Taxonomic characters and physiological responses to temperature and photoperiod of two Lithocolletis species mining apple leaves

Taxonomic characters and physiological responses to temperature and photoperiod of two Lithocolletis species mining apple leaves

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The taxonomic characters of two *Lithocolletis* sp. mining the lower apple leaf surface have been studied. From this work it is concluded that *L. blancardella* FAB. attacks apple orchards in the lake Léman area in western Switzerland, while *L. pomonella* ZELL. was found south of the Alps in heavily infested Valtellina (Italy) orchards. The effects of constant temperatures and photoperiod on developmental time of immature stages (eggs, sap feeders, tissue feeders and pupae) of both species were compared. All. *L. pomonella* life stages have lower developmental thresholds, and their development rates generally reach a maximum at higher temperatures than corresponding stages of *L. blancardella*. In the temperature range of 10 °C to 30 °C, the developmental rates of all life stages of both species, except *L. pomonella* pupae, is best described by nonlinear models. The life stages of both species respond in a similar manner when exposed to short day. Late life stages (tissue feeders and particularly pupae) have longer developmental times, but the response is temperature dependent. The longevity of the females after mating is similar. *L. pomonella* females, however, produce more eggs at 21 °C and have a higher fecundity rate than *L. blancardella* females.

BAGGIOLINI (1959, 1960) reported outbreaks of *Lithocolletis blancardella* FAB. mining apple leaves from the western part of Switzerland. This species was considered to be the most common *Lithocolletis* sp. mining the lower apple leaf surface in neglected orchards in various fruit growing areas in Switzerland (BAUM-GÄRTNER *et al.*, 1981). Infestations observed south of the Alps in the Valtellina area (Lombardy, Italy) were thought to be caused by the same species (SÜSS & LOZZIA, 1974).

HERING (1966), however, reared only *L. pomonella* ZELLER from apple leaves collected in the northern part of Italy. Thus a study was undertaken to reexamine the species responsible for outbreaks both north (Switzerland: lake Léman area) and south (Italy: Valtellina) of the Alps. Once 2 different species were found it was attempted 1) to redescribe morphological characters to separate the two species, 2) to study their development in relation to temperature and photoperiod and 3) to compare their fecundity and adult longevity. The results are considered to be a base both for the understanding of the miners' population dynamics and for their distribution in various fruit growing areas.

The biology and population dynamics of *L. blancardella* infesting apple orchards in the Canadian province of Québec has been presented in detail by POTTINGER & LE ROUX (1971). Briefly, the pest has 5 distinct stages: eggs (E) laid on the lower apple leaf surface, sap feeding larvae (A) forming a flat mine through the spongy mesophyll adjacent to the lower epidermis, tissue feeding larvae (B) constructing the conspicuous spotted tentiform mine type, the pupae (P) found inside the mines, and the adult stage (F). For technical and ecological reasons it is referred to the developmental stages (called also life stages) rather than to the actual instars.

MATERIAL AND METHODS

Preparation of male genitalia

Adults from both the lake Léman area and the Valtellina were reared separatively as described below. Abdomens of male moths were treated with K(OH) at 60 °C during 1 h and then dissected to separate the external genitalia from the abdominal segments. The genitalia were subsequently washed in alcohol 70%, cellosolve (Ethylglycol) and xylol, and finally mounted in caedax.

The development of immature stages in relation to temperature and photoperiod

Overwintering *Lithocolletis* sp. mining the lower apple leaf surface were collected in fall 1979 near Nyon (Canton Vaud, Switzerland) and Valtellina (Lombardy, Italy) and kept in darkness at 2 °C. About 150 adults of each species were released, upon emergence at 21 °C, separately into 2 oviposition cages (wire nettings covered by organdy cloth with the bottom left open, diameter 40 cm, height 80 cm). One Golden Delicious apple tree (one year old) grafted on M9 rootstock was placed in each cage for one day at 21 °C. All plants were grown in 10 l plastic containers filled with soil having a relatively high percentage of organic matter. The trees were replaced daily and exposed to various experimental conditions.

Two plants per treatment (at 30 °C only 1 plant) and per species were exposed to the following conditions:
Long day (16/8): 10 °C, 15 °C, 21 °C, 26 °C, 30 °C, and short day (8/16): 15 °C, 21 °C, 26 °C, receiving a radiation of 10.5 to 17.5 watts per m². The number of eggs per tree varied between 10 and 60. *Lithocolletis* individuals on all plants were checked daily for their development, except the short day experiments in which late life stages were checked twice weekly. Leaves that abscised prematurely were examined in the same way.

