Colorado Potato Beetle (Coleoptera: Chrysomelidae) Survival and Fecundity after Short- and Long-Term Rearing on Artificial Diets[1]

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Abstract Survival, fecundity, and development time of Colorado potato beetles, *Leptinotarsa decemlineata* (Say), reared for a single generation or multiple generations (7 to 9) on one of three artificial diets or potato foliage and then switched back to potato foliage were measured. There were no consistent differences in any of the variables among diets regardless of whether the insects had been reared for one or many generations on artificial diets. Adult survival was about 80% at 10 wks after eclosion and <10% after 30 wks. Females produced an average of 977 and 796 eggs by week 16 after being reared for a single and for many generations, respectively, on artificial diets. Egg hatch rates ranged from 81% at week 3 to 14% on week 15, resulting in an average larval production of 516 and 552 by week 16 for females reared for a single and for many generations, respectively, on artificial diets. These results suggest that Colorado potato beetles reared on artificial diets can be used in subsequent tests on foliage without significant carryover effects from either short- or long-term rearing on these diets.

Key Words *Leptinotarsa decemlineata,* Colorado potato beetle, artificial diet, potatoes, survivorship, fecundity

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is a serious pest of potatoes (*Solanum tuberosum* L.), tomatoes (*Lycopersicon esculentum* Mill.), and eggplant (*Solanum melongena* L.) in North America and Europe. The ability of this insect to rapidly become resistant to insecticides (Bishop and Grafius 1996) impedes the development of effective pest management programs. A continuous supply of reared insects is needed for laboratory and out-of-season research efforts, for screening candidate chemical and microbial control agents, and as hosts for the production of natural enemies. Currently, most Colorado potato beetles are reared on foliage from host plants grown in a greenhouse. The availability of a cost-effective artificial diet could reduce rearing expenses.

Artificial diets for Colorado potato beetle have been developed by several researchers (Wardojo 1969, Hsaio and Frankel 1968, Domek et al. 1997). However, none of these diets could economically support the continuous rearing of all Colorado potato beetle life stages. In an unpublished report (*circa* 1990), O. T. Forrester (USDA, APHIS, Mission, TX) described the development and evaluation of a semi-

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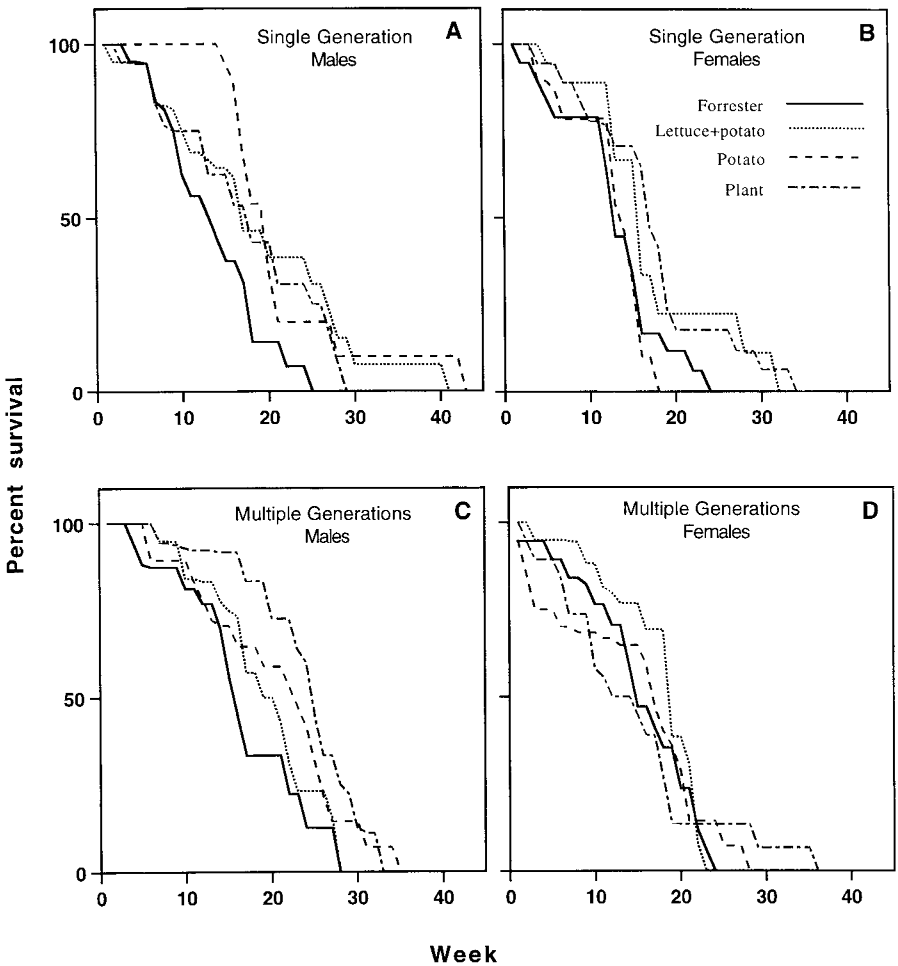

