ASPECTS OF THE CHEMICAL ECOLOGY OF LYGAEID BUGS
(ONCOPELTUS FASCIATUS AND LYGAEUS KALMII KALMII)
FEEDING ON MILKWEEDS (ASCLEPIAS SPECIES) IN
CENTRAL CALIFORNIA

by

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B.Sc., University of British Columbia, 1975

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We accept this thesis as conforming
to the required standard

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April, 1977
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Date April 29/77
Frontispiece. Adult *Oncopeltus fasciatus* and *Lygaeus kalmii kalmii* (center) on a dehiscent pod of *Asclepias fascicularis* in Napa County, California.
ABSTRACT

A plant-insect allomonal system was investigated, involving seed bugs (Lygaeidae) on milkweeds (*Asclepias* spp.). The ability of the insects to sequester secondary compounds from host plants was studied in detail in central California.

A colorimetric assay was used to quantify the amount of cardenolides (cardiac glycosides) in the lygaeid bugs *Oncopeltus fasciatus* and *Lygaeus kalmii kalmii* and nine species of milkweed host plants. The cardenolide content of individual adult insects, determined in microgram equivalents of digitoxin, varied from zero to over 300 μg per insect. Sources of variation of cardenolide content in the insects included interspecific and intraspecific differences in the cardenolide content of the host plant species, and also differences in the content of plant organs on which insects were feeding.

When reared in the laboratory on a diet of milkweed seeds, the uptake of cardenolides by the bugs was proportional to the cardenolide content of the seeds. However, field collected lygaeids contained fewer cardenolides than were available in the plants on which they were feeding. This suggests that several ecological parameters, such as different reproductive phenologies and morphologies of the different host species, may interfere with the bugs' acquisition of cardenolides.

*O. fasciatus* and *L. k. kalmii* differ in their feeding requirements, host plant utilization, and ability to sequester cardenolides from their hosts. However, the larval growth and development of
O. fasciatus and L. k. kalmii in the laboratory on seeds of different milkweed species did not vary with plant species or cardenolide content of the seeds. Therefore, seeds of all plant species were equally suitable as food sources for the bugs.

The colonization pattern of O. fasciatus on species of Asclepias in north central California suggests that this species does not maximize its opportunities to sequester large quantities of cardenolides from potential hosts. Over 50 per cent of field collected lygaeids in this study contained less than 50 µg of cardenolide. The ecological significance of cardenolide sequestration by lygaeids as a defensive strategy may depend on several modes of pharmacological activity in potential vertebrate predators, not simply emetic potential.

Alternately, the presence of volatile secretions from the scent glands and histamine-like compounds in the dorso-lateral space fluid of O. fasciatus and L. k. kalmii could be more important than sequestered cardenolides as anti-predator strategies.

The data suggest that the effectiveness of cardenolide sequestration as a chemical defense strategy may vary over the geographical range of these insect species, and may vary in the course of a season at a particular location. Therefore, cardenolide sequestration does not appear to be a crucial aspect in the feeding and population ecology of these lygaeid species at least in central California.
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I INTRODUCTION

Many aposematic insects have been hypothesized to serve as models in mimicry rings or complexes because they have the ability to sequester and store bitter-tasting or toxic chemicals from their host plants. These chemicals can act as deterrents to vertebrate predators, thus providing a form of defense which would not be available to the insects had they fed on a food source lacking these compounds (Rothschild 1972).

One such class of chemicals is the cardenolides (cardiac glycosides and their genins), (Figure 1), produced by several families of angiosperms (Singh and Rastogi 1970, Patterson et al. 1975). These heart poisons, which are highly toxic to many vertebrates (Hoch 1961, Detweiler 1967, Thorp and Cobbin 1967), also induce emesis (Parsons and Summers 1971, Borison and Wang 1953), and are very bitter (Reichstein et al. 1968, Brower and Glazier 1975).

Temperate and subtropical milkweeds (Asclepiadaceae) are rich sources of cardenolides (Roeske et al. 1976), and support a well-defined insect fauna (Price 1975, Weiss and Dickerson 1921). It has been established that certain brightly-coloured insects which feed on milkweed at some stage in their life cycle are able to sequester and store cardenolides derived from this group of plants. In some cases, the insects are capable of storing large quantities of these compounds in their bodies, which may have the potential to serve as a chemical defense against vertebrate predators (von Euw et al. 1967, 1971, Duffey and Scudder 1972, Brower 1969, Abushama and Ahmed 1976). Well
FIGURE 1: Structures of some cardenolides.

1. General structure
   (R = one or more sugar moieties in the glycoside;
    R = H in the genin)

2. Calotropin - calactin (configurational isomers)
   (after Roeske et al. 1976)

3. Digitoxin
documented examples of insects which store cardenolides obtained from milkweeds are the monarch butterfly, *Danaus plexippus* L. (Brower 1969) and an African grasshopper, *Poekilocerus bufonius* (Klug) (von Euw et al. 1967).

Brower et al. (1972) and Brower and Moffitt (1974) have shown that there is a wide variation in the cardenolide content of field-collected monarch butterflies. The cardenolide content can be related to the plant species fed upon by the larvae of the butterfly (Brower et al. 1975, Roeske et al. 1976). Further, Brower (1969) and Roeske et al. (1976) have demonstrated that the emetic potential of the monarch butterfly to a vertebrate predator, the blue jay (*Cyanocitta cristata bromia* Oberholser), is determined by the host plant species upon which the larvae fed. The existence of a relationship between host plant species, cardenolide content and emetic potential of a milkweed-feeding insect has thus been well established.

As in the monarch butterfly, the ability of the milkweed bugs *Oncopeltus fasciatus* Dallas and *Lygaeus kalmii kalmii* Stål to sequester and store cardenolides from milkweed plants in the laboratory has been well documented (Duffey and Scudder 1972, 1974). Laboratory studies of *P. bufonius*, *D. plexippus* and the lygaeids *O. fasciatus* and *L. k. kalmii* suggest that none of these insects are capable of synthesizing cardenolides, and so must obtain these chemicals from their host plants (von Euw et al. 1967, Brower 1969, Duffey and Scudder 1972).

Both *O. fasciatus* and *L. k. kalmii* have a close feeding association with members of *Asclepias* L. in temperate North America
(Ralph 1976, Scudder and Duffey 1972, Eggermann and Bongers 1971, Simanton and Andre 1936, Andre 1934). Over thirty of the 108 described species of *Asclepias* in North America have been shown to contain cardenolides (Duffey and Scudder 1972, Roeske *et al.* 1976), and Roeske *et al.* (1976) have documented many examples of intraspecific variation in cardenolide content. It has been suggested that such variation could give rise to a palatability spectrum among lygaeids feeding on these plants (Duffey and Scudder 1972).

In this thesis I have investigated the gross cardenolide content of *O. fasciatus* and *L. k. kalmii* and their hosts (*Asclepias* spp.) in central California, an area with a diverse milkweed flora. The purpose of this study was 1.) to establish a relationship between the cardenolide content of the host species and that of the lygaeids feeding on it; 2.) to investigate the effects of cardenolides on the growth and development of lygaeids; 3.) to determine if cardenolides play a role in the colonization pattern of lygaeids on a milkweed species complex; and 4.) to assess the role of cardenolides in defense strategies of lygaeids.
II MATERIALS AND METHODS

A. Field Study of Milkweeds and Lygaeids

Throughout the summer of 1976, milkweed stands were examined in ten counties in north and central California. Most observations and collections were made in two areas of the state: the north bay area, represented by Yolo, Solano, Napa and Contra Costa counties, and the Fresno area, represented by Fresno and Tulare counties (Figure 2). Many of the locations of milkweed stands were provided by other workers, however, new locations were found during routine collecting trips.