Interpretation of the results

Temperature influence on developmental rates

The developmental rates of all immature life stages (E, A, B, P) were plotted against temperature (fig. 9, fig. 10). Visual examination shows the rates to be linearly related to temperatures from 10 °C to 21 °C. Developmental thresholds (thr) and standard errors (tab. 1) were calculated after CAMPBELL *et al.* (1974). Thresholds of corresponding life stages of both species were compared with a t-test (tab. 1), while the linearity of the rates up to 30 °C was tested after SACHS (1978). To describe nonlinear relationships, SIDDIQUI's *et al.* (1973) symmetrical curve (eq. 1)

$$r(t) = b \cdot e^{-a\,(c-t)^2} \tag{1}$$

while r(t) = temperature (t) dependent developmental rate, assumed to be 0
   when t less than the threshold thr
   b = maximum rate
   c = corresponding temperature
   a = constant

was compared with LOGAN's *et al.* (1976) asymmetrical temperature response function (eq. 2):

$$r(t) = a\,(e^{b(t-thr)} - e^{b(Tm-thr) - \frac{1}{c}(Tm-t)}) \qquad (2)$$

while r(t) = temperature (t) dependent developmental rate
      thr = temperature threshold calculated in the linear model as explained above
      Tm = lethal temperature estimated from preliminary experiments with no egg survival (33 °C)
      a = developmental rate at the base temperature
      b = rate increase to optimum temperature
      c = temperature range over which the negative influence on the rate becomes dominant

The parameters a, b, c of both functions were calculated with JENNRICH's (1979) program. With 3 parameters estimated (a, b, c) both nonlinear models (eq. 1, 2) were compared with their residual sums of squares (RSS) (LOGAN *et al.* 1976) given in tab. 2. The model with smaller RSS was used to describe the developmental rates whenever a nonlinear model was required (tab. 1).

Photoperiodic influences

The duration of certain life stages was considerably extended when exposed to short day conditions. This influence on a particular life stage (i) at a given temperature (t) was described by

$$f_{i,t} = \frac{DS_{i,t}}{DL_{i,t}} \qquad (3)$$

while DS = duration of a group feeding on the same plant at short day (8/16)
      DL = duration of a group feeding on the same plant at long day (16/8)

With 2 plants at each temperature regime (15 °C, 21 °C, 26 °C) 2 f-values were obtained generally for a given developmental stage (i) of each species. Only 2 pupae of *L. pomonella* developed to adults at 26 °C and short day conditions. Because this group was considered to be too small for a DS-estimate, it was excluded from the analysis. Log (f) transformed values were analyzed in a 3-way non-orthogonal analysis of variance to test for temperature (fixed factor), life stage (fixed factor), and species (fixed factor) effects (tab. 3).

Fecundity rate

Pairs of newly emerged adults (overwintering generation) of both species were fed a 10% sugar solution and kept at 21 °C in glass vials until mated. Male and female were transferred into a smaller oviposition cage (diameter 10 cm, height 13 cm) placed around some leaves on an apple tree. Insects were moved to a new set of leaves daily. Adult survival and the number of eggs laid was recorded daily for 7 pairs of each species. The pattern of daily fecundity rates was described by a nonlinear regression using JENNRICH's (1979) program.

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The identity of the Lithocolletis species

