

**CONSTANT-TEMPERATURE DEVELOPMENT RATES OF PRE-IMAGINAL
COLORADO POTATO BEETLES (*Leptinotarsa decemlineata* (SAY),
COLEOPTERA: CHRYSOMELIDAE) FROM MANITOBA AND
BRITISH COLUMBIA**

LACTIN, D.J. AND N.J. HOLLIDAY

Department of Entomology, The University of Manitoba
Winnipeg, Manitoba R3T 2N2

ABSTRACT

Colorado potato beetle eggs and larvae from Winnipeg, Manitoba (49° 54' N, 97° 9' W) (two collections), and Winfield, British Columbia (50° 02' N, 119° 24' W) were reared at a range of constant temperatures. In all stages, development was fastest between 29 and 31°C. Developmental responses to temperature did not differ significantly among the three collections. Developmental rates of larvae in the present study are similar to those in the literature, but egg development was faster at low temperatures and increased more slowly with temperature than did published values. However, if the experiment-wise error rate is protected at 0.05, none of the comparisons is significant.

INTRODUCTION

To construct a predictive model of Colorado potato beetle population trends under field conditions, the relationship of developmental rate to temperature must be quantified. Although several groups of researchers have measured Colorado potato beetle developmental rates under constant conditions, the study was repeated here because developmental rates may vary among populations of different geographic origins (Tauber *et al.* 1988) and because specimens used in the present trials were collected ca. 7° latitude (ca. 800km) north of those tested in the other trials (Walgenbach and Wyman 1984, Wisconsin; Ferro *et al.* 1985, Massachusetts; Logan *et al.* 1985 and Groden and Casagrande 1986, Rhode Island; and Tauber *et al.* 1988, coastal and inland New York state).

METHODS

All trials were conducted in environmental chambers during fall and winter 1990 and 1991 at the University of Manitoba (Winnipeg), under a 14L:10D diel cycle. The following rearing procedures were the same in all trials. Adult Colorado potato beetles were reared in transparent plastic boxes (10 x 10 x 30cm), with moistened paper towels and excised 'Russet Burbank' potato foliage *ad libitum*. Eggs and the first, second and third instars were reared in 10cm x 1.5cm plastic petri dishes with fresh excised potato leaflets and moist paper towel. Fourth instars were reared in 20 cm diameter x 3 cm petri dishes with fresh excised potato leaflets and a 2-cm layer of moistened perlite as a pupation medium. Leaflets in petri dishes were replaced at least daily and the paper towels or perlite were kept moist with distilled water.

To obtain egg masses, boxes with adults were established at the desired rearing temperature and inspected twice daily. Egg masses produced in known intervals were collected and placed in petri dishes. Eggs were considered individuals; this is distinct from Logan *et al.* (1985), who assigned this status to egg masses.

Petri dishes with eggs or larvae were inspected at least once each day. Dates and times of inspection (± 15 min) were recorded for each dish. The number of eggs or larvae hatched, moulted, dead, or unchanged, was noted at each inspection. The fourth instar was considered to end when larvae entered the soil; the subterranean non-feeding 'prepupal' stage (*sensu* Groden and Casagrande 1986) was disregarded. At each temperature and instar, rearing groups were assembled from larvae which hatched or moulted in the same interval.

Adult beetles were collected near Winnipeg, Manitoba, Canada, (49° 54' N, 97° 9' W) in the spring of 1990 and 1991, and Winfield, British Columbia, Canada (50° 02' N, 119° 24' W) in the spring of 1992. The three lineages descending from the collections taken from these two founder populations will be called isolates.

In all isolates, adults of the first filial generation (F1) produced the larvae used in the trials. F1 adults from both Winnipeg isolates were maintained in diapause at 5° C until required. Diapausing F1 adults were transported from Winfield to Winnipeg in soil in a cooler with ice, in October 1991. In each isolate, 20 to 30 adults provided the eggs used; sex ratios of these samples, and the relative contributions by the individuals, are not known.

Developmental rates were measured for eggs and all larval instars of each isolate. Some methods varied among years. In 1990 only the Manitoba isolate was tested. Rearing temperatures were 17°, 21°, 25°, and 29° C.

