degree requirements are tabulated for each month and then translated back into days for 1976/77 in tab. 4. The listed mean durations refer to eggs which were laid on the first day of the month, with all days of the month contributing the same number of day-degrees. (For a comparison with tab. 2 it must be considered that the larvae of the October sleeves e.g. developed during November–December.) For eggs the durations correspond to the amount of heat received, and the highest mean of 11.3 days is found in January. For larvae two attempts are made for extrapolating the data, which cover the period October to March, back to June–July, when the low fecundity of the females and the high mortality of the larvae precluded any evaluation of experimental data. The first method (tab. 4) assumes that the day-degrees' requirements do not increase beyond the expected mean for October. In the second method the regression equation is utilized to calculate extrapolated values for the summer months. With the exception of June, when Manaki and Tsounati olives start to be susceptible for infestation, the calculated mean durations in days differ so little from the values obtained with the first method that for practical purposes both methods can be assumed to give the same results.

For the measurement of the duration of each larval instar 31 sleeves were used where all instars had been sampled. With the Spearman-Kärber technique the durations expressed in day-degrees were calculated. 27.5% of the total larval development was consumed by the first stage; L_2 utilized 28.1% and L_3 44.4%. In the course of the season no change in this distribution was apparent. These data were based on larvae, whose stage was determined from a visual estimation of the size of the larva and the frass tunnel. A check for accuracy by means of micropreparations of cleared larvae among a batch of 213 larvae revealed that all 62 designated as L_1 larvae indeed had the mouth hooks of the first instar. Among the 86 supposed second instars 23 (or 26.7%) despite their considerable body size still belonged to the first instar. The 65 supposed L_3 on the other hand included only

Tab. 4: Calculated developmental times (d.t.) for eggs and larvae of *D. oleae* for Chania, Crete in 1976/77.

Month	Eggs: 68.0°d constant		Larvae: °d requirements changing[1]				
				°d June-Oct. constant		°d June-Oct. extrapolated	
	monthly mean °d above 6°C per day[2]	mean d.t. in days	monthly mean °d above 10°C per day	°d needed	mean d.t. in days	°d needed	mean d.t. in days
June	16.0	4.2	12.0	146.2	12.2	183.4	15.3
July	18.5	3.7	14.5	146.2	10.1	174.3	12.0
Aug.	18.4	3.7	14.4	146.2	10.2	164.9	11.5
Sept.	16.8	4.0	12.8	146.2	11.4	155.5	12.2
Oct.	13.6	5.0	9.6	146.2	15.2	146.2	15.2
Nov.	9.8	6.9	5.9	137.4	23.3		
Dec.	7.7	8.8	4.0	128.3	32.1		
Jan.	6.0	11.3	2.5	118.9	47.6		
Feb.	8.0	8.5	4.4	109.5	24.9		
March	6.5	10.5	3.4	101.1	29.7		
April	9.2	7.4	5.4	92.6	17.1		

[1] °d requirements for the larvae (y) calculated for each month for eggs laid on the first day of the month, according to $y = 146.46 - 0.3027\ x$, with $x = 1$ on Oct. 1

[2] °d per day = daily mean temperature minus 6°C

one (or 1.5%) oversized L_2. In the view of these results the percent distribution for the durations of the different stages had to be slightly adjusted.

In conclusion it can be stated that the egg development of *D. oleae* in the field lasted 68°d above a lower thermal threshold of 6°C. The total larval development required from 146°d above 10°C in October to 93°d in March–April, with the L_1 taking up 30%, the L_2 25%, and the L_3 45%.

Microclimate in the olive tree

The results concerning the speed of development were obtained from artificial infestations in sleeves, which were installed at the periphery of the tree, while temperature was recorded in the shady interior of the tree. Two questions therefore arise: How do the sleeves influence the environment? How does the temperature inside an olive in the outer canopy, where the bulk of the olives are found, compare with the recorded temperature in the centre of the tree?