Once a milkweed stand had been located, several characteristics were noted, including approximate number of plants in the population, degree of flowering or fruit production, and the presence or absence of lygaeids and/or other insect herbivores.

As the population dynamics of *O. fasciatus* on milkweed was currently under investigation in northern California (K.E. Evans, unpublished results), no formal attempt was made to follow the phenology and population dynamics of milkweed-feeding lygaeids. However, several milkweed stands in the vicinity of Davis (Yolo Co.) were visited on a weekly basis, and at one location (Woodland, Yolo Co.) the total numbers of *O. fasciatus* adults and larvae were recorded by direct census. This was accomplished by counting all the bugs within a well defined stand, a task aided by the conspicuous coloration and behaviour of these insects (Root and Chaplin 1976).

The fruiting season of different *Asclepias* species was estimated
FIGURE 2: Map of California showing localities where milkweed plants were examined and plant material collected.

"NORTH BAY AREA"
1 Napa County
2 Yolo County
3 Solano County
4 Contra Costa County

"FRESNO AREA"
5 Fresno County
6 Tulare County

SF = San Francisco   LA = Los Angeles

Milkweed species:

- Asclepias fascicularis Dcne.
- A. erosa Torrey
- A. eriocarpa Benth.
- A. californica greenei Woodson
- A. cordifolia (Benth.) Jeps.
- A. vestita vestita Hook & Arn.
- A. speciosa Torrey
- A. solanoana Woodson
- A. curassavica L.
Map of California showing localities where milkweed plants were examined and plant material collected.
by personal observations, field notes (S.P. Lynch, pers. comm.) and by extrapolation from the flowering periods of Asclepias species as given by various authors in the taxonomic literature (Woodson 1954, Munz 1968, Abrams 1951).

B. Extraction of Cardenolides from Plant Material

Plant material was usually collected at locations where insects were taken, to provide direct comparisons between the cardenolide content of the plant and those of the insects where possible. To minimize possible differences when comparing equivalent organs from different milkweed species, it was important to attempt to take plant material of a similar developmental stage. Thus, mature or lower leaves of several individual plants within a stand were collected by cutting whole leaves at the petiole. Umbels were taken in the same manner, when in full bloom only. Seed samples consisted of mature seeds (those from dehiscent pods or those in which the seeds had darkened in color immediately prior to dehiscence) from several pods, usually from different plants. Care was taken not to select samples from plant organs on which lygaeids or other insect herbivores had been directly feeding.

On return to the laboratory, plant material for analysis was kept cool and dry, but was not frozen. Although Roeske et al. (1976) have suggested that cardenolides may be subject to spontaneous breakdown, the results obtained in this thesis suggest that any losses incurred were no greater than those of Roeske et al. (1976) who stored all field collected plant material on dry ice. Plant samples were dried for 12
hours at 80°C in a forced draft oven prior to extraction.

The extraction of cardenolides from dried plant material was based on the procedures of Duffey and Scudder (1972). To maximize the extraction efficiency for quantitative analysis, plant material, especially dry seeds, required mechanical breakdown to increase the exposure of the cardenolide-containing tissues to the extracting solvents.

Milkweed seeds were ground to a fine powder with a commercial grinder (Crescent Dental Mfg. Co.), and 0.2 grams placed in a 12 ml screw-cap test tube. The seed material was extracted with 4 ml of petroleum ether, the test tube being shaken vigorously after addition of the solvent, then allowed to settle for 6 hours. This initial extraction removed large quantities of lipids from the seeds which would otherwise have interfered with the spectroassay. This hydrocarbon extract, removed by pipette, was discarded because it was found to lack cardenolides when tested by both spectrophotometric and thin-layer chromatographic means.

The seed residue was then extracted with 2 ml of hot chloroform-methanol (10:1 by volume), shaken vigorously, and allowed to stand for 12 hours. The chloroform phase was then decanted, and the residue re-extracted with 3 ml of 95 per cent aqueous ethanol for 12 hours. The ethanol extract was added to the chloroform extract, the pooled extracts evaporated to dryness at room temperature and redissolved in 4 ml of 95 per cent ethanol. One-half or 1.0 ml aliquots (depending on the intensity of a preliminary colour reaction) of pooled extract
were used for the spectro assay of cardenolides.

Dried milkweed leaves and umbels contained much less lipid material; direct extraction with ethanol provided sufficiently pure samples. The petroleum ether extraction was therefore unnecessary.

Dried milkweed leaves or umbels were ground to a fine powder in a commercial grinder. Two-tenths of a gram extracted once with 4 ml of hot 95 per cent ethanol were allowed to stand 12 hours before 0.5 of 1 ml aliquots were taken directly for cardenolide estimation. Oxidative degradation of cardenolides was kept to a minimum by analyzing the final extracts within 24 hours.

For leaf and umbel samples, a single estimation of each sample was made. Since the lygaeid bugs O. fasciatus and L. k. kalmii are closely associated with the seeds of milkweed plants, it was important to obtain concise estimations of the cardenolide content of these organs. Therefore, up to five extractions of each seed sample was made.

C. Colorimetric Assay of Cardenolides

The colorimetric assay used was that of Brower et al. (1972) as modified by Brower and Moffitt (1974) and Brower et al. (1975). This assay, first developed by Rabitsch and Tambor (1969) is based on the blue colour formed when 2,4,2',4'-tetranitrodiphenyl (TNDP) is combined with the cardenolide in the presence of a base (-OH ions in solution). The colour is formed by the reaction of the reagents with the unsaturated lactone (butenolide) ring at the C-17 position on the cardenolide genin.

Cardenolide estimations were based on a standard of digitoxin
(Nutritional Biochemical Corp., Cleveland) (Figure 1); all determinations are expressed as equivalents of digitoxin.

Using the procedure outlined by Brower et al. (1972), digitoxin was shown to obey Beer's Law within a range of 10 to 250 micrograms in 4 ml. If the absorbance of a sample indicated a quantity in excess of 250 micrograms, the sample solution was diluted to provide a quantity within this range.

Readings were taken on a Beckman Model B spectrophotometer using Beckman quartz sample cuvettes. The maximum colour intensity was reached after 40 to 45 minutes. At this point the absorbance was recorded, and the concentration calculated from the linear relationship established between concentration and absorbance using the digitoxin standard.

The cardenolide estimations reported here are directly comparable to those of Roeske et al. (1976). The procedures of Roeske et al. (1976) were closely followed to allow such comparisons, as this latter study provides the only quantitative data on the cardenolide content of North American milkweeds of the genus *Asclepias*. The data obtained in the present research are in close agreement with that of Roeske et al. (1976) for plant organs and species which are common to both studies.

D. Extraction of Cardenolides from Lygaeids

Adult *O. fasciatus* and *L. k. kalmii* were collected on milkweed plants (*Asclepias* spp.) in central California between April and September, 1976.

Field-caught lygaeids were maintained in controlled environmental
cabinets at 27°C on a 13.5:10.5 LD light regime, and provided with distilled water and husked sunflower seeds. Field-caught lygaeids were assayed for cardenolides within two weeks of their capture. A control experiment indicated that there was no significant loss of cardenolides in lygaeids feeding on sunflower seeds (which lack cardenolides) for this length of time after having previously fed on a cardenolide-containing diet. Maintaining field-caught insects in the laboratory on sunflower seeds prior to the cardenolide assay had two advantages: first, this period allowed the insects to clear the gut, so that cardenolide estimations would not be influenced by gut contents, and second, reproductive adults could provide eggs for laboratory rearing of lygaeids on milkweed seeds.