The identification of the *Lithocolletis* species mining the lower surface of the apple leaves has been based mainly on the morphology of the external male genitalia (fig. 1-8). The right costa (R) of *L. blancardella* FAB. (fig. 1-14) bears a rather long, thin and sharp pointed process, the top of which reaches almost the terminal portion of the valves (claspers), while the left costa (L) is relatively small. The right costa (R) of *L. pomonella* ZELL. (fig. 5-8) is much shorter than in *L. blancardella* and bears a short process which is sometimes strongly curved and
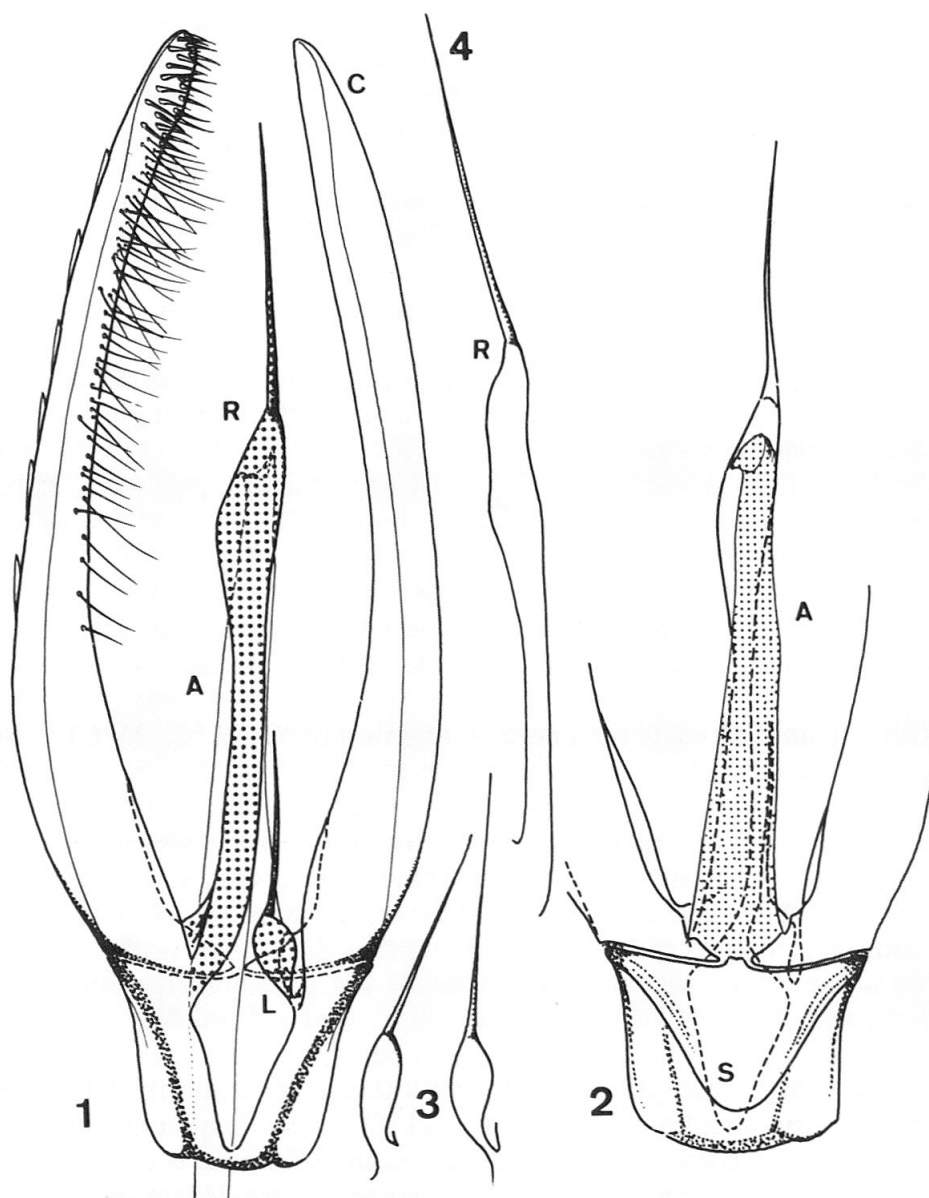

Fig. 1-4: Male genitalia of *Lithocolletis blancardella* as seen dorsally (1) and ventrally (2), with details of the left (3) and right (4) costa (A, aedaegus; C, claspers; R and L, right resp. left costa; S, saccus).

pointed (fig. 8) and reaches in general ⅔ of the valve (clasper) length. The left costa (L) is long as compared with that of *L. blancardella* and is generally more elongate. The male genitalia of the two species show additional differences like the form of saccus (S) or the position of the aedaegus (A), but these characters are less useful for the species differentiation than are the shape of the costae. It is very difficult to separate the two species according to observed differences of the wings: colour, basal streak and costal markings are very similar in both species and one can never be sure to have identified correctly. The name of *L. pomonella* for the second species observed in apple orchards has been selected on the basis of characters presented by HERING (1966). It could not be clarified whether the species is synonymous of *L. cydoniella* DENIS & SCHIFFM. as stated by POVOLNY (1967), hence the systematics of *Lithocolletis* associated with Pomoideae needs reconsideration.