During the whole season, air temperatures in different parts of the canopy of an olive tree were therefore compared with the shade temperature in the interior of the same tree for minimum temperatures at sunrise and maximum temperatures at noon on sunny days (tab. 5). At maximum temperatures, shade temperatures on the north side of the tree were only slightly above the temperatures in the interior of the tree, but striking differences occurred throughout the year between the sunny south side and the interior. The largest single difference of 8.1°C was recorded at the end of June. At sunrise, on the other hand, temperatures throughout the lower exterior canopy differed little, with a tendency to be higher than the temperature in the interior of the canopy. Inside the sleeves the air temperature curve in general was dampened, the maximum being up to several °C lower than the external air temperature on the south side, the minimum being a fraction of a °C higher.

In tab. 6 pulp temperatures are compared with the reference air temperature in the interior of the tree. The temperature experienced by the larvae in the pulp usually is about 1°C higher than the reference. This difference becomes several °C where the olive is directly exposed to the sun on sunny days. Thus the mean pulp temperature at noon in exposed olives on the south side was 3.6°C higher than in the shady centre. In general pulp temperatures respond slowly to changes in the air temperature, and therefore show a smoother course during the day. By protecting the olives with sleeves the daily temperature fluctuations are further dampened in comparison to exposed olives on the outside of the canopy. In the north the maximum is thereby lowered by 0.2°C, the minimum lifted by 0.3°C.

Comparing tab. 6 with tab. 5 gives no indication that pulp temperatures regularly surpass air temperatures because of direct irradiation on the south side of the tree. On the north side and during minimum temperatures the pulp is however on the average 0.5 to 1.0°C warmer than the surrounding air.

The surface temperature on the epidermis of the fruit proved to be highly variable according to wind and exposure. Even in highly irradiated olives in the south the temperature difference between epidermis and pulp in general surpassed only a fraction of a °C, the maximum recorded being 3.0°C above the pulp temperature.

All these measurements had been done on green Tsounati olives, which are more easily handled than the black olives of other varieties. In March however

Tab. 5: Air temperatures in different parts of the canopy of an olive tree in Chania, Crete in 1977/78. Means of 10 measurements on sunny days.

Date	At maximum temperatures (1-2 p.m.)						At minimum temperatures (6-8 a.m.)					
	wind speed km/hr.	temp. of tree interior in °C	difference (in °C) between interior temp. and: N side open	N side within sleeve	S side open	S side within sleeve	wind speed km/hr.	temp. of tree interior in °C	difference (in °C) between interior temp. and: N side open	N side within sleeve	S side open	S side within sleeve
28 July	2.80	31.8	0.5	0.6	4.9	2.5	0.01	18.8	0.6	0.5	0.5	2.1
9 Aug.	2.73	31.1	0.3	0.2	4.8	1.6	0.06	18.6	0.5	0.5	0.2	0.6
25 "	5.58	28.3	0.8	0.6	3.6	1.8	0.73	22.8	0.0	0.2	0.0	0.2
9 Sept.	2.48	29.0	0.2	0.3	4.8	2.4	0.05	21.5	-0.3	0.2	0.1	0.2
7 Oct.	1.49	21.1	0.2	1.0	3.6	2.4	0.00	11.5	-0.3	0.5	0.0	0.5
10 Nov.	1.22	18.4	0.6	0.9	1.6	1.8	0.00	7.7	0.0	0.3	-0.1	0.3
15 Dec.	0.38	12.5	0.0	0.1	5.7	3.2	0.40	7.1	0.1	0.3	0.3	0.6
12 Jan.	0.32	14.5	0.2	0.5	1.0	1.6	0.01	3.9	0.1	0.2	-0.2	-0.1
2 March	2.02	18.9	1.0	1.8[1]	2.9	1.8	0.21	10.6	0.0	0.0	-0.1	0.4
mean			0.4	0.7	3.7	2.1			0.1	0.3	0.1	0.5

[1]sleeve installed in NW where the last fruit were found

some Tsounati fruit started turning violet, which enabled one to measure the temperatures on carefully chosen pairs of olives of the same size and exposure on the same small branch, one olive being green the other turning darker. Surface temperatures on the coloured olives in south exposure was higher (by 0.70°C ± 0.25°C SE, N = 23, t = 2.77*); so was the more constant pulp temperature (0.47°C ± 0.10°C SE, t = 4.80*). Both differences were significant; but they were outweighed by the effects of position.