Fig. 1. Percent of surviving Colorado potato beetle adults reared for a single (A,B) or multiple (C,D) generations on three artificial diets or on potato foliage.

defined artificial diet based on yeast and oats, and including dried potato leaf and tomato fruit powders, on which Colorado potato beetles were successfully reared through eight continuous generations. Development time, prepupal weight, and survival of diet-reared insects were reported to compare favorably with those reared on potato foliage. R. A. Bell (USDA, ARS, Beltsville, MD) subsequently developed two additional semi-defined Colorado potato beetle diets based on the Forrester diet but containing fewer ingredients. Twelve generations were successfully reared (Gelman et al. 2001). In one of these diets, most of the potato leaf powder was replaced with lettuce leaf powder. Lettuce for producing the leaf powder is available year-round at produce markets. Larval development time for insects reared on these artificial diets was 0.8 to 1.5 days longer, weights of newly-emerged adults were 18 to 22% lower,

Table 1. Survival of Colorado potato beetle adults reared for a single or multiple generations on three artificial diets or on potato foliage

		Males			Females		
		Percent survival at week:					
Generation	Diet	10	20	30	10	20	30
1	Forrester	62.5	14.3	0	79.0	11.8	0
1	Lettuce + potato	75.0	38.5	7.7	78.6	0	0
1	Potato	100	33.3	10.0	77.8	17.7	6.3
–	Plant	75.0	42.9	0	88.9	22.2	11.1
$P > \chi^2 =$		0.05	0.39	0.49	0.91	0.48	0.18
7	Forrester	81.3	33.3	0	76.5	23.5	0
9	Lettuce + potato	84.2	50.0	0	88.2	38.5	0
9	Potato	89.5	58.8	14.3	68.4	28.6	0
–	Plant	92.9	72.7	12.5	57.9	13.3	6.7
$P > \chi^2 =$		0.78	0.34	0.22	0.22	0.49	0.25

and first instar mortality was 2 to 6 times higher than for insects reared on potato foliage. However, Gelman et al. (2001) presented evidence that the differences between insects reared on artificial diets and plants were less significant in later generations of continuously reared Colorado potato beetles.

The focus of this study was to compare diet- and plant-reared Colorado potato beetles after they were reared for one generation or for many continuous generations on artificial diet and then switched back to plants. Larval and adult survivorship, development time, and fecundity were assessed as indicators of Colorado potato beetle quality. We expect that researchers with only limited access to greenhouses may rear Colorado potato beetles on artificial diet for subsequent testing on potted plants or in the field.

Materials and Methods

Insects. The Colorado potato beetles used in this study were from a colony started in 1996 from insects collected in the field at the Beltsville Agricultural Research Center, Beltsville, MD. Once each year, additional field-collected adults were infused into the colony to maintain genetic diversity. The colony was reared on potted potato plants (var. Kennebec) grown in a greenhouse and maintained at 25 ± 2°C, 60% RH, and 16:8 (L:D) photoperiod in a controlled-environment chamber.