Time limitation precluded following individuals from egg to pupation. Consequently, a stock colony was reared at 29° C in plastic boxes of the type used to rear adults, and used to supply larvae of the desired instar as follows.

First instar larvae all originated in the 29° C chamber. Egg masses at 29° C were inspected twice daily; larvae which hatched in a given interval were assigned randomly into groups of 10 - 15, and each group was assigned randomly to a nominal rearing temperature.

Data obtained for the first instar appeared anomalous, even after adjusting for time spent in 29° C, and were disregarded.

The supply of other instars at each rearing temperature was obtained by transferring larvae of the previous instar from the stock colony into the rearing temperature, and rearing these to the desired instar before initiating rate measurement. The interval from transfer to moult into the desired instar always exceeded 24 h.

In 1991 both the Manitoba and British Columbia isolates were tested. Rearing temperatures were 17°, 21°, 24°, 27°, 29°, 31° and 33° C. Colorado potato beetles reared at temperatures $\geq 24^\circ$ were inspected twice daily.

In 1991, larvae remained at the same temperature throughout their lives wherever possible. Exceptions occurred at 31° and 33° C, due to high mortality rates; replacement larvae of a particular instar were obtained by moving larvae of the preceding instar from 29° C into the nominal rearing temperature and awaiting moult to the desired instar.

ANALYSIS

Only surviving larvae were included in the analysis. Egg or instar developmental time (the stadium, in days) was estimated to be from the midpoint of the two inspections bracketing oviposition or moult into the instar, to the midpoint of the two inspections bracketing moult into the next instar (Logan *et al.* 1985). Developmental rate was calculated for each larva as the reciprocal of the stadium.

Temperature-dependence of developmental rates was modelled using equation (1) (Logan *et al.* 1976).

$$r_j(T) = \Psi \left[e^{\rho T} - e^{\left(\rho T_{\max} - \frac{(T_{\max} - T)}{\Delta} \right)} \right] \quad (1)$$

Where T is °C above some arbitrary base temperature, usually the lowest used in the trial (in this trial, 17°); $r_j(T)$, constrained to be non-negative, is the developmental rate for life stage j at temperature T; T_{\max} is the upper temperature at which development ceases; Ψ may be a basal developmental rate, usually calculated at the lowest rearing temperature; ρ "can be interpreted as a composite Q_{10} for critical enzyme-catalysed, biochemical reactions" (Logan *et al.* 1976, p. 1134); and Δ measures the upper temperature range in which developmental rate diminishes. Parameters were estimated using iterative nonlinear regression (PROC NLIN, DUD algorithm; SAS Institute 1988).

Development functions for each instar obtained in the present study were compared to a literature data set consisting of pooled results from five studies of Colorado potato beetle developmental rate (Walgenbach and Wyman 1984; Logan *et al.* 1985; Ferro *et al.* 1985; Groden and Casagrande 1986 [only beetles reared on *Solanum tuberosum*]; Tauber

et al. 1988). Alone among these groups of authors, Tauber *et al.* (1988) included the subterranean prepupal stage in the fourth instar; because of this difference in method, their fourth-instar data are not included in the following comparisons.

Direct comparison of parameter estimates by t-tests, is invalid for two reasons. First, PROC NLIN merely finds a best fitting curve; where parameter estimates are strongly correlated (as in the present case), a change in the estimate of one parameter can be compensated by a change in that of one or more others, with no apparent change to the curve. The parameters estimates combine to provide a good fit to the data, but they are not necessarily the best estimates of the parameters. The second reason is that the standard errors produced by PROC NLIN are only approximate. For these reasons, conclusions based on direct comparison of parameter estimates are of limited merit. Direct comparison of trends is more appropriate.

Therefore, analysis of covariance (ACOVA) was used in statistical comparison of data sets. ACOVA requires that the functions compared be linear, but the developmental rate function (above) is nonlinear and apparently cannot be converted to a linear form, so the analyses considered data only from the approximately linear portion of the curve ($\leq 29^{\circ}\text{C}$).