Though fruit weight, oil and water contents (tab. 6) could possibly influence the temperature response, no correlations were evident from the records. Similarly no influence of the wind could be detected, because the wind pattern was very constant throughout the experiment, namely no wind around sunrise and a slight wind at noon. More important, none of the single temperature differences recorded in tab. 5 and tab. 6 showed a clear trend throughout the season from the hot and dry summer through the wet and cold winter up to March when the last olives dropped. There was however an indication that the difference in maximum pulp temperatures between the north and the south side of the canopy decreased with the decreasing mean shade temperatures. Thus, at the 31.8°C recorded in the shady interior of the canopy on 28 July 1977 the difference in pulp temperatures was 3.8°C in unprotected olives, whereas at 14.5°C on 12 Jan. 1978 the difference was 0.8°C. This indicated that the influence of the shading was greater at high temperatures.

In conclusion it was estimated that the mean temperatures experienced by the eggs and larvae in the sleeves did not deviate significantly from the uncaged olives. The dampening effect of the sleeves was barely measurable. Irrespective of the season, pulp temperatures in olives which were not directly exposed to the sun were about 1°C higher than the temperatures measured in the shady interior of a tree. Slightly higher temperatures were experienced in fully exposed olives at noon, specially if they had a dark skin.

DISCUSSION

As far as the egg development is concerned it is possible to explain the data from the sleeve cage experiment with a constant temperature sum, namely 68°d, if the lower thermal threshold is defined as 6°C. The faster development in the black olives may be connected to the greater accumulation of solar radiation especially near the epidermis of the olives, which results in a higher temperature in comparison to the green olives. In a study near Athens temperature differences of several °C were recorded between black and green olives (LAUDÉHO *et al.*, 1978). On oleasters in Crete, however, the same small difference of about 0.5°C was measured in carefully chosen pairs of black and green olives (F. BIGLER, pers. comm.) as found in this study.

In the case of the larval development no single pair of heat sum and lower thermal threshold explains the data satisfactorily. A fixed lower thermal threshold of 10°C was therefore chosen which gave the smallest variation in day-degrees. The day-degree requirement for completion of all three larval instars was thereby found to diminish irrespective of the variety from 146°d in October to 63% of this value in March–April.

The fact that the physiological time needed to complete the larval development declines linearly while the overall temperature pattern through winter is U-

Tab. 6: Pulp temperatures in comparison to air temperature in the interior of the tree, in Chania, Crete 1977/78.

Date	Weight of 100 fruit in g	Water content in %	Oil content in % wet weight	Difference between tree interior, in °C at maximum temperatures and: N side open	N side sleeve	S side open	S side sleeve	at minimum temperatures and: N side open	N side sleeve	S side open	S side sleeve	means S·SE = 0.2 max.	min.
28 July	60.4	55.7	2.8	0.6	0.7	4.4	2.2	1.5	2.0	1.7	2.4	2.0	1.9
9 Aug.	66.5	49.3	5.0	0.4	0.3	4.1	2.8	1.7	2.1	1.7	2.0	1.9	1.9
25 "	83.3	40.6	8.0	0.7	0.6	4.0	3.0	0.2	0.3	0.1	0.1	2.1	0.2
9 Sept.	85.0	43.7	11.1	0.5	0.7	4.3	3.4	0.4	0.7	0.8	0.9	2.2	0.7
7 Oct.	121.6	54.2	13.9	0.2	0.7	2.4	2.3	0.3	0.7	0.6	0.9	1.4	0.6
10 Nov.	119.0	52.9	16.7	0.7	1.0	2.1	2.2	0.8	1.3	0.4	0.4	1.5	0.7
15 Dec.	141.8	44.8	24.7	0.3	0.5	4.9	3.7	0.2	0.5	0.7	0.8	2.4	0.5
12 Jan.	129.9	49.6	25.7	1.9	1.9	2.7	2.4	1.3	1.2	1.5	1.2	2.2	1.3
2 March	110.4	50.6	23.4	1.7	2.7	3.2	2.8	0.8	1.2	1.2	1.3	2.6	1.1
means			S·SE[1]										
time of day x sleeve x direction			0.1	0.8	1.0	3.6	2.8	0.8	1.1	1.0	1.1		
time of day x direction			0.1	0.9		3.2		0.9		1.0			
time of day x sleeve			0.1	2.2	1.9	←	←	0.9	1.1	←	←		