Artificial diets. We tested three artificial diets, all of which are described in detail by Gelman et al. (2001): (1) the original Forrester diet; (2) the potato diet which is based on the Forrester diet but contains fewer ingredients; and (3) the lettuce + potato diet which is the same as the potato diet but with 80% of the potato leaf powder replaced by lettuce leaf powder. Briefly, the diet ingredients were mixed with boiling

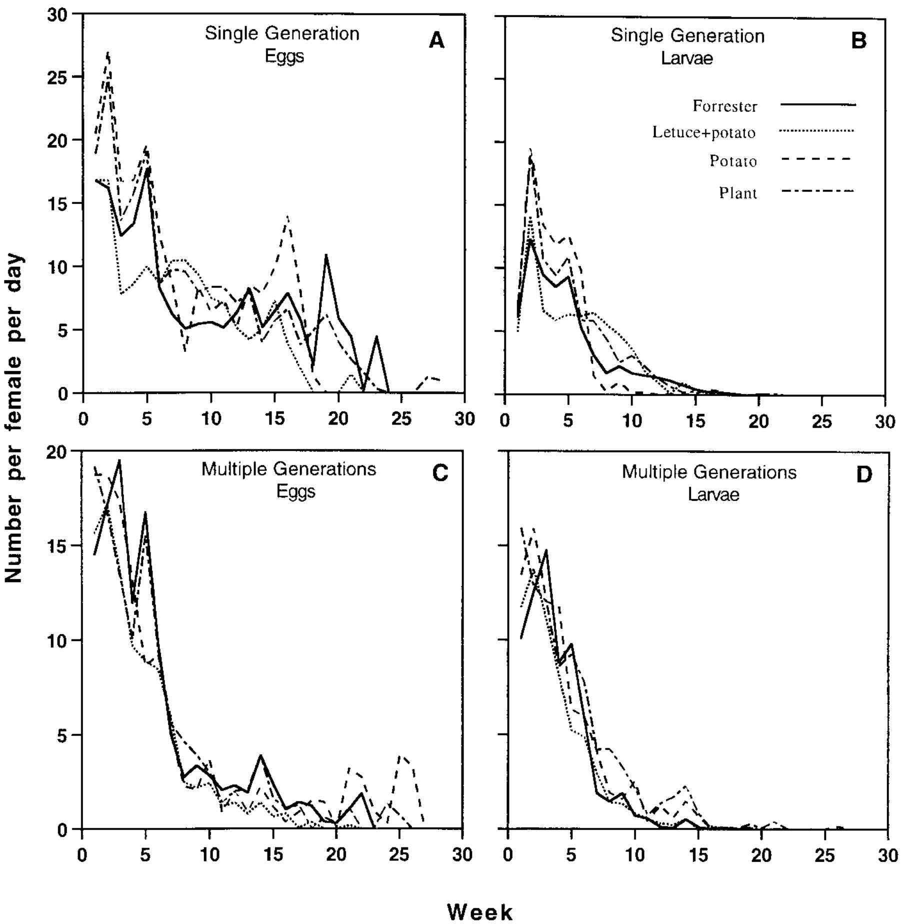

Fig. 2. Number of Colorado potato beetle eggs (A,C) and larvae (B,D) produced per female per day by insects reared for a single (A,B) or multiple (C,D) generations on three artificial diets or on potato foliage.

water in a blender. While still hot, the liquid diet was poured into plastic trays to a depth of 12 mm and allowed to cool and solidify. Once cool, the diet was stored in a refrigerator for a maximum of 2 days until needed.

Rearing on artificial diet. Details of the rearing procedure can be found in Gelman et al. (2001). Briefly, Colorado potato beetle neonates were placed in groups of 10 on a cube (approximately 18 × 12 × 6 mm) of diet in a plastic Petri dish (50 × 9 mm). When the larvae reached the third stadium, they were transferred to a larger Petri dish (60 × 20 mm) that contained a larger diet cube (approximately 18 × 12 × 12 mm). Diet cubes were replaced with fresh cubes three times per week. When the larvae reached the prepupal stage they were transferred to 170-mL plastic containers half filled with a slightly-moistened commercial potting mixture (primarily peat moss,

Table 2. Fecundity of Colorado potato beetles reared for a single or multiple generations on three artificial diets or on potato foliage