RESULTS AND DISCUSSION

Developmental rates and standard errors from the present study are plotted in Fig. 1. The model used to describe these relationships between temperature and developmental rate (Logan *et al.* 1976) is composed of two components, which sum to give the overall response. All data contribute to the estimation of both components, and therefore the descending portion of the response can be estimated even in the absence of data in this region. The extrapolated sections of the curves are drawn to facilitate comparison with the literature data, but should be interpreted cautiously.

ACOVA on the linear portions of the developmental rate curves revealed no difference in slope ($F_{2,9} < 0.7$, $p > 0.05$) or intercept ($F_{2,9} < 1$, $p > 0.05$) among isolates in any instar, so data are pooled for analysis. Parameter estimates of the fitted models for the pooled data from the present study, and from the literature, are listed in Table 1. The estimate of the parameter Ψ depends on the basal temperature chosen; to simplify comparison among data sets, this temperature has been standardized at 17°C in the table.

At 31°C , eggs and first instar larvae survived better than did later instars; this trend accounts for the diminution of sample sizes with age at this temperature (Table 2). At 33°C , all larvae ($n \approx 15$ per temperature) died within 1 - 2 days and none moulted. This is a noticeable departure from the results of Logan *et al.* (1985), Ferro *et al.* (1985) and Groden and Casagrande (1986) who were able to measure larval development at 33°C , although survival was poor in all cases.

impeded by the rarity of chance meetings between bearers of the trait (Wright 1932), or by dilution by immigrant genetic material. Adaptation is expected to occur most rapidly in small, isolated populations under strong selective forces (Wright 1968). Because Colorado potato beetles often occur in great numbers, and disperse relatively well as adults (Johnson 1969), extensive gene flow among populations occurs (Hsiao 1985). Hence, lack of local adaptation in thermal physiology is not unexpected.

ACKNOWLEDGEMENTS

We thank D. Holder for technical assistance and D. Murphy for statistical advice.

REFERENCES

- Ferro, D.N., J.A. Logan, R.H. Voss and J.S. Elkinton. 1985. Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feeding rates. *Environmental Entomology*, 14: 343-348.
- Groden, E. and R. A. Casagrande. 1986. Population dynamics of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) on *Solanum berthaultii*. *Journal of Economic Entomology*, 79: 91-97.
- Hertz, P.E., R.B. Huey, and E. Nevo. 1983. Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. *Evolution*, 37: 1075-1084.
- Hsiao, T.H. 1985. Ecophysiological and genetic aspects of geographic variation in the Colorado potato beetle. *Proceedings of the Symposium on the Colorado potato beetle, XVIIth International Congress of Entomology*. D.D. Ferro and R.H. Voss (eds.). University of Massachusetts. Amherst.
- Johnson, C.G. 1969. *Migration and dispersal of insects by flight*. Methuen, London.
- Logan, J.A., D.J. Wollkind, S.C. Hoyt, and L.K. Tanigoshi. 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. *Environmental Entomology*, 5: 1133-1140.
- Logan, P.A., R.A. Casagrande, H.H. Faubert and F.A. Drummond. 1985. Temperature-dependent development and feeding of immature Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Environmental Entomology*, 14: 275-283.

- SAS Institute. 1988. SAS/STAT User's Guide. Release 6.03 Edition. Cary, NC: SAS Institute, Inc. 1028 pp.
- Sidak, Z. 1967. Rectangular confidence limits for the means of multivariate normal distributions. *Journal of the American Statistics Society*, 62: 626-633.
- Tauber, C.A., M.J. Tauber, B. Gollands, R.J. Wright, and J.J. Obrycki. 1988. Preimaginal development and reproductive responses to temperature in two populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America*, 81: 755-763.
- Ushakov, B.P. 1964. Thermostability of cells and proteins of poikilotherms and its significance to speciation. *Physiology Review*, 44: 518-560.
- Van Damme, R. D. Bauens and R.E. Verheyen. 1990. Evolutionary rigidity of thermal physiology: the case of the cool temperate lizard *Lacerta vivipara*. *Oikos*, 57: 61-67.
- Walgenbach, J.F. and J.A. Wyman. 1984. Colorado potato beetle (Coleoptera: Chrysomelidae) development in relation to temperature in Wisconsin. *Annals of the Entomology Society of America*, 77: 604-609.
- Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. *Proceedings of the Sixth International Congress of Genetics*. 1: 356-366.
- Wright, S. 1968. *Evolution and the genetics of populations, Vol. 1. Genetic and Biometric foundations*. University of Chicago press. Chicago.