All *Lithocolletis* collected in 1979 in apple orchards along the lake Léman (Switzerland) belong to *L. blancardella,* whereas those from the Valtellina region (Italy) belong to *L. pomonella*. HERING (1966) also reported *L. pomonella* from apple orchards in northern Italy.
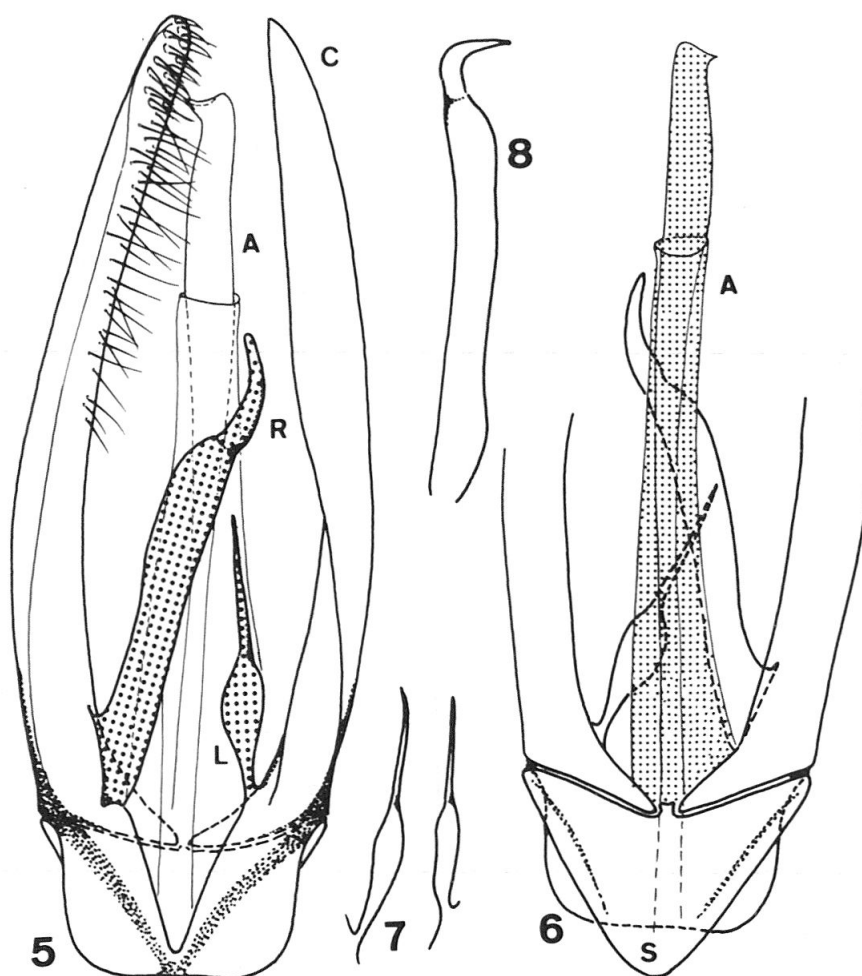

Fig. 5-8: Male genitalia of *Lithocolletis pomonella* as seen dorsally (5) and ventrally (6), with details of the left (7) and right costa (8) (A), aedaegus; C, claspers; R and L, right resp. left costa; S, saccus).

The developmental rates of all life stages except *L. pomonella* pupae have to be described by nonlinear models in the range 10 °C to 30 °C (tab. 1). In two cases the model proposed by LOGAN *et al.* (1976) has lower residual sums of squares (tab. 2) then SIDDIQUI's *et al.* (1973) symmetrical response curve. Thus *L. blancardella* pupae and *L. pomonella* sap feeders are described by eq. 2 (tab. 1). In comparing the two models it must be noted, however, that in LOGAN's *et al.* (1976) equation both the base temperature and the lethal temperature (eq. 2) are given, while SIDDIQUI's *et al.* (1973) curve is calculated on a empirical basis only.