[1]critical interval (Scheffe test)

shaped with a minimum in January lends support to the conclusion that this shortening of the development is not due to temperature influence. It may be caused by an improvement of the nutritional value of the olives in the course of the season and under the conditions around Chania. With the shortest development occurring in the February sleeves, it may be concluded that these larvae were in the nutritionally most favourable olives. The slightly longer development times found in the March sleeves would indicate nutritional deterioration of the olives. The fact that in the oleaster fruit larval development takes longer as the season progresses from December to April (F. BIGLER, pers. comm.) indicates that the olives may actually pass through an optimum in nutritional value for *D. oleae* larvae. The data presented here are therefore only representative for conditions where this nutritional value improves almost up to the time of fruit drop.

Since mortality may interfere with the determination of the duration of a life stage (HOWE, 1967), it must be recalled that most larvae in this study were not allowed to finish their development. Though they might die before leaving the olives, their presence in the dissected fruit contributed to the evaluation of the mean larval duration. As long as the larval mortality is evenly distributed over the stages and within the stages, no bias would occur from this reasoning. If however the slowest larvae die preferentially, the method utilizing dissections instead of direct observation of e.g. third instar larvae leaving the olives, will give an overestimate of the mean duration. In this study a parallel set of sleeves, which was harvested only after all larvae had left the fruit, gave the following mortalities for the period from October to April: 0% to 2.4% of all eggs died, and larval mortalities ranged from 8.9% to 20.4%, affecting all stages roughly according to their duration (unpubl. results). Mortality therefore should not have influenced the estimate for the durations in this study.

The results on the speed of development can now be used in connection with local weather data to interpret fluctuations in egg and larval population densities through the season. From a known egg population at one point in time it becomes possible to predict the period when the different larval stages will be found. The difference between the expected number and the actual number of larvae found permits an estimate of mortality. With an appropriate computer programme such mortality calculations become possible even in a continuously changing population with heavily overlapping generations (T.R.E. SOUTHWOOD, pers. comm.), as is the case with *D. oleae* in Crete. Since the Stevenson screens used to shelter a hygrothermograph approach the conditions given in the shaded interior of a dense tree, the data presented here can be used in connection with official weather data.

Temperatures from official weather stations or from the shaded interior of the tree, though valuable for monitoring, often do not represent the temperatures under which the insects live (for a review see BURRAGE, 1976). While big fruit like melons may reach up to 24°C above ambient temperature in the sun (BARBER & SHARPE, 1971), no great heat accumulation has been found in olives. The mean temperatures actually experienced by eggs and larvae within olives in the outer canopy are on the average 1°C higher than the ones registered in the interior of the tree. Up to October this translates into a difference of 1°d every day. From November to January, when minimum temperatures drop with increasing frequency below the lower thermal threshold, this daily difference becomes smaller, but the total is higher because of the long duration. For comparisons with laboratory studies it is therefore recommended to utilize the following developmental

times which are based on pulp temperatures: eggs about 75°d above 6°C, and larvae between 160°d above 10°C in October and 110°d in April.

These data are averages calculated from sleeves installed in all directions around the tree. The difference in pulp temperature between extremely exposed olives in the south and shaded olives in the north on the average reached 2.8°C at noon and practically disappeared at night (tab. 6). Since the mean temperature (for 10 days) stayed above the lower temperature threshold throughout the winter 1976/77 (fig. 1), eggs and larvae on the south side daily received an average of 1.4°d more heat than their counterparts on the north side of the same tree. Similarly it was shown that *D. oleae* larvae on a sunny November day in Athens received 1.63 times more day-degrees in the south of the canopy than on the north side (LAUDÉHO *et al.*, 1978). From the durations given in tab. 4 it is then estimated that in October exposed larvae in the south may pupate an average of 2.2 days earlier than in the north. If half of the days are assumed to be sunny, the difference in the date of pupation of larvae which hatched at the same time, one in the south, the other one in the north of the same canopy, becomes 5.6 days in December and 13.3 days in January. Obviously this increased speed in the larval development on sunny branches can contribute heavily to the observed overlap of the generations in the field.