Only adult insects were used for the cardenolide assay. Duffey and Scudder (1974) demonstrated that cardenolides are accumulated throughout the juvenile life of the lygaeid *O. fasciatus*, with over half of the adult cardenolide complement sequestered by the fifth and final larval instar. While some quantities of cardenolides are sequestered in the adult stage, the rate of uptake is less than that in the late instar larvae. The collection of only adult insects provides an estimate of the ultimate quantity of cardenolides stored by these milkweed feeding insects under natural conditions, and allowed direct comparisons between different populations of lygaeids.

To investigate the cardenolide content of lygaeids reared exclusively on milkweed seeds, first instar larvae of *O. fasciatus* and *L. k. kalmii* were reared to the adult stage of the seeds of different
milkweed species. The quantities of cardenolide found in these adult bugs were determined and provide an estimate of the ultimate amount that these insects might sequester in the field, had they had an unlimited supply of mature seeds during their larval life. Further, since the milkweed seeds used in this study were subjected to quantitative cardenolide analysis, it was possible to examine the relationship between the cardenolide content of the seed diet and the content of bugs feeding on such seeds. The conditions under which the lygaeids were reared are described in detail in the following section.

Ideally, physiological effects of cardenolide sequestration should be studied by including differing known concentrations of single isolated cardenolides in a standardized, chemically inert diet which could be offered to the insects. This study has been much simpler in a technical sense, because the diet (i.e. milkweed seeds) contains cardenolides in a pre-packaged form, and the insects will readily feed on the seeds. Further, the cardenolides ingested by the bugs from milkweed seeds are in fact those compounds, occurring in the actual concentrations and relative frequencies that the insects would encounter in the natural environment. In light of the fact that the diet led to over a ten-fold difference in cardenolide content of the resultant adult insects, it is felt that the results are valid evidence that *O. fasciatus* are highly tolerant of the compounds as they are encountered in the field.

The method of Scudder & Duffey (1972) was used for extracting cardenolides from insects. Insects, either live or dried, were placed
individually in 2 ml of chloroform-methanol (2:1 by volume), allowed to stand for 24 hr, then removed from this solvent and placed in an additional 2 ml of chloroform-methanol (2:1). After a further 24 hr, the two extracts were pooled, evaporated to dryness, and dissolved in 1 ml of 95 per cent ethanol. One half of this final solution was used for the spectro assay of gross cardenolide content in each insect. More than 95 per cent of an insect's cardenolide complement is extracted using this procedure since repeated extractions failed to produce more than 5 per cent of the total quantity estimated. The spectro assay was identical to that used for estimating gross cardenolide content of plant samples.

Unlike other studies of the cardenolide content of insects, in which the quantities reported have been based on a fixed weight of tissue (Brower et al. 1975, Rothschild et al. 1975) or many insects pooled (von Euw et al. 1967), cardenolide determinations in this study were based on whole individual insects, irrespective of their body weight. Two reasons for estimating the cardenolide content of whole insects were because it allowed an examination of the composition of localized insect populations which in nature consist of individuals of different weights and body sizes, and of more importance, the individual insect is the unit that must be sampled by a predator.

E. Laboratory Rearing of Lygaeids

*O. fasciatus* and *L. k. kalmii* were reared in the laboratory on the seeds of different species of *Asclepias* which they might encounter in the field and seeds of *Helianthus annus* (which lack cardenolides).
This rearing aimed to

1. assess, in a gross sense, the nutritional value of each milkweed species to the insects. These data are related to the pattern of colonization of lygaeids on different milkweed species in the field;

2. determine if differing concentrations of cardenolides in the diet (seeds) affect growth and development of larvae;

3. examine the relationship between cardenolide content in the diet (seeds) and the cardenolide content of insects reared on specified diets (i.e. different milkweed species).

Separate rearing experiments were carried out in 1975 and 1976. In the autumn of 1975, _O. fasciatus_ and _L. k. kalmii_ were reared in an environmental cabinet set at 25±1°C, 60±10% RH, and continuous light. Eggs of _O. fasciatus_ were obtained from a laboratory culture which had been established at least three years earlier. Eggs of _L. k. kalmii_ were obtained from a culture initiated in the summer of 1975 from adults collected at Manning Park and the lower Okanagan Valley, B.C. Larvae were reared in clear plastic petri dishes, approximately 9 cm in diameter, 2 cm in height, and enclosed by a lid with a screened vent, 3 cm in diameter. An excess of milkweed seeds of one species per container was added every three days during larval development; dechlorinated water was constantly available from a section of cotton dental roll which was moistened daily.

Collection dates and localities of the _Asclepias_ seeds utilized are as follows. Collected in September 1975: _A. fascicularis_, Napa Co. Calif., _A. eriocarpa_, Tulare Co., Calif., _A. speciosa_,
Cache Creek, B.C. Seeds of *A. erosa* were collected in September 1974 in Tulare Co. California, and those of *A. cordifolia* collected in July 1974 in Placer Co., California. All seeds were dried at room temperature and stored in plastic bags. Shelled sunflower seeds (*Helianthus*) were purchased locally.

Within 12 hr of hatching ten first instar larvae of one species were placed together in one container; two replicates were established for each different seed diet. The insects were checked every 12 hr for individuals which had moulted to the next larval instar. Those which had moulted were weighed, and the wet weight and time of moulting noted. The insects were then placed back into their original container. The density of insects was kept constant.

In the autumn of 1976, *O. fasciatus* were reared in an environmental cabinet set at 21°C±1, 60±10% RH and a 16:8 LD light regime. This temperature represents the mean ambient temperature at Davis, California between the months of April and September. Eggs of *O. fasciatus* were obtained from the offspring of adults which were collected in Napa County, California in August 1976.

In this experiment, larvae were reared in styrofoam containers approximately 9 cm in diameter, 6 cm in height, and enclosed by a clear plastic lid with a screened vent, 3 cm in diameter.

An excess of milkweed seeds of one species per container was added every five days during larval development, and distilled water was constantly available from a plastic culture tube plugged with cotton, inserted through the side of the rearing container.

As in the previous experiment, within 12 hours of hatching ten first instar larvae were placed in one container, with two replicates established for each seed diet. The larvae were checked every 12 hours for individuals which had moulted from fifth instars into adults. These teneral adults were weighed live, their hemelytra marked with ink, and then replaced in their original containers. Teneral adults were permitted to feed on their respective diets for an additional 10-14 days. They were then frozen, dried for 24 hours at 60°C in a forced draft oven, and kept over dessicant for a further 24 hours before being reweighed.

It was felt that obtaining dry weights of the adult insects might provide more accurate data than wet weights. Further, the additional feeding time in the adult stage allowed the insects to stabilize their body weights, and perhaps their cardenolide contents as well.

All weights were measured on a Mettler Micro Gram-Atic electrobalance. The pronotal width, an index of adult body size, was measured using a dissecting microscope fitted with an eyepiece graticule.

Additional *O. fasciatus* and *L. k. kalmii*, used in cardenolide
determinations, were reared from egg to adult in an environmental
cabinet set at 27°C, 70% RH and a 13.5:10.5 LD light regime (this
cabinet was shared with other researchers who determined the rearing
conditions).

In the figures, error is expressed as ±2 standard errors of
the mean (roughly equivalent to 95 per cent confidence limits). Where
error bars do not overlap, means are considered to be significantly
different.
III RESULTS

A. Milkweed Flora and Lygaeid Fauna

Nine species of *Asclepias* were encountered in central California during the summer of 1976 (Figure 2). Most of the species occurred in areas disturbed by man, viz. roadsides, railway cuts, and in fields lying fallow. Most species are members of semi-arid chaparrel plant communities (Munz 1968); exceptions were the montane *A. cordifolia*, which occurred in yellow pine forest communities, and the relatively rare *A. solanoana*, which was seen on south-facing serpentine slopes only.