Generation	Diet	Cumulative number of eggs per female $\bar{x}$ ± SEM) at: 5 weeks	16 weeks	Cumulative number of larvae per female ($\bar{x}$ ± SEM) at: 5 weeks	16 weeks
1	Forrester	545.0 ± 50.9ab	931.0 ± 184.1	322.6 ± 52.8	480.0 ± 97.8
1	Lettuce + potato	428.6 ± 50.9a	810.0 ± 184.1	259.6 ± 52.8	459.0 ± 97.8
1	Potato	716.8 ± 50.9c	1172.0 ± 253.5	472.8 ± 52.8	556.5 ± 138.3
–	Plant	649.0 ± 50.9bc	993.8 ± 200.8	351.0 ± 52.8	569.4 ± 80.0
$P > F =$		0.02	0.65	0.10	0.82
7	Forrester	439.0 ± 99.2	723.0 ± 225.7	305.7 ± 73.2	418.1 ± 152.0
9	Lettuce + potato	533.8 ± 114.5	705.0 ± 225.7	400.4 ± 84.5	494.3 ± 152.0
9	Potato	662.8 ± 114.5	889.6 ± 184.3	522.7 ± 84.5	631.2 ± 124.1
–	Plant	541.2 ± 99.2	868.3 ± 225.7	424.2 ± 73.2	665.0 ± 152.0
$P > F =$		0.57	0.89	0.35	0.66

Numbers within a column followed by the same letter are not significantly different at a 0.05 comparison-wise error rate.

perlite, and vermiculite) covered with a plastic lid into which 10 to 12 pin-sized holes were drilled. Newly-emerged adults were placed in groups of 10 in Petri dishes (100 × 20 mm) each containing a diet cube that was replaced three times per week. A circular piece of paper towel was placed beneath the lid of the Petri dish as an oviposition substrate. To minimize egg consumption by adults, the paper towels were checked twice daily for egg masses. Egg masses were cut from the towel and placed in a clean Petri dish lined with filter paper. All rearing was done in environmental chambers at 25 ± 1°C, 75-80% RH, and 16:8 L:D photoperiod

Production of insects for experiments. The following procedures were used to produce insects for use in the experiments. For generation one adults, eggs obtained from the colony were hatched and neonates were randomly assigned to one of the three artificial diets or to potato foliage (var. Kennebec). Larvae assigned to artificial diets were reared as described in the previous section. Larvae assigned to plants were placed on bouquets of foliage contained in a 250 × 300 × 100 mm plastic crisper and placed in the same environmental chamber. As adults were produced, they were placed in mixed-sex groups of 10 in Petri dishes containing the same diet on which they had been reared or, in the case of larvae reared on plant bouquets, on bouquets of potato foliage in a 300 mm^3 cage. After 1 wk, adults were ready for use in experiments. To produce generation 1 larvae, eggs were obtained from adults that had been reared on artificial diet for 1 generation. Larvae hatching from these eggs were used in the experiments. To produce larvae from foliage-fed adults, eggs were collected from the colony. To produce generation 7 (Forrester diet) or 9 (potato and lettuce + potato diets) adults, eggs were collected from cultures of beetles reared for 6 (For-