As regards literature records for the incubation period of eggs in the field, two to four days are indicated for summer (MARTELLI, 1908; SILVESTRI, 1914), 10 during fall (MARTIN, 1948; MARTELLI, 1965), and 12–19 days for late fall and winter (BERLESE, 1907; MARTELLI, 1908). These results are compatible with the findings in Crete (tab. 4). Laboratory experiments with constant temperatures indicate a duration of only one to two days at 18°C (BERLESE, 1907), 1.5–2 days at 23–25°C (SACANTANIS, 1953) on olives, and in more elaborate studies, 2.3 days at 25°C for a laboratory culture of *D. oleae* with artificial egging devices (TSIROPOULOS, 1972; TSITSIPIS, 1977). From the data reported here one would expect an egg duration of 3.9 days at 25°C. At temperatures below 15°C, however, the relative difference between the results from the laboratory culture and the field results become much smaller. It seems that eggs of laboratory flies, on artificial diet for many generations, exhibit a higher threshold (about 8°C) and lower heat requirements (TSIROPOULOS, 1972; TSITSIPIS, 1977) than the eggs from wild flies under field conditions. This difference may be partly attributed to a selection of the laboratory stock for life at the relatively high constant temperature of 25°C, as well as for faster development. This speeding-up is comparable to the faster deposition of the total egg complement already observed in the same culture (ECONOMOPOULOS *et al.*, 1976).

For the larval development, on the other hand, the increase in the speed of development in riper fruit is more striking in the field than in the laboratory. In experiments on detached fruit the problem of fruit preservation may obscure such differences in developmental times. Thus it was observed that in the laboratory larval development is generally one day shorter in black ripe olives than in green ones (TZANAKAKIS, 1971). Also the 9–14 days required according to variety in the laboratory at 25°C (TZANAKAKIS, 1971), or the 8–10 days at 23–25°C, 75% RH (SACANTANIS, 1953) both correspond to the rather long developmental times found in fall or early winter in this field study.

It is concluded that the speed of development as determined under natural conditions differs as follows from laboratory data: The egg development takes less time under constant conditions than expected from the field data. This may be

attributable to a selection for faster breeding in the culture, where the most accurate assessments have been made. On the other hand the egg development is short in relation to both the duration allowed for oviposition and the sampling frequency possible in a sleeve cage experiment. The field data for eggs therefore do not have the same relative accuracy as for larvae. The slow development reported for larvae in collected olives may be partly attributed to the deterioration of the infested olives during storage. Under conditions where the nutritional value of the infested olives is still improving up to fruit drop, the larval development is much faster in spring, as found in this study. This is of importance for the evaluation of the capacity for increase of the fly population on the left over fruit after harvest.

The method described to calculate the lower thermal threshold and day-degree requirement from field observations could be refined by the use of a computer by utilizing daily running maximum and minimum temperatures connected by a sine curve (ALLEN, 1976) together with better adapted equations for the temperature dependence (STINNER *et al.,* 1974), and a much narrower spacing of the lower temperature threshold values to be checked. The use of a simple temperature summation model is only possible under conditions as those in Crete, where the immatures of *D. oleae* did not suffer any lethal temperatures during the experiment from fall to spring. Otherwise upper and lower temperature thresholds have to be introduced into the calculations. The results obtained in this example seem to make it a promising technique for supplementing laboratory data on temperature dependence, or even to create such data anew where the equipment does not allow laboratory tests, or where the insect and plant material defies the use of temperature cabinets. This new method has the advantage of utilizing field data in connection with artificially caged populations, but involves the use of a new, and less obvious definition of the lower temperature threshold.

Furthermore it is hoped that the data reported here will contribute to improve decision making in pest management. The developmental times of eggs and larvae reported here will help to decide whether under the given climatic conditions a last spray before harvest is still necessary. This last spray has to be timed so that harvest is accomplished before the larvae leave the fruit. The reason for this timing lies in the observation that the living infestation of *D. oleae* does not increase fruit drop (unpubl. results) or lower the value of the oil olives at harvest. Exit holes, however, produce a substantial increase in the acidity of the oil. Therefore they constitute a net loss to the farmer (NEUENSCHWANDER & MICHELAKIS, 1978).

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