Table 1. Parameter estimates from nonlinear regression of developmental rates of Colorado potato beetle eggs and larvae on constant temperature. Model used (equation 3 in text) is equation 6 in Logan *et al.* (1976). For comparison purposes, base was temperature standardized at 17°C for estimation of Ψ in both data sets.

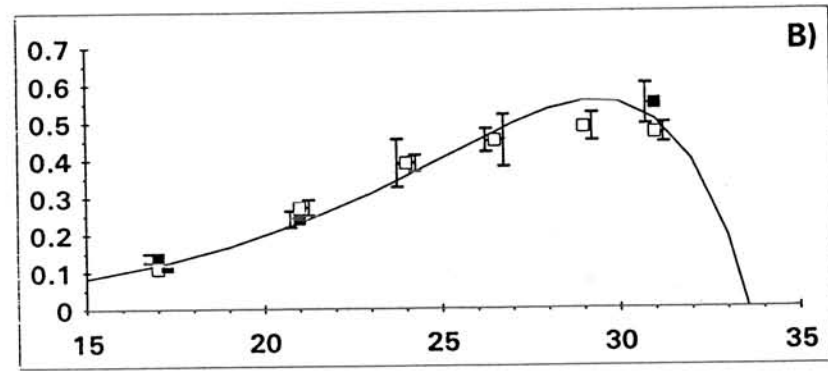
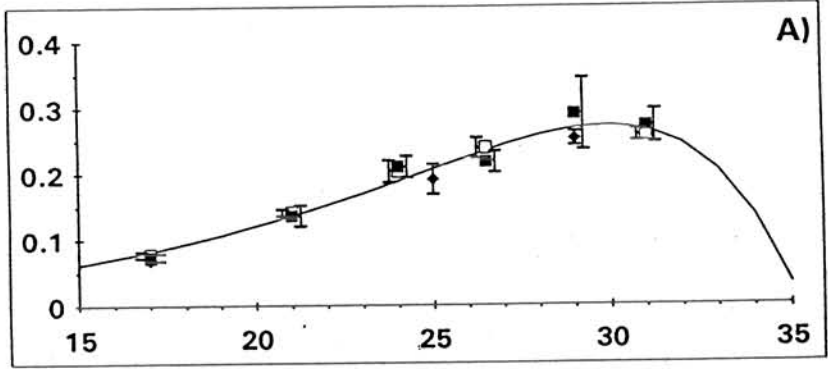
		Ψ		ρ		T_{max}		A		R^2
INSTAR	EGGS	EST	S.E.	EST	S.E.	EST	S.E.	EST	S.E.	
a) Present study.										
	1	0.146	0.00	0.169	0.000	35.18	1.88	4.68	11.52	0.99
	2	0.189	0.00	0.153	0.008	34.47	0.89	3.84	0.55	0.99
	3	0.501	0.00	0.191	0.042	37.14	3.70	4.87	0.91	0.98
	4	0.150	0.07	0.162	0.026	33.68	1.60	3.29	0.72	0.99
		0.150	1.08	0.134	0.221	36.54	4.42	4.78	11.50	0.99
b) Pooled literature review data.										
INSTAR	EGGS	EST	S.E.	EST	S.E.	EST	S.E.	EST	S.E.	R^2
	1	0.328	0.00	0.127	0.000	38.65	0.378	6.89	0.487	0.99
	2	0.349	0.00	0.146	0.469	37.46	2.37	5.53	20.4	0.96
	3	3.645	0.00	0.145	0.000	37.15	1.21	6.77	1.20	0.98
	4	0.213	0.04	0.104	0.056	35.75	1.486	3.63	3.39	0.97
		0.132	0.25	0.151	0.166	34.49	0.832	4.06	5.56	0.98

Table 2. Sample sizes (N) and survivorship (S) in developmental rate trials. (isolates: MB, Manitoba; BC, British Columbia).