In general, *L. pomonella* life stages have lower developmental thresholds than corresponding life stages of *L. blancardella* (tab. 1), differing significantly ($P < 0.05$) only for tissue feeders. However, maximum developmental rates of life stages of *L. pomonella* occur at higher temperature than those of *L. blancardella*. This is shown by both the parameter c of eq. 1 and 2 and the linear model used to describe the pupal developmental rate for *L. pomonella* (tab. 1). Furthermore, *L. blancardella* sap feeders have a decreasing developmental rate at temperatures above a calculated maximum value of 23 °C (tab. 1) and this contribute to the
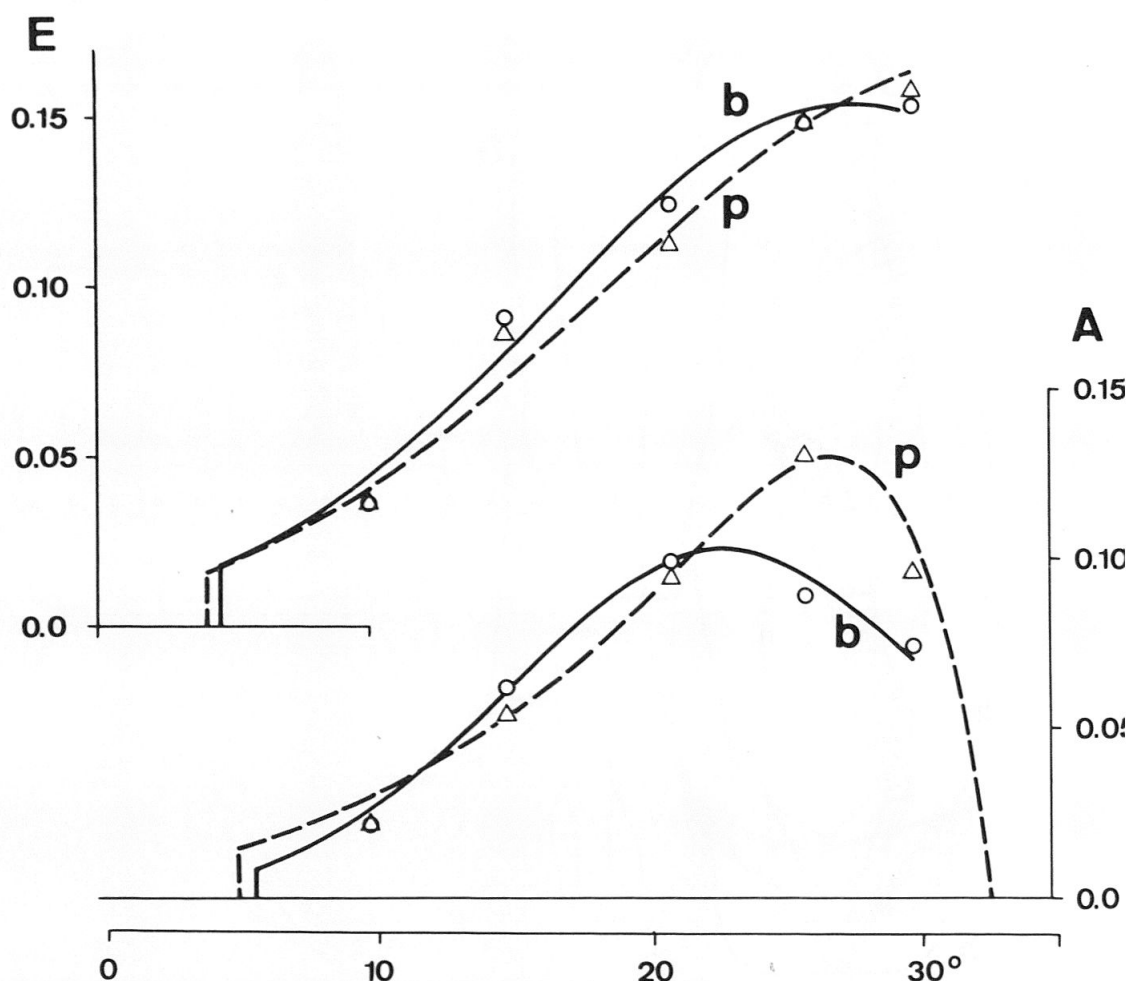

Fig. 9: Developmental rates for eggs (E) and sap feeders (A) of *Lithocolletis blancardella* (b) and *L. pomonella* (p). Models and parameters are given in eq. 1 and 2 and tab. 1 respectively.

Table 1: Temperature dependent developmental rates for eggs (E), sap feeders (A), tissue feeders (B) and pupae (P) of *Lithocolletis blancardella* and *L. pomonella* (THR, temperature thresholds in linear models describing the rates between 10 °C and 21 °C; SE, standard error; Test 1, comparison of corresponding life stages (s = different with $P < 0.10$); Test 2, test for linearity of developmental rates in the temperature range 10 °C to 30 °C; s = nonlinear with $P < 0.01$); S, model after SIDDIQUI *et al.*, 1973; L, model after LOGAN *et al.*, 1976; Li, linear model; a, b, c, parameters as given by equations 1 or 2; T, day-degrees (above the threshold TR).