The most common species, with the widest range of habitats, was *A. fascicularis*. *A. erosa* which is an economic pest in California, occasionally occurred in large populations on cultivated land.

Different species of *Asclepias* were occasionally found growing in sympatry. On a ranch in Fresno county, *A. v. vestita*, *A. cordifolia*, *A. eriocarpa* and *A. fascicularis* were found within a 200 meter radius.

The lygaeid *O. fasciatus* which immigrates into central California every summer from overwintering sites in the south (probably Mexico), showed two contrasting colonization patterns on species of *Asclepias* in the two basic study areas (Table I). In the north bay area, *O. fasciatus* was only found on *A. fascicularis*. even in locations where other broadleaf milkweeds such as *A. speciosa* and *A. eriocarpa* were growing sympatrically. In contrast, *O. fasciatus* colonized all of the milkweed species in the Fresno area, with the notable exception of *A. cordifolia*. 
TABLE I: Occurrence of *O. fasciatus* and *L. k. kalmii* on different species of *Asclepias* in two areas of California, summer 1976.

<table>
<thead>
<tr>
<th>Milkweed species</th>
<th>North Bay Area (Yolo, Capa, Solano, Contra Costa counties)</th>
<th>Fresno Area (Fresno, Tulare counties)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fascicularis</em></td>
<td>0†</td>
<td>0</td>
</tr>
<tr>
<td><em>A. cordifolia</em></td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td><em>A. eriocarpa</em></td>
<td>L</td>
<td>0</td>
</tr>
<tr>
<td><em>A. californica greenei</em></td>
<td>L</td>
<td>0</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>L</td>
<td>-</td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><em>A. vestita vestita</em></td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

† 0 = *O. fasciatus*, L = *L. k. kalmii*
During 1976 ten stands of *A. cordifolia* were examined in five counties, yet *O. fasciatus* was never observed on this plant. Even where *A. cordifolia* occurred in sympatry with *A. vestita* and *A. eriocarpa* and where both of these latter species supported *O. fasciatus*, none of the insects were found on the *A. cordifolia*.

The timing and duration of flowering periods varies among milkweed species. This leads to temporal differences in the fruiting season of the respective species. Also, flowering and fruiting season varies with elevation by as much as six weeks. *A. cordifolia* and *A. californica greenei* had early, highly synchronized flowering and fruiting seasons, completing the reproductive cycle by releasing the comose seeds in June and July (Figure 3). *A. v. vestita* also had a rather short fruiting season during mid summer, completing its cycle in August. In contrast, *A. fascicularis, A. eriocarpa, A. erosa* and *A. speciosa* did not have synchronized flowering and fruiting periods which led to an extended fruiting season. *A. fascicularis* and *A. erosa* had the longest flowering periods, bearing both flowers and mature seed pods on the same plant as late as mid-September.

At the Woodland *A. fascicularis* site, *O. fasciatus* did not appear until mid-June, and remained in low numbers until the second generation reached its peak in early September (Figure 3). Examinations of populations of *O. fasciatus* at other localities on *A. fascicularis* strongly suggested that this was the pattern of population growth in most of north central California in 1976 (K.E. Evans, unpubl. results). In 1976 the populations of *O. fasciatus* did not reach high densities in
FIGURE 3: Fruiting season in native Californian *Asclepias* species and the population trend of *O. fasciatus* at one milkweed stand.

Stage of fruit development:
- immature
- dehiscent

Milkweed species:
- V = *Asclepias vestita vestita*
- CA = *A. californica greenei*
- SP = *A. speciosa*
- EC = *A. eriocarpa*
- CO = *A. cordifolia*
- ES = *A. erosa*
- F = *A. fascicularis*

As fruiting season can vary with elevation, seasons depicted here are for plants growing at elevations below 500 meters above sea level. *O. fasciatus* population data from a weekly census of a stand of *A. fascicularis* in Woodland, Yolo County. Population trends of *O. fasciatus* on *A. fascicularis* at several localities in Napa county are very similar.
this area until A. cordifolia and A. californica greenei had dispensed most, if not all of their seed crop (Figure 3).

B. Cardenolide Content of Plant Material

The cardenolide content of plant samples of Asclepias from California varies over a wide range. (Tables II, III). Within species variation in cardenolide content of milkweed seeds was about equal to between species variation. Most of the seed samples analysed had cardenolide contents between 1.2 and 4.3 mg per gram of dry tissue. However, three species of Asclepias had cardenolide contents which occur notably outside of this range: These are A. fascicularis (from 0.25 to 0.44 mg per gram) A. v. vestita (7.1 mg per gram for the single sample analysed) and A. curassavica (7.9 mg per gram, single estimation) (Figure 4).

Both temporal and geographical intraspecific differences in the cardenolide content of the milkweed seed occur (Table II). For example, the cardenolide content of seeds of A. erosa, collected in the same area, but in two different years varied considerably, as did the content of seeds of A. californica greenei, collected in the same season from two geographically isolated populations. However, the two samples of A. fascicularis collected in 1975 from different counties had similar cardenolide contents, whereas the 1976 sample from adjacent Yolo county contained a somewhat lower amount of cardenolide than the former two (Table II).

Variation also existed in the cardenolide content of different organs of a single host plant species (Table III). No standard
TABLE II: Gross Cardenolide content of seed of *Asclepias* species from California.

1 county in California and year of collection of milkweed seeds
2 mg equivalents of digitoxin per gram of dried seed
3 for each collection locality, seeds from several plants were pooled and 1.0 g of seed ground to a powder; sample size
   \[ N = \text{number of 0.2 g aliquots taken from the 1 g sample} \]
4 cultivated plants; this species is not native to California
5 Cache Creek, British Columbia, Canada
<table>
<thead>
<tr>
<th>Milkweed Species</th>
<th>Collection Data</th>
<th>Cardenolide Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
</tr>
<tr>
<td>A. californica greenei</td>
<td>Contra Costa</td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>Fresno</td>
<td>1976</td>
</tr>
<tr>
<td>A. cordifolia</td>
<td>Napa</td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>Placer</td>
<td>1974</td>
</tr>
<tr>
<td>A. curassavica</td>
<td>Yolo</td>
<td>1976</td>
</tr>
<tr>
<td>A. eriocarpa</td>
<td>Fresno</td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>Yolo</td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>Fresno</td>
<td>1975</td>
</tr>
<tr>
<td>A. erosa</td>
<td>Tulare</td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>Tulare</td>
<td>1974</td>
</tr>
<tr>
<td>A. fascicularis</td>
<td>Yolo</td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>Napa</td>
<td>1975</td>
</tr>
<tr>
<td></td>
<td>Solano</td>
<td>1975</td>
</tr>
<tr>
<td>A. solanoana</td>
<td>Tehama</td>
<td>1973</td>
</tr>
<tr>
<td>A. speciosa</td>
<td>Solano</td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>B.C.</td>
<td>1975</td>
</tr>
<tr>
<td>A. v. vestita</td>
<td>Fresno</td>
<td>1976</td>
</tr>
</tbody>
</table>
TABLE III: Gross cardenolide content of plant samples of *Asclepias* from California.

Leaf = mature or lower leaves
Umbel = flowers at time of peak flowering
Seed = seeds from dehiscent pods or darkened seeds from pods close to maturity

All plant material collected in summer, 1976.