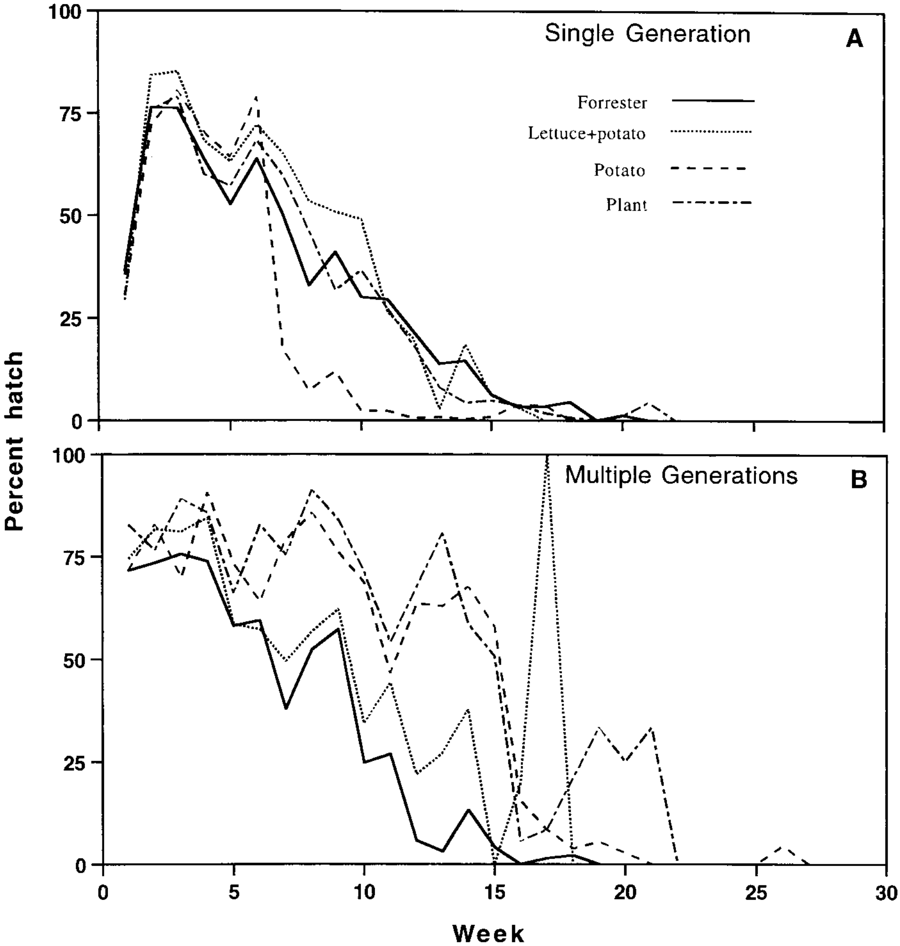

Fig. 3. Percent hatch of eggs from Colorado potato beetles reared for a single (A) or multiple (B) generations on three artificial diets or on potato foliage.

rester diet) or 8 (potato and lettuce + potato diets) generations on the specified diet. Only six generations of beetles reared on the Forrester diet were obtained because the culture reared on this diet was inadvertently lost after two generations due to an equipment malfunction and had to be re-initiated. Eggs for the production of foliage-fed adults were obtained from the main colony. Hatching larvae were reared and adults produced for experimental use as described for the generation 1 adults. To produce generation 7 or 9 larvae, egg masses were obtained from adults in cultures reared for 7 or 9 generations on artificial diet, and hatching larvae were used in the experiments. To produce larvae from foliage-fed adults, eggs were collected from the colony.

Experimental procedure. To measure fecundity and survival, adults were placed in single-sex groups of five on a potted potato plant covered with a nylon organza cage (600 mm long × 600 mm diam) held in place by a metal hoop frame. Four replicates of each treatment and sex were set up and placed in an environment-controlled room at 25 ± 2°C, 60% RH, and 16:8 (L:D) photoperiod. Plants were checked three times per week and replaced once per week. Each time the plants were checked, Colorado potato beetle mortality and egg production were recorded and all egg masses were collected. The eggs were counted and placed in Petri dishes on filter paper in an environmental chamber at 25 ± 1°C, 75-80% RH, and 16:8 (L:D) photoperiod until they hatched. The number of hatching larvae was recorded. To measure larval survival and development rate, larvae were placed in groups of 20 on potted potato plants covered with organza cages. The plants were checked daily (generation 1) or every other day (generation 7 or 9) and the number and stadia of larvae on each plant were recorded. Plants were replaced weekly. Each treatment was replicated four times. When larvae were predominantly fourth instars, the number of larvae was reduced to five per plant to limit defoliation. In culling larvae, the proportion of insects in each stadium was kept constant.

Data analysis. Because some of the adults lived for such an extended period of time (over 40 wks), insects from different cages were eventually combined to reduce the resources needed to maintain the experiment. Unfortunately, this disturbed the replicated structure of the experiment, in some cases preventing the use of ANOVA and the calculation of standard errors based on observed variability among replicates. Thus, differences among the diets in male and female survivorship at 10, 20, and 30 wks were tested using a Chi-square test (Proc Freq, SAS Institute 1985). Differences among the diets in egg and larval production were replicated and were tested by ANOVA (Proc Mixed, SAS Institute 1996). Egg hatch rate was plotted against time for insects reared for a single or multiple generations on artificial diets and foliage and differences among the slopes were tested by determining the significance of the diet x time interaction for the linear portion of the graph (wks 5 to 15) using an analysis of variance (ANOVA) (Proc GLM, SAS Institute 1985). Differences in larval development time among the diets were determined by testing the following dependent variables by ANOVA: (1) the percentage of fourth instars on day 9 or 10; and (2) the percentage of adults on day 28 or 29. Percentage values were transformed to arcsine ($\sqrt{x}$) prior to analysis. A plot of the residuals verusus the predicted values indicated that the transformation improved variance homogeneity. When the ANOVA indicated significant treatment effects, means were separated at a comparison-wise error rate of 0.05 using the least significant difference (LSD) procedure (SAS Institute 1985).