Species	life stage	THR ± SE	Test 1	Test 2	Model	a	b	c
L. blancardella	E	4.5 ± 0.4	-	s	S	0.004	0.154	27.73
	A	5.7 ± 0.5	-	s	S	0.008	0.103	23.05
	B	5.2 ± 0.5	s	s	S	0.004	0.164	27.42
	P	6.5 ± 0.5	-	s	L	0.028	0.100	2.637
L. pomonella	E	3.9 ± 0.4	-	s	S	0.003	0.163	31.54
	A	5.1 ± 0.7	-	s	L	0.126	0.165	5.893
	B	3.6 ± 0.9	s	s	S	0.004	0.156	27.60
	P	5.8 ± 0.5	-	-	Li[1]			

[1] TR = 6.7 °C, T = 129.5

Table 2: Residual sums of squares (RSS) in the symmetrical model S (SIDDIQUI *et al.*, 1973) and the asymmetrical model L (LOGAN *et al.*, 1976) describing nonlinear developmental rates of different life stages (E = eggs, A = sap feeders, B = tissue feeders, P = pupae) of *Lithocolletis blancardella* and *L. pomonella*.

Species	life stage	RSS (S)	RSS (L)	RSS (L) / RSS (S)
L. blancardella	E	0.0596	0.0810	1.36
	A	0.0271	0.0814	3.00
	B	0.0448	0.0568	1.27
	P	0.0485	0.0428	0.88
L. pomonella	E	0.0929	0.1045	1.12
	A	0.1324	0.1156	0.87
	B	0.0906	0.0914	1.01

lengthening of the larval stage during the summer months as observed by BAUM-GÄRTNER *et al.* (1981). These results indicate that *L. blancardella* is adapted to a narrower temperature regime than *L. pomonella*. To date, the ambient temperatures were considered to represent the temperatures experienced by the life stages inside the mine. However, measurements with thermocouples inserted inside the mines indicate that the mine temperature varies sometimes considerably. Further studies are necessary to compare the temperature environment of leaf miners and the leaves they inhabit.

Table 3: Temperature (Temp), life stage (Age) and species (Spec) effects on the relative lengthening of *L. blancardella* and *L. pomonella* developmental stages under short day conditions. Log(f)-values are analyzed in a non-orthogonal 3-way analysis of variance.

Source of variation	Sum of Squares	DF	Mean Square	F	Signif. of F
Main effects	3.588	6	0.598	344.432	0.001
Temp	0.030	2	0.015	8.521	0.002
Age	3.481	3	1.160	668.336	0.001
Spec	0.000	1	0.000	0.012	0.913
2-way interactions	0.162	11	0.015	8.501	0.001
Temp Age	0.124	6	0.021	11.940	0.001
Temp Spec	0.003	2	0.002	0.897	0.422
Age Spec	0.016	3	0.005	3.157	0.044
3-way interactions	0.011	5	0.002	1.303	0.297
Temp Age Spec	0.011	5	0.002	1.303	0.297
Explained	3.761	22	0.171	98.483	0.001
Residual	0.040	23	0.002		
T o t a l	3.801	45	0.084		

Table 4: The relative lengthening (f-values) in the development of immature *Lithocolletis* life stages (E = eggs, A = sap feeders, B = tissue feeders, P = pupae) when exposed to short day conditions (number of replicates in parenthesis).

Temperatures °C	Life stages			
	E	A	B	P
15	1.14(4)	1.00(4)	1.63(4)	5.46(4)
21	0.91(4)	1.29(4)	2.39(4)	6.59(4)
26	1.00(4)	1.40(4)	2.82(4)	4.37(2)

Photoperiodic influences

When exposed to short day the life stages of both species responded the same way (tab. 3 and 4).

The factor «life stage» is a major contributor to the total variation in the transformed f-value (tab. 3) describing the short day effect (eq. 3). The later the life stage the more it appears sensitive to short day conditions (tab. 4). Short day as observed in late fall at Swiss latitude is therefore concluded to be mainly responsible for the dormancy of pupae as observed in the field. The exact nature of this phenomenon (MÜLLER, 1970) remains to be determined. Eggs appear to be affected by short day conditions at most in the temperature range 15 °C to 21 °C. At 26 °C larvae respond mainly to the daylength, while pupae are primarily sensi-