\(^1\)mg equivalents of digitoxin per gram of dried tissue; data for leaf and umbel represents single estimations

\(^2\)data from TABLE II
<table>
<thead>
<tr>
<th>Milkweed Species</th>
<th>Locality</th>
<th>Leaf</th>
<th>Umbel</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. californica greenei</em></td>
<td>Contra Costa Co.</td>
<td></td>
<td></td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Fresno Co</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. eriocarpa</em></td>
<td>Fresno Co</td>
<td>3.7</td>
<td>5.5</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Fresno Co</td>
<td></td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Yolo Co</td>
<td>1.7</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>Tulare Co</td>
<td>3.0</td>
<td>0.89</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Tulare Co</td>
<td></td>
<td></td>
<td>1.9</td>
</tr>
<tr>
<td><em>A. fascicularis</em></td>
<td>Yolo Co</td>
<td>0.22</td>
<td>0.73</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Fresno Co</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Napa Co</td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td><em>A. v. vestita</em></td>
<td>Fresno Co</td>
<td>6.8</td>
<td>2.6</td>
<td>7.1</td>
</tr>
</tbody>
</table>
FIGURE 4: Gross cardenolide content of seed of Asclepias species from California.

Data from TABLE II; error is expressed as twice the standard error of the mean. Interspecific and intraspecific differences are depicted.
relationship between the relative amounts of cardenolide present and the plant organs occurred, at least in the few species examined here (Figure 4). Umbels contained the greatest concentrations of cardenolides in two of the species examined (A. fascicularis and A. erosa), while the umbels contained the lowest concentrations of cardenolides in two other species examined (A. eriocarpa and A. v. vestita) (Figure 5).

C. Cardenolide Content of Field Collected Lygaeids

There was a wide variation in the cardenolide content of the milkweed bugs O. fasciatus and L. k. kalmii collected on Asclepias species in the field in central California (Table IV). Some insects were found to either lack cardenolides or have levels of cardenolides below the colorimetric detection limit. Other insects were found to contain up to 372 μg of cardenolide per individual. Differences in cardenolide content between samples of lygaeid bugs taken on a particular host species indicated that interspecific differences in the cardenolide content of the host species cannot alone account for the variability in the insects, even between populations feeding on the same plant organ.

Both O. fasciatus and L. k. kalmii sequestered the largest quantities of cardenolides from the three broadleaf milkweed species A. v. vestita, A. erosa and A. eriocarpa (Figures 6,7). Of the five localities where both O. fasciatus and L. k. kalmii were collected and assayed, L. k. kalmii sequestered larger quantities of cardenolides than O. fasciatus in four (Figure 8). In two localities, (near Piedra, Fresno Co. on A. eriocarpa; and near Ducor, Tulare Co. on A. erosa),
FIGURE 5: Gross cardenolide content of plant samples of *Asclepias* from California.

Data from TABLE III.
FIGURE 5

EC eriocarpa
ES erosa
F fascicularis
V vestita

l leaf
u umbel
s seed

plant organ
species

gg per gram DRY TISSUE
TABLE IV: Gross cardenolide content of *O. fasciatus* and *L. k. kalmii* collected on *Asclepias* in central California in 1976.

1Voucher specimens of flowers of the milkweed species are in the possession of the author

2County in California, month of collection, and part of plant on which insects were predominantly feeding

3Expressed as equivalents of digitoxin; NIL = no cardenolides detected, S.E. = standard error of the mean, N = number of insects assayed from site

4 Plants at this locality were taxonomically between *A. v. vestita* and *A. v. parishii* Woodson.
<table>
<thead>
<tr>
<th>Milkweed Species</th>
<th>Collection Data</th>
<th>Cardenolide Content (µg per insect)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean + S.E.</td>
<td></td>
</tr>
<tr>
<td><strong>ONCOPELTUS FASCIATUS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. californica greenei</td>
<td>Fresno May pod</td>
<td>5.5 ± 5.5</td>
<td>6</td>
</tr>
<tr>
<td>A. eriocarpa</td>
<td>Fresno May umbel</td>
<td>20 ± 8.8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Fresno July umbel</td>
<td>107 ± 16</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Fresno August pod</td>
<td>82 ± 8.3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Fresno August stem</td>
<td>15 ± 7.0</td>
<td>6</td>
</tr>
<tr>
<td>A. erosa</td>
<td>Tulare July pod</td>
<td>159 ± 26</td>
<td>9</td>
</tr>
<tr>
<td>A. fascicularis</td>
<td>Napa June umbel</td>
<td>37 ± 7.7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Fresno July pod</td>
<td>61 ± 10</td>
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</tr>
<tr>
<td></td>
<td>Tulare July pod</td>
<td>13 ± 8.3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Yolo August pod</td>
<td>11 ± 2.8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Solano August pod</td>
<td>18 ± 5.4</td>
<td>9</td>
</tr>
<tr>
<td>A. vestita vestita</td>
<td>Fresno May umbel</td>
<td>17 ± 14</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fresno July pod</td>
<td>195 ± 32</td>
<td>9</td>
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<tr>
<td><strong>LYGAEUS KALMII KALMII</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>A. californica greenei</td>
<td>Contra Costa May umbel</td>
<td>5.6 ± 4.3</td>
<td>9</td>
</tr>
<tr>
<td>A. cordifolia</td>
<td>Placer June pod</td>
<td>3.3 ± 1.7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Napa June umbel</td>
<td>NIL</td>
<td>2</td>
</tr>
<tr>
<td>A. eriocarpa</td>
<td>San Luis Obispo June umbel</td>
<td>NIL</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Yolo June umbel</td>
<td>17 ± 8.4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fresno July umbel</td>
<td>152 ± 13</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Fresno July umbel</td>
<td>162 ± 22</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Yolo August umbel</td>
<td>93 ± 23</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Fresno August stem</td>
<td>116 ± 23</td>
<td>9</td>
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<tr>
<td>A. erosa</td>
<td>Tulare July umbel</td>
<td>74 ± 23</td>
<td>9</td>
</tr>
<tr>
<td>A. fascicularis</td>
<td>Tulare August pod</td>
<td>205 ± 31</td>
<td>8</td>
</tr>
<tr>
<td>A. v. vestita</td>
<td>Yolo August pod</td>
<td>5.7 ± 1.4</td>
<td>6</td>
</tr>
<tr>
<td>A. v. vestita</td>
<td>San Luis Obispo June pod</td>
<td>51 ± 23</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fresno July pod</td>
<td>256 ± 35</td>
<td>9</td>
</tr>
</tbody>
</table>
FIGURE 6: Gross cardenolide content of field collected

0. fasciatus.

CA = Asclepias californica greenei
EC = A. eriocarpa
ES = A. erosa
F = A. fascicularis
V = A. v. vestita

Data from TABLE IV.
FIGURE 6

ONCOPELTUS FASCIATUS

m may
jn june u umbel
jl july p pod
a august s stem

μg per insect

species CA EC ES F V
organ p u u p s p u p p p p u p
month m m jl a a jl jl a a m jl
FIGURE 7: Gross cardenolide content of field collected

L. k. kalmii.

CA = Asclepias californica greenei
CO = A. cordifolia
EC = A. eriocarpa
ES = A. erosa
F = A. fascicularis
V = A. v. vestita

Other symbols (for plant organs, month of collection) as in FIGURE 6.
FIGURE 8: Comparison of gross cardenolide contents of *O. fasciatus* and *L. k. kalmii* collected from the same localities.