INSTAR	°C	1990		1991			
		MB		BC		MB	
		N	S	N	S	N	S
EGGS	17	111	13	107	64	119	64
	21	68	14	141	34	106	67
	24	.	.	156	53	112	51
	25	54	10
	27	.	.	195	45	68	21
	29	83	20	9	0	194	47
	31	.	.	143	27	114	13
FIRST	17	.	.	118	29	51	12
	21	.	.	49	35	67	49
	24	.	.	87	45	63	40
	25
	27	.	.	81	51	34	15
	29	51	27
	31	.	.	110	68	33	7
SECOND	17	42	10	39	16	11	1
	21	18	12	37	27	48	31
	24	.	.	39	25	38	26
	25	19	11
	27	.	.	58	39	22	11
	29	26	9	.	.	28	15
	31	.	.	64	27	18	9
THIRD	17	23	12	15	9	.	.
	21	19	15	26	20	31	30
	24	.	.	26	22	26	24
	25	17	12
	27	.	.	39	30	13	9
	29	23	14	.	.	15	12
	31	.	.	39	21	6	3
FOURTH	17	23	13	12	8	.	.
	21	26	22	22	18	30	28
	24	.	.	21	17	23	21
	25	28	23
	27	.	.	32	23	9	9
	29	39	27	.	.	14	2
	31	.	.	9	5	1	0

Figure 1. Mean developmental rates of pre-imaginal Colorado potato beetles from Manitoba (MB) and British Columbia (BC).
 A) eggs; B) first instar; C) second instar; D) third instar; E) fourth instar.
 SYMBOLS: Diamond: MB isolate 1990.
 Open square: BC isolate 1991.
 Filled square: MB isolate 1991.
 Standard error bars for the BC isolate 1991 and the MB isolate 1991 are offset left and right, respectively.

STADIA / DAY



REARING TEMPERATURE [°C]