Results and Discussion

Percent survival of males and females reared for a single or multiple generations on artificial diets and for insects reared continuously on plants is shown in Figure 1. In general, survival through time was similar among insects that had fed on different diets. A more detailed examination of the differences in survival among beetles reared on the different diets is presented in Table 1. The only significant Chi-square value was for 10-wk mortality of males reared for a single generation on artificial diet (χ^2 = 7.7; df = 3; P = 0.05). It appears that first-generation males reared on the Forrester diet may have had a lower survival rate than males reared on the other diets (Fig. 1A). A similar, although less pronounced, trend occurred among males that had been reared for multiple generations on artificial diet, although the Chi-square values were

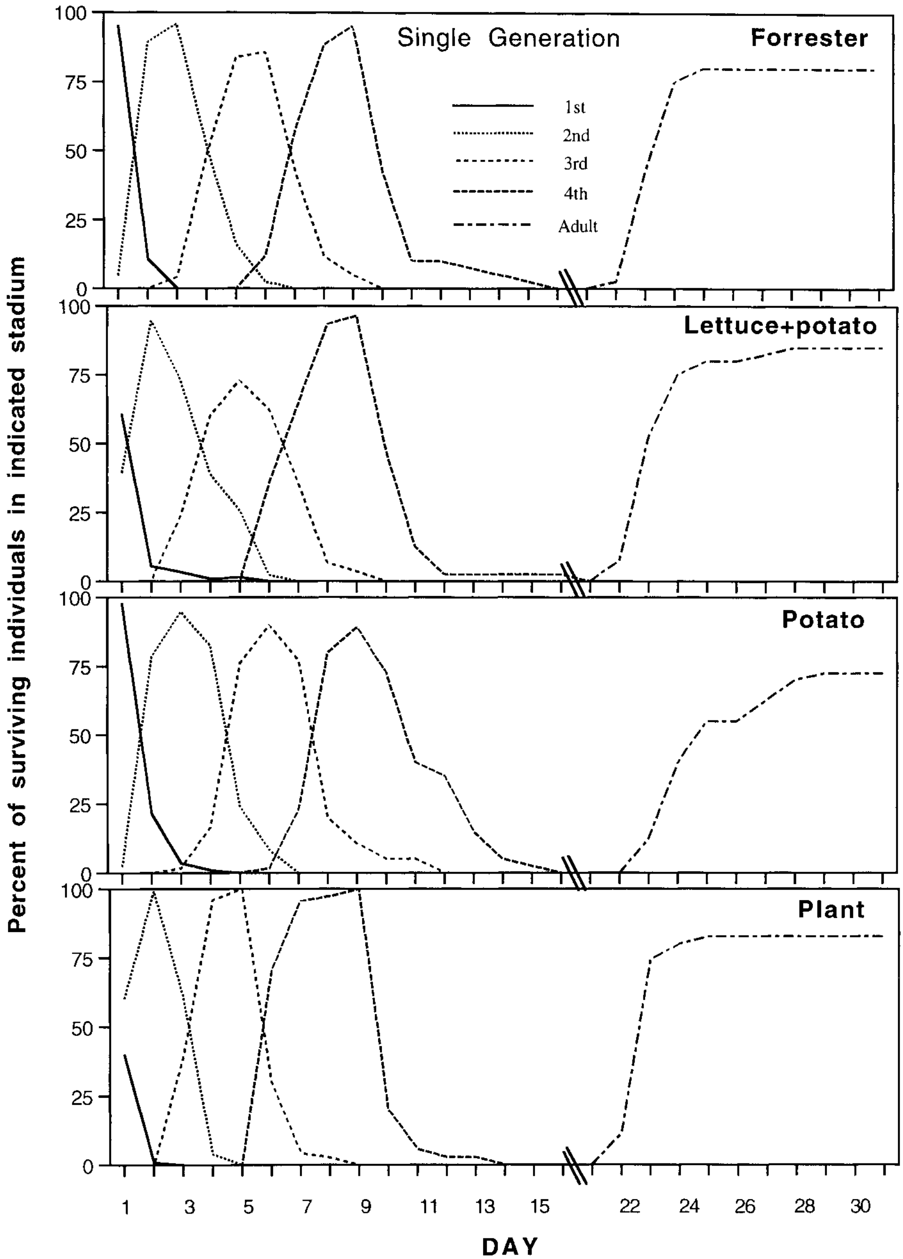

Fig. 4. Percent of Colorado potato beetles in different stadia over time when reared for a single (A) or multiple (B) generations on three artificial diets or on potato foliage.