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tive at 21 °C. Thus high temperatures affect particularly late life stages. This response may be important for the two leaf miners as only the pupae overwinter. Further studies at temperatures below 15 °C and variable daylength are necessary for a full understanding of their reactions to autumn conditions.
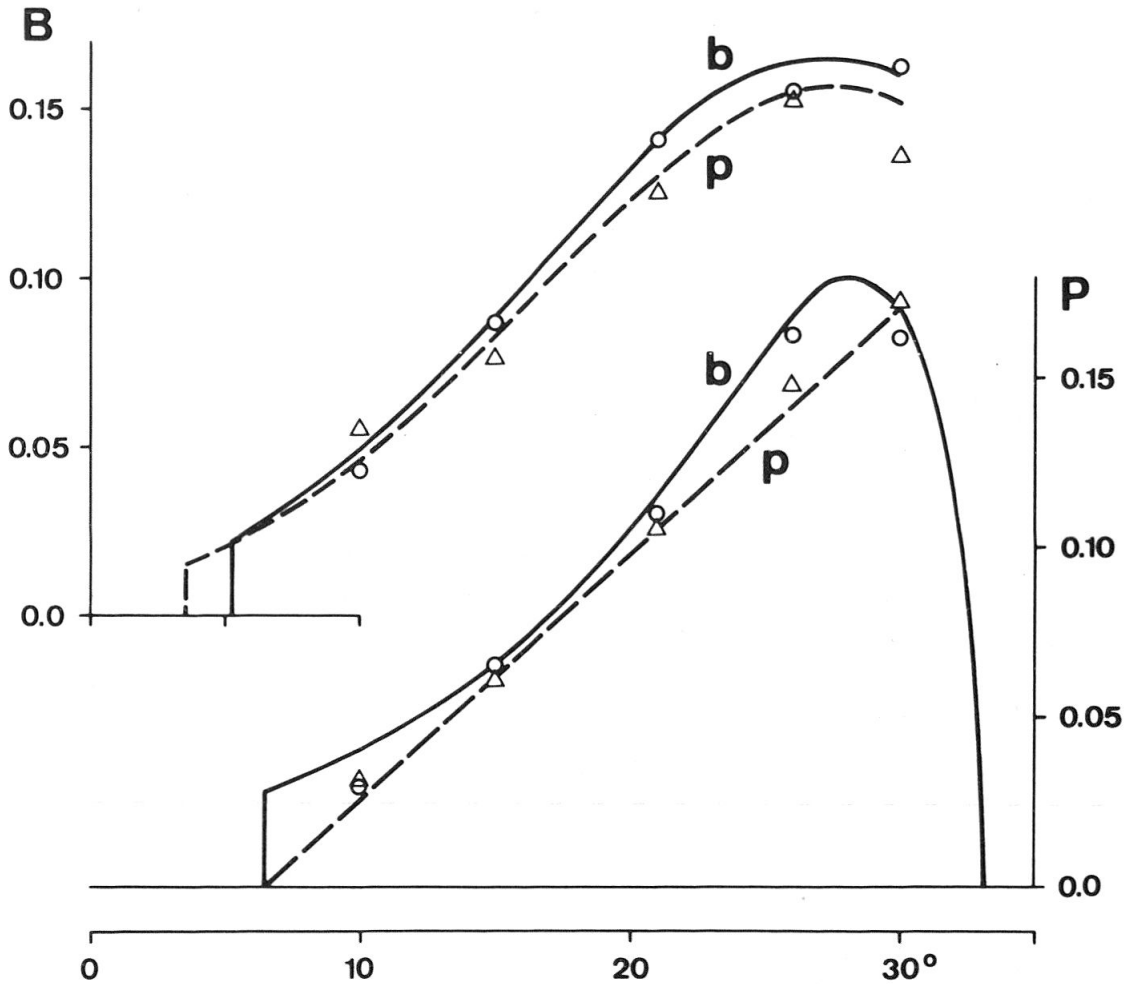

Fig. 10: Developmental rates for tissue feeders (B) and pupae (P) of *Lithocolletis blancardella* (b) and *L. pomonella* (p). Models and parameters are given by eq. 1 or 2 and tab. 1 respectively.