F = *Asclepias fascicularis*
ES = *A. erosa*
EC = *A. eriocarpa*
V = *A. v. vesita*
p = dehiscent pods and seed
ip = immature pods
u = umbels
s = stems

Date from TABLE IV.
L. k. kalmii sequestered significantly more cardenolide than did O. fasciatus.

There is evidence of temporal variation in the cardenolide content of the O. fasciatus (Table V). Adults of O. fasciatus collected in Fresno county in May from the three broadleaf milkweed species A. californica greenei, A. eriocarpa and A. v. vestita contained small quantities of cardenolides. However, adult insects collected on A. eriocarpa and A. v. vestita later in the season from the same localities had much higher levels of cardenolides in their bodies. On the other hand, this trend did not exist among adults of O. fasciatus collected on A. fascicularis from June through August. Therefore, if temporal variation in the cardenolide content of lygaeids is the result of some aspect of the phenologies of their respective hosts (e.g. availability of pods and seed), this situation is limited to the cardenolide-rich broadleaf species of milkweed in California. In general, both O. fasciatus and L. k. kalmii collected in May and June contained substantially lower levels of cardenolides than conspecifics taken later in the season on the same host species (Figures 6 and 7).

The frequency distribution of cardenolide content in all the field-collected lygaeids in this study is strongly skewed toward the left, or low end of the X-axis (Figure 9). Of the 211 individual lygaeids assayed, 52 per cent of those had less than 50 micrograms of cardenolide in their bodies, and 71 per cent had less than 100 micrograms. The overall range in cardenolide content of the field-collected lygaeids fell within the range of cardenolide contents in laboratory-reared bugs.
TABLE V: Temporal variation in gross cardenolide content of field caught *O. fasciatus* from Central California.

<table>
<thead>
<tr>
<th>Milkweed Species</th>
<th>May-June</th>
<th>July-August</th>
<th>Lab Reared</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. californica greenei</em></td>
<td>5.5</td>
<td>-</td>
<td>251</td>
</tr>
<tr>
<td><em>A. eriocarpa</em></td>
<td>20</td>
<td>107, 82</td>
<td>149</td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>-</td>
<td>159</td>
<td>151</td>
</tr>
<tr>
<td><em>A. fascicularis</em></td>
<td>37</td>
<td>61, 13, 11, 18</td>
<td>34</td>
</tr>
<tr>
<td><em>A. v. vestita</em></td>
<td>17</td>
<td>195</td>
<td>281</td>
</tr>
</tbody>
</table>

1 mean in µg equivalents of digitoxin per insect
2 data from Table IV
3 reared from egg to adult exclusively on seed from respective milkweed species; data from Table VI.
FIGURE 9: Gross cardenolide content of field collected lygaeids: distribution of classes based on cardenolide content.

Total sample size of 211 represents all individual insects used to formulate date in Table IV. While not a random sample of lygaeids on milkweed in California, these insects constitute a representative sample owing to the disproportionate numbers of insects on different Asclepias species.

Of the total sample, 52 per cent contained less than 50 µg per insect, and 71 per cent contained less than 100 µg per insect.
D. Cardenolide Content of Laboratory-Reared Lygaeids

Table VI shows analyses of the cardenolide content of lygaeids reared from egg to adult in the laboratory on seed from different species of *Asclepias* at 25°C. *O. fasciatus* and *L. k. kalmii* sequester and store small quantities of cardenolides from *A. fascicularis*, moderate amounts from *A. erosa, A. cordifolia* and *A. eriocarpa*, and relatively large quantities from *A. californica greenei* and *A. v. vestita*. There is a positive correlation between cardenolide content of laboratory reared insects and the cardenolide content of the seeds of their respective host species (Figure 10, Table VI).

*L. k. kalmii* appears to be more efficient at sequestering these compounds as it stored larger quantities of cardenolides than *O. fasciatus* when both insects were reared on the same seed diet (Figure 10). Similarly, *O. fasciatus* reared at 21°C was more efficient at sequestering cardenolides than those reared at 25°C (Figure 10).

When the data for adult *O. fasciatus* reared at 21°C are examined in detail, it is apparent that the cardenolide content of adult bugs is significantly different between the sexes in all but one milkweed seed diet (Table VII). Females were more efficient at sequestering cardenolides than males on all eight species of *Asclepias* (Figure 11).

E. Larval Growth and Development of Lygaeids on Different Species of Milkweed in the Laboratory

Both *O. fasciatus* and *L. k. kalmii* were successfully reared from eggs to adults on a diet of milkweed seeds from several different species of *Asclepias* as well as *Helianthus* (Compositae). None of the milkweed
TABLE VI: Gross cardenolide content of O. fasciatus and L. k. kalmii reared in the laboratory on milkweed seed.

<table>
<thead>
<tr>
<th>Milkweed Species</th>
<th>Collection Data</th>
<th>Collection Data</th>
<th>O. fasciatus mean + SE</th>
<th>L. k. kalmii mean + SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. californica greenei</td>
<td>Contra Costa 1976</td>
<td>4.3</td>
<td>251 ± 29</td>
<td>9</td>
</tr>
<tr>
<td>A. cordifolia</td>
<td>Placer 1974</td>
<td>1.7</td>
<td>125 ± 19</td>
<td>9</td>
</tr>
<tr>
<td>A. eriocarpa</td>
<td>Fresno 1975</td>
<td>3.4</td>
<td>149 ± 13</td>
<td>9</td>
</tr>
<tr>
<td>A. erosa</td>
<td>Tulare 1974</td>
<td>1.9</td>
<td>151 ± 23</td>
<td>9</td>
</tr>
<tr>
<td>A. fascicularis</td>
<td>Napa 1975</td>
<td>0.4</td>
<td>34 ± 5.1</td>
<td>9</td>
</tr>
<tr>
<td>A. vestita vestita</td>
<td>Fresno 1976</td>
<td>7.1</td>
<td>281 ± 20</td>
<td>18</td>
</tr>
</tbody>
</table>

1 Californian collection data for milkweed seed fed to insects.
2 mg equivalents of digitoxin per gram of dried seed; data from Table II.
3 μg equivalents of digitoxin per insect: N = no. of insects in each sample, S.E. = standard error of the mean.
FIGURE 10: Relationship between cardenolide concentration in the diet (milkweed seeds) and the cardenolide content of adult O. fasciatus and L. k. kalmii.

Insects assayed for cardenolides were reared from egg to adult exclusively on seeds of respective species of Asclepias. Values for sexes combined.

Insect data from Tables VI and VIII, seed data from Table II; \( r \) = correlation coefficient, \( m \) = slope. Differences between the sexes (Figure 11) contribute to the large standard errors.