Figure 1. Continued

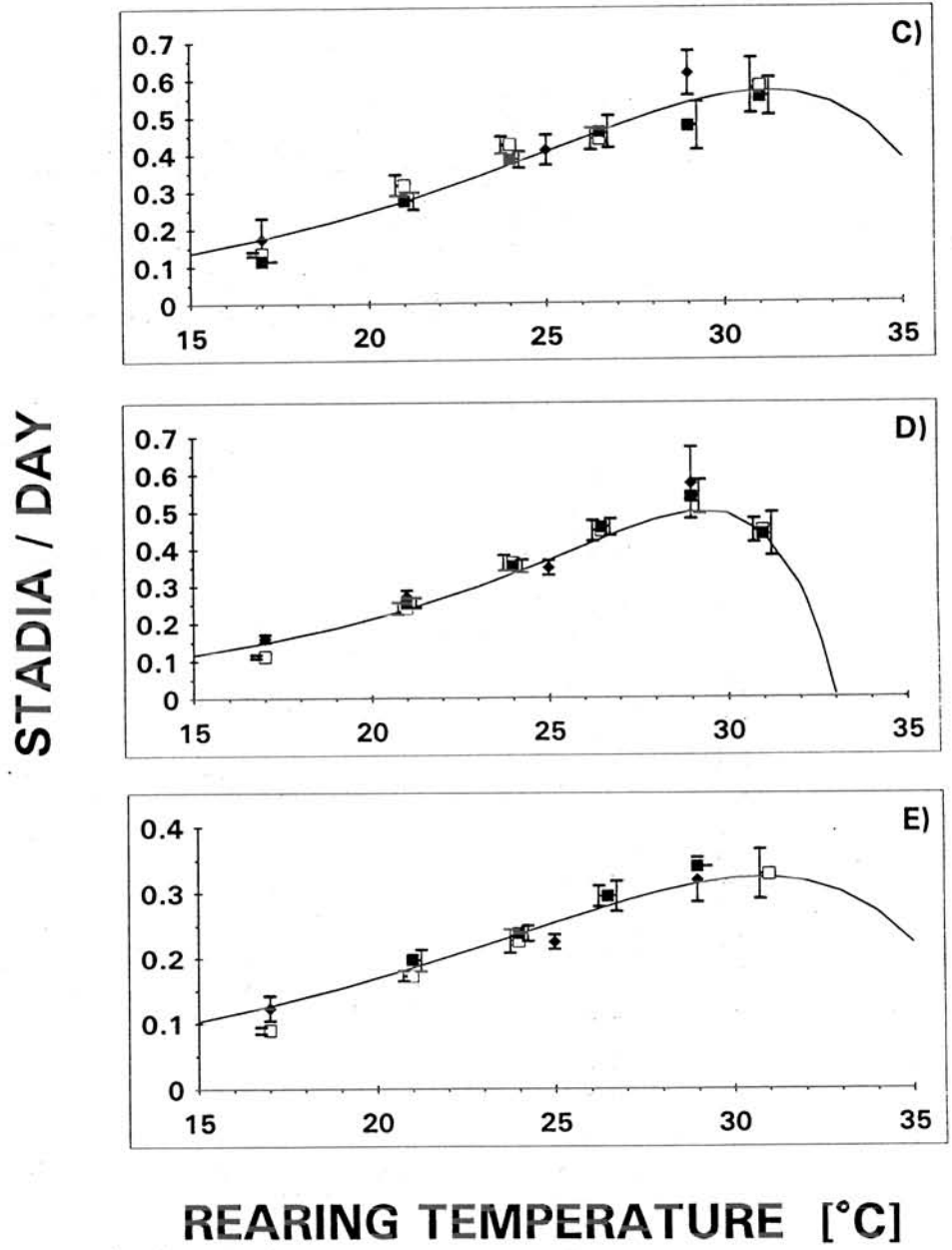
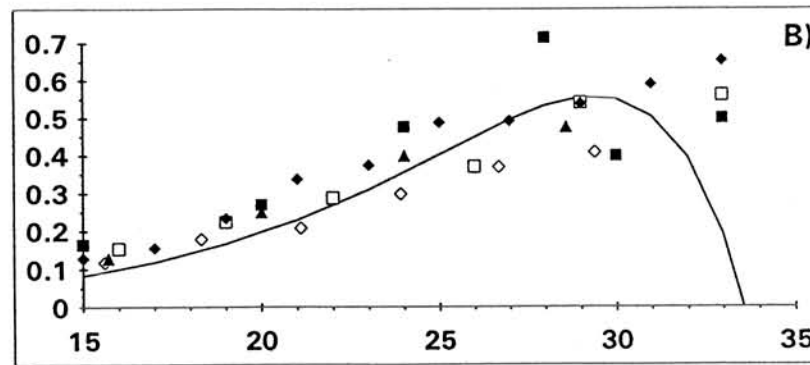
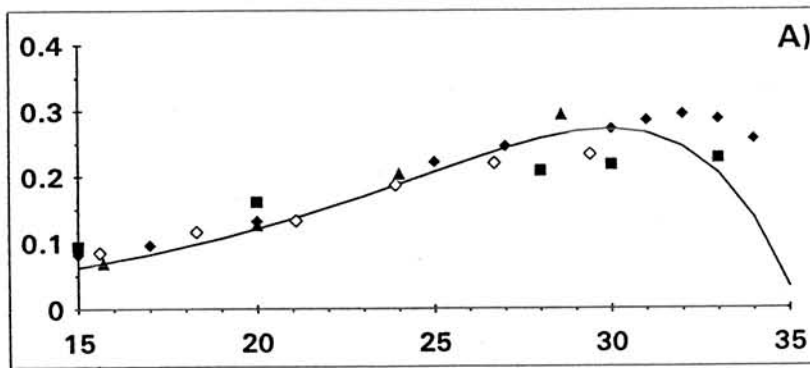


Figure 2. Developmental rates of pre-imaginal Colorado potato beetles. Comparison of regression description of data from present study, to 5 sets of published data.
 A) eggs; B) first instar; C) second instar; D) third instar; E) fourth instar.
 SYMBOLS: Filled square: Ferro *et al.* 1985
 Open square: Groden and Casagrande 1986
 Filled diamond: Logan *et al.* 1985.
 Open diamond: Tauber *et al.* 1988
 Filled triangle: Walgenbach and Wyman 1984

STADIA / DAY



REARING TEMPERATURE [°C]

Figure 2. Continued