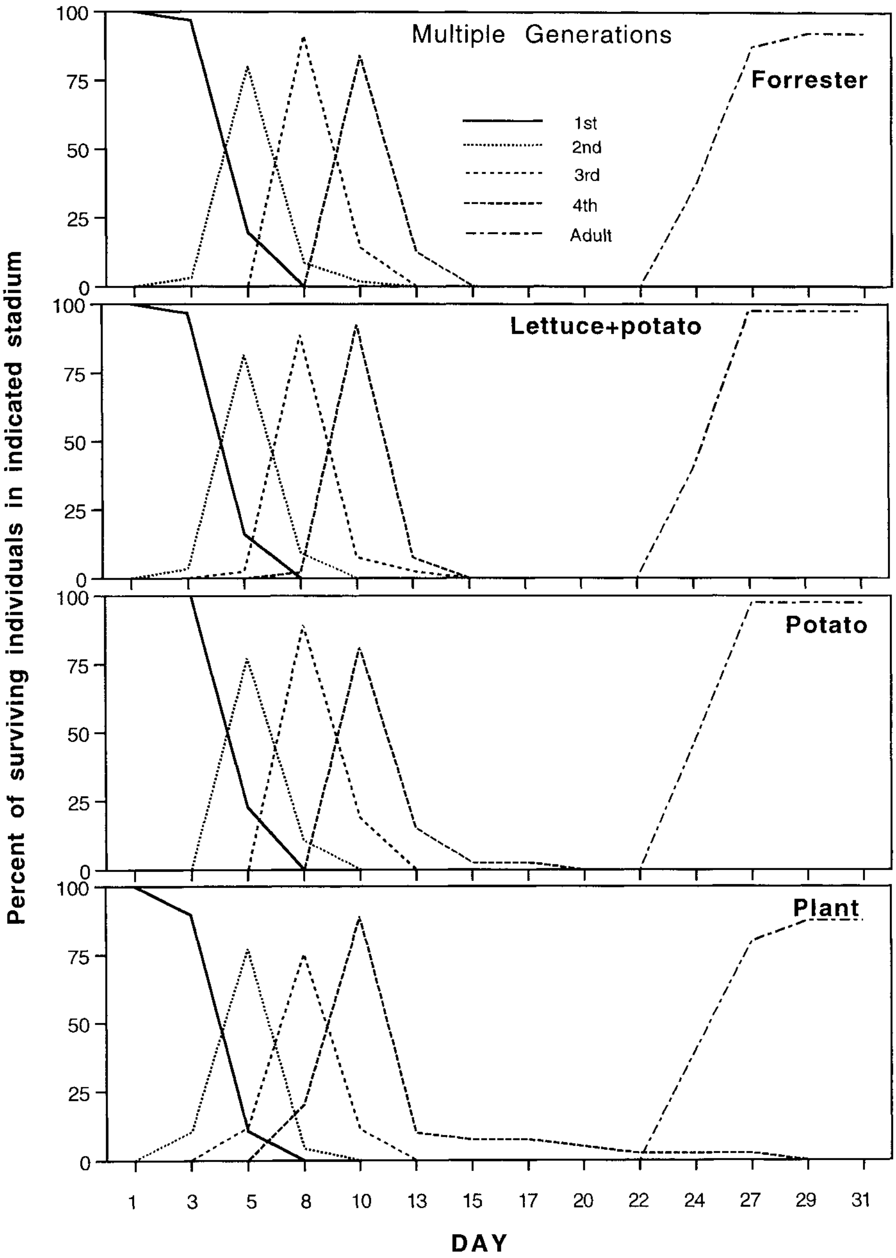

Fig. 4. (continued)

not significant (Fig. 1C). In general, survival was high during the first 10 wks (overall mortality at wk 10 = 20.7%), but substantial mortality occurred during the next 10 wks. Very few insects survived to wk 30.