- O. fasciatus reared at 21°C
- O. fasciatus reared at 25°C
- L. k. kalmii reared at 25°C

O. fasciatus sequesters more cardenolides at 21°C owing to a longer duration of larval development (approx. 40 days to the adult molt) compared to conspecifics reared at 25°C (approx. 22 days to the adult molt). L. k. kalmii appears to be more efficient at sequestering cardenolides than O. fasciatus at the same temperature.
TABLE VII: Gross cardenolide content of *O. fasciatus* reared in the laboratory at 21°C on milkweed seed.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Seed¹ mean ± S.E.</th>
<th>N</th>
<th>Adult <em>O. fasciatus</em>²,³ mean ± S.E.</th>
<th>♂♀ mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fascicularis</em></td>
<td>0.25 ± 0.03</td>
<td>3</td>
<td>42.3 ± 2.5</td>
<td>48.7 ± 3.8⁴</td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>2.4 ± 0.07</td>
<td>3</td>
<td>139 ± 20</td>
<td>204 ± 14</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>2.7 ± 0.15</td>
<td>3</td>
<td>151 ± 20</td>
<td>250 ± 14</td>
</tr>
<tr>
<td><em>A. cordifolia</em></td>
<td>3.1 ± 0.15</td>
<td>3</td>
<td>152 ± 27</td>
<td>363 ± 30</td>
</tr>
<tr>
<td><em>A. eriocarpa</em></td>
<td>3.4 ± 0.57</td>
<td>3</td>
<td>75 ± 6.7</td>
<td>158 ± 9.3</td>
</tr>
<tr>
<td><em>A. californica</em></td>
<td>4.3 ± 0.40</td>
<td>5</td>
<td>215 ± 5.6</td>
<td>387 ± 32</td>
</tr>
<tr>
<td><em>A. vestita</em></td>
<td>7.1 ± 0.29</td>
<td>5</td>
<td>291 ± 81</td>
<td>649 ± 58</td>
</tr>
<tr>
<td><em>A. curassavica</em></td>
<td>7.9 ± 0.31</td>
<td>4</td>
<td>386 ± 23</td>
<td>707 ± 51</td>
</tr>
</tbody>
</table>

¹mg per gram of dried seed; data from Table II  
²ug per insect  
³N = 3 for both sexes  
⁴difference between sexes not significant
FIGURE 11: Relationship between cardenolide concentration in the diet (milkweed seeds) and the cardenolide content of adult male and female *O. fasciatus* reared at 21°C.

Data from Table VII. $r =$ correlation coefficient, $m =$ slope.
FIGURE 11

- female
- male

\[ r = +0.966 \]
\[ m = +92.0 \]

\[ r = +0.858 \]
\[ m = +42.5 \]
species tested appear to present a nutritional barrier to the growth and development of *O. fasciatus* and *L. k. kalmii*.

When reared at 25°C, *O. fasciatus* completed larval development significantly faster on seeds of *A. fascicularis* than on the other milkweed seeds provided (Figure 12). There were no significant differences among these bugs when reared on seeds of other milkweeds, viz. *A. erosa*, *A. cordifolia*, *A. eriocarpa* and *A. speciosa* (Table VIII). Survival to the adult stage was 75 per cent or greater on all diets. However, developmental duration of the larvae was significantly longer for bugs reared on seeds of *Helianthus annus*.

The small milkweed bug *L. k. kalmii* also developed faster on the seeds of *A. fascicularis* than on the seeds of other milkweeds. In contrast to *O. fasciatus*, *L. k. kalmii* developed significantly faster on sunflower seeds than on the milkweed seeds with the exception of *A. fascicularis* (Figure 12). Survival to the adult stage on all diets was lower than that of *O. fasciatus* (Table IX).

At 21°C there were few significant differences in the larval developmental times of *O. fasciatus* reared on eight different species of *Asclepias* (Table X). As the cardenolide content of these insects had been determined (Table VII), it was possible to compare the cardenolide content of the insects with the larval duration. In this study, no significant correlation was found between these two indices (Table XIV, Figure 14).

There were no significant differences in the wet weights of teneral *O. fasciatus* of one sex when reared on the five different species
FIGURE 12: Relationship between duration of larval development and teneral wet weight of *O. fasciatus* and *L. k. kalmii* reared in the laboratory at 25°C on seeds of different species of *Asclepias* and *Helianthus*.

*Asclepias* spp. *H. annus*

- • ☢️ *O. fasciatus*
- ○ • *L. k. kalmii*

Data from Tables VIII and IX:

- f *A. fascicularis*
- es *A. erosa*
- c *A. cordifolia*
- ec *A. eriocarpa*
- s *A. speciosa*
FIGURE 12

WET WEIGHT (mg)

LARVAL DURATION (days)
TABLE VIII: Duration of larval development and teneral adult wet weight of *O. fasciatus* reared in the laboratory at 25°C on the seeds of different species of *Asclepias* and *Helianthus*.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Developmental Duration (days)</th>
<th>Wet Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± S.E.</td>
<td>N</td>
</tr>
<tr>
<td><strong>A. cordifolia</strong></td>
<td>21.9 ± 0.28</td>
<td>19</td>
</tr>
<tr>
<td><strong>A. eriocarpa</strong></td>
<td>21.7 ± 0.37</td>
<td>18</td>
</tr>
<tr>
<td><strong>A. erosa</strong></td>
<td>22.4 ± 0.17</td>
<td>19</td>
</tr>
<tr>
<td><strong>A. fascicularis</strong></td>
<td>19.5 ± 0.12</td>
<td>16</td>
</tr>
<tr>
<td><strong>A. speciosa</strong></td>
<td>22.9 ± 0.63</td>
<td>17</td>
</tr>
<tr>
<td><strong>H. annus</strong></td>
<td>24.4 ± 0.61</td>
<td>15</td>
</tr>
</tbody>
</table>

*N = total number of insects completing development to the adult stage (both sexes combined)*

See materials and methods (Section D) for collection data for milkweed seeds.

Survival is the percentage of larvae that successfully complete the final molt into the adult stage. Teneral adults were weighed within 12 hours of the adult molt.
TABLE IX: Duration of larval development and teneral adult wet weight of *L. k. kalmii* reared in the laboratory at 25°C on the seeds of different species of *Asclepias* and *Helianthus*.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Developmental Duration (days) mean ± S.E.</th>
<th>N</th>
<th>Survival (%)</th>
<th>Wet Weight (mg) σ+♀ mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. cordifolia</em></td>
<td>28.5 ± 0.16</td>
<td>16</td>
<td>80</td>
<td>43.6 ± 1.0</td>
</tr>
<tr>
<td><em>A. eriocarpa</em></td>
<td>29.6 ± 1.6</td>
<td>6</td>
<td>30</td>
<td>44.1 ± 2.9</td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>29.3 ± 0.88</td>
<td>14</td>
<td>70</td>
<td>47.2 ± 1.7</td>
</tr>
<tr>
<td><em>A. fascicularis</em></td>
<td>25.8 ± 0.12</td>
<td>17</td>
<td>85</td>
<td>44.1 ± 0.8</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>29.1 ± 1.5</td>
<td>13</td>
<td>65</td>
<td>41.6 ± 1.6</td>
</tr>
<tr>
<td><em>H. annus</em></td>
<td>26.5 ± 0.43</td>
<td>16</td>
<td>80</td>
<td>42.9 ± 1.4</td>
</tr>
</tbody>
</table>

N = total number of insects completing development to the adult stage (both sexes combined).

See materials and methods (section D) for collection data for milkweed seeds.

Survival is the percentage of larvae that successfully completed the final molt to the adult stage. Teneral adults were weighed within 12 hours of the adult molt.
TABLE X: Influence of sequestered cardenolides on larval development of *O. fasciatus* reared in the laboratory at 21°C on the seeds of *Asclepias*.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Cardenolide Content&lt;sup&gt;1,2&lt;/sup&gt; mean ± S.E.</th>
<th>Developmental Duration (days) mean ± S.E.</th>
<th>Survival&lt;sup&gt;3&lt;/sup&gt; N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fascicularis</em></td>
<td>45.5 ± 3.5</td>
<td>39.3 ± 0.28</td>
<td>20</td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>171 ± 18</td>
<td>40.5 ± 0.27</td>
<td>18</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>200 ± 25</td>
<td>39.2 ± 0.37</td>
<td>19</td>
</tr>
<tr>
<td><em>A. cordifolia</em></td>
<td>258 ± 51</td>
<td>40.3 ± 0.26</td>
<td>16</td>
</tr>
<tr>
<td><em>A. eriocarpa</em></td>
<td>117 ± 19</td>
<td>40.5 ± 0.33</td>
<td>18</td>
</tr>
<tr>
<td><em>A. californica greenei</em></td>
<td>301 ± 41</td>
<td>40.3 ± 0.20</td>
<td>18</td>
</tr>
<tr>
<td><em>A. v. vestita</em></td>
<td>470 ± 92</td>
<td>40.7 ± 0.25</td>
<td>19</td>
</tr>
<tr>
<td><em>A. curassavica</em></td>
<td>547 ± 76</td>
<td>39.8 ± 0.25</td>
<td>18</td>
</tr>
</tbody>
</table>

<sup>1</sup>μg per adult insect

<sup>2</sup>data for both sexes combined, N = 6 (data from Table VII)

<sup>3</sup>survival is the percentage of larvae that successfully completed the adult molt

See materials and Methods (section D) for collection data for milkweed seeds.
of Asclepias at 25°C (Table XIII). However, there were significant differences between the sexes when reared on the same diet, the females being significantly heavier than their male counterparts on all the diets tested. The ratio of mean female wet weight to mean male wet weight for O. fasciatus in this study fell within the range of 1.2:1 to 1.3:1.