The fecundity rate

In this experimental procedure the preoviposition period of the females was not studied in detail but estimated to 1–3 days at 21 °C. There was not much variability in the daily egg production at 21 °C between the different females of each pair. The fecundity rates in fig. 11 are therefore considered to reflect the reproductive capacity of both species when fed a sugar solution. *L. pomonella* produces more eggs at a faster rate than *L. blancardella* (mean fecundity 153.7 with standard error 18.0 for *L. pomonella* and mean fecundity 102.1 with standard

error 15.0 for *L. blancardella)*. The fecundity of the latter species is higher than observed by POTTINGER & LE ROUX (1971) in an insectary (mean 44–67). Female longevity after mating of 21 °C of both species appears to be very similar. Therefore the survival rate was calculated with the pooled data of both species (fig. 11).
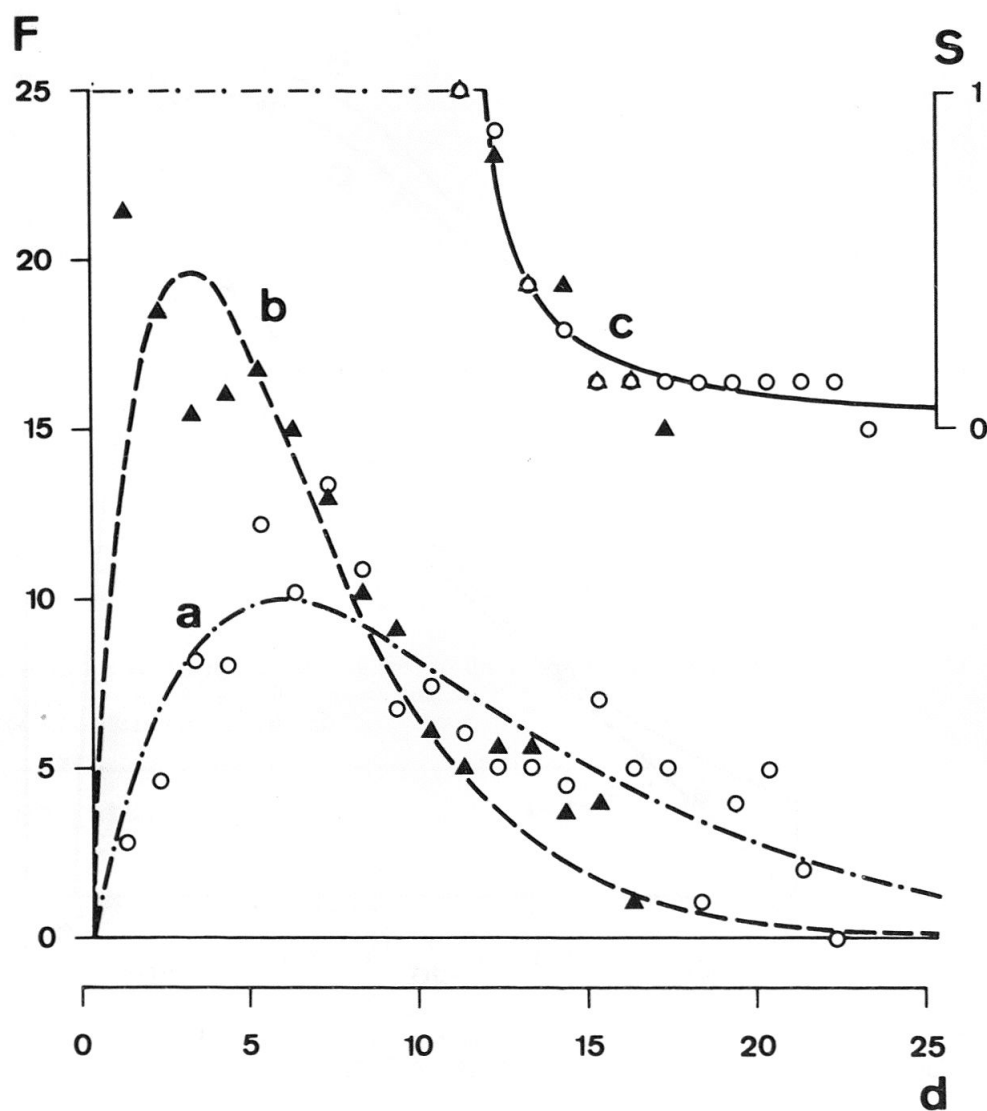

Fig. 11: Daily fecundity rate (F) of *Lithocolletis blancardella* [a = $4.92 \, d \div (1.2^d)$ for d $<$ 25] and *L. pomonella* [b = $18.55 \, d \div (1.41^d)$ for d $<$ 25], and survivorship (S) calculated from the pooled data (14 females of both species ($1 \geqq c = 0.8 \, (d-11)^{-0.96}$, for d $>$ 11) (d = days at 21 °C after mating).

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