The number of eggs and larvae produced by females reared on artificial diets for a single or for multiple generations and for females continuously reared on plants are shown in Figure 2. Fecundity values through time for insects that had fed on different diets were similar. A more detailed examination of differences in egg and larval production among the diet treatments is provided in Table 2. The *F*-value was significant only for eggs from females reared for a single generation on artificial diets at 5 wks (F = 6.1; df = 3,8; P = 0.02). Multiple range tests indicated that the egg production of females reared on the potato diet was significantly higher than that of those reared on the Forrester and lettuce + potato diet, and that the egg production of females reared on plants was significantly higher than that of those reared on the lettuce + potato diet. These differences were no longer evident by wk 16. Among females reared for a single generation on artificial diets, the total number of eggs produced per female ranged from 810 to 1172 and the total number of larvae produced ranged from 480 to 569 at 16 wks. Among females that had been reared continuously for multiple generations on artificial diets, the total number of eggs produced per female ranged from 705 to 890 and the total number of larvae produced per female ranged from 418 to 665 at 16 wks. Egg production was higher among females from colonies that had been reared for only a single generation on artificial diets than among females reared for multiple generations. However, because egg production by females from the main colony that had been reared continuously on plants was also higher in the single-generation test compared to the multiple-generation test, it is likely that the difference is due to external factors such as the condition of the host plants used in the test rather than any effects from continuous rearing on artificial diets. Since insects from all diet treatment were tested on foliage at the same time, any potential variation in host plant condition over time should not have contributed to differences among the diet treatment effects.

Hatch rates over time for the different treatments are shown in Figure 3. From wk 5 to wk 15, the slopes are not significantly different at the 0.05 level among the diet treatments [F = 2.1; df = 3,393; P = 0.1 (single generation) and F = 1.0; df = 3,332; P = 0.4 (multiple generations)]. The reason for the sudden drop in the hatch rate of eggs produced by females that had been reared on the potato diet for a single generation at wk seven is not known, but the lack of a similar effect in eggs from females that had been reared on the potato diet for multiple generations suggests that it is probably not a result of the diet. As seen in Table 2, the reduced hatch rate of eggs from potato diet-fed females did not significantly reduce the cumulative number of larvae produced by wk 16 relative to the other treatments. In general, egg viability was high initially ($\bar{x}$ at week 3 = 80.7%) and then dropped off gradually. By week 15, average egg viability was 13.8%.

Development times for each of the four larval instars and for adult eclosion are shown in Figure 4. Development times were very similar and did not appear to be affected by previous rearing on artificial diet. To test for differences in development times, a one-way ANOVA was performed on the percent of larvae that had reached the fourth instar by day 9 (single generation) or day 10 (multiple generations), and on the percent of individuals that had eclosed to adult by day 28 (single generation) or day 29 (multiple generations). No significant differences were detected (F = 2.5; df = 3,23; P = 0.09 for day 9 (single generation), F = 0.5; df = 3,24; P = 0.69 for day 10 (multiple generations), $F = 0.7$; df = 3,23; $P = 0.58$ for day 28 (single generation), $F = 1.5$; df = 3,24; $P = 0.23$ for day 29 (multiple generations).

Gelman et al. (2001) found that Colorado potato beetles reared on potato foliage were heavier and oviposited more eggs that those reared on either the potato or the lettuce + potato diets. However, in this study there was no consistent evidence of differences in survival, fecundity, or development time of beetles raised on plants or any of the three artificial diets and then switched back to plants. These results suggest that Colorado potato beetles reared on the artificial diets tested in this study can be used in subsequent tests on foliage without significant carryover effects from either short- or long-term rearing on these artificial diets.

The development of an artificial diet for rearing Colorado potato beetles is an important advance that should lead to significant cost reductions and improvements in rearing methods. Additional modifications in diet recipe and rearing method including aggregate rearing, diet preservation, and additional substitutions to replace components that are costly or difficult to obtain will result in further improvements. Our results show that prior rearing on artificial diets does not affect survivorship, fecundity, or development time when beetles are switched back to potato foliage. Further tests on the effects of artificial diets on Colorado potato beetle performance and on the results of bioassays in which insecticides are incorporated into different artificial diets are underway.

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