Oncopeltus fasciatus reared on sunflower seeds were significantly lighter than those reared on the different milkweed seeds (Figure 12). The female to male weight ratio was similar to that of O. fasciatus reared on the milkweed seed diets.

Like O. fasciatus, there were no significant differences in teneral wet weight of L. k. kalmii reared on the different species of Asclepias (Table IX). Differences between the sexes were not investigated.

In contrast to O. fasciatus, teneral adults of L. k. kalmii reared on sunflower seeds are no lighter than conspecifics reared on milkweed seeds (Figure 12). This finding, together with the relatively rapid larval development of L. k. kalmii on sunflower seeds, strongly indicate that this insect is capable of better growth and development on this non-asclepidaceous host than is O. fasciatus. However, O. fasciatus develops faster and attains greater body weights at all larval instars compared to L. k. kalmii when both are reared on the seeds of A. fascicularis under the same conditions (Figure 13).

When O. fasciatus was reared on eight different species of Asclepias at 21°C, there were some significant differences between teneral wet weights within the sexes (Table XI). However, all
FIGURE 13: Weight gain in O. fasciatus and L. k. kalmii during larval development, reared in the laboratory at 25°C on the seeds of A. fascicularis.

A. fascicularis seed collected in Napa county in September, 1975.
FIGURE 13

- *O. fasciatus*
- *L. k. kalmii*

Age (days) vs. Wet Weight (mg)
FIGURE 14: Relationship between gross cardenolide content and duration of larval development of *O. fasciatus* reared in the laboratory at 21°C on the seeds of different species of *Asclepias*.

Data from Table X; m = slope.
TABLE XI: Adult body weight of *D. fasciatus* reared in the laboratory at 21°C on the seeds of different species of *Asclepias*.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Teneral Wet Weight (mg)</th>
<th></th>
<th>Adult Dry Weight (mg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± S.E.</td>
<td>n</td>
<td>mean ± S.E.</td>
<td>n</td>
</tr>
<tr>
<td><em>A. fascicularis</em></td>
<td>58.6 ± 1.5</td>
<td>14</td>
<td>68.2 ± 3.6</td>
<td>6</td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>59.9 ± 1.7</td>
<td>6</td>
<td>68.3 ± 2.4</td>
<td>12</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>57.9 ± 1.4</td>
<td>11</td>
<td>68.6 ± 5.0</td>
<td>8</td>
</tr>
<tr>
<td><em>A. cordifolia</em></td>
<td>60.7 ± 1.4</td>
<td>9</td>
<td>72.3 ± 3.3</td>
<td>7</td>
</tr>
<tr>
<td><em>A. eirocarpa</em></td>
<td>60.2 ± 1.8</td>
<td>13</td>
<td>76.7 ± 1.6</td>
<td>4</td>
</tr>
<tr>
<td><em>A. californica greenii</em></td>
<td>62.1 ± 1.5</td>
<td>7</td>
<td>75.0 ± 1.2</td>
<td>11</td>
</tr>
<tr>
<td><em>A. v. vestita</em></td>
<td>57.3 ± 1.2</td>
<td>10</td>
<td>73.3 ± 1.7</td>
<td>9</td>
</tr>
<tr>
<td><em>A. curassavica</em></td>
<td>53.2 ± 1.2</td>
<td>10</td>
<td>64.5 ± 2.0</td>
<td>8</td>
</tr>
</tbody>
</table>

1 bracket ends join significantly different means

2 no significant difference between sexes

Teneral wet weight determined within 12 hours of the adult molt. Adult dry weight determined between 10 and 14 days following the adult molt.
differences were owing to the light weights of both males and females reared on _A. curassavica_. As in the 25°C rearing experiment, females were significantly heavier than males on all diets tested. Adult dry weights yielded the same relationship as the teneral wet weights. There were no significant correlations between teneral wet weight or adult dry weight and cardenolide content for either sex (Table XIV).

Ratios of mean female wet weight to mean male wet weight were similar to those in the 25°C rearing experiment, falling within a range from 1.14 to 1.28 (Table XI).

Measurement of adult pronotal width showed no significant differences for insects of the same sex reared on the different diets (Table XII). Thus, there were no correlations between pronotal width and cardenolide content of the adult insects (Table XIV). Females were significantly larger than males on all diets.

In most group rearings, females were significantly heavier, larger, and contained more cardenolide per insect than the males (Tables XI, XII, VII, Figures 15, 11). The differences between the sexes in wet weight of tenerals does not appear to be related to sex differences in cardenolide content of the adult insects. _O. fasciatus_ reared on sunflower seeds exhibit a female to male weight ratio equal to that of milkweed-reared conspecifics which varied with respect to cardenolide content (Table XIII).

As there were no significant correlations between cardenolide content and body weight, body size or larval developmental duration (Table XIV), it appears that there are no measurable detrimental affects
FIGURE 15: Relationship between gross cardenolide content and adult body weight of *O. fasciatus* reared in the laboratory at 21°C on the seeds of different species of *Asclepias*.

WET = teneral wet weight (within 12 hours of the adult molt)

DRY = adult dry weight (10 to 14 days following the adult molt).

Data from Table XI; m = slope.
TABLE XII: Pronotal width of adult *O. fasciatus* reared in the laboratory at 21°C on the seeds of different species of *Asclepias*.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>♂ mean ± S.E.</th>
<th>♀ Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fascicularis</em></td>
<td>3.7 ± .06</td>
<td>4.1 ± .04</td>
</tr>
<tr>
<td><em>A. erosa</em></td>
<td>3.6 ± .04</td>
<td>4.1 ± .04</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>3.6 ± .06</td>
<td>3.9 ± .08</td>
</tr>
<tr>
<td><em>A. cordifolia</em></td>
<td>3.6 ± .03</td>
<td>4.0 ± .06</td>
</tr>
<tr>
<td><em>A. eriocarpa</em></td>
<td>3.6 ± .06</td>
<td>4.1 ± .05</td>
</tr>
<tr>
<td><em>A. californica</em></td>
<td>3.7 ± .06</td>
<td>4.1 ± .04</td>
</tr>
<tr>
<td><em>A. vestita</em></td>
<td>3.6 ± .06</td>
<td>4.0 ± .09</td>
</tr>
<tr>
<td><em>A. curassavica</em></td>
<td>3.6 ± .04</td>
<td>4.0 ± .05</td>
</tr>
</tbody>
</table>

\(^1\)N = 5 for all samples except *A. eriocarpa* reared females (N = 4).
TABLE XIII: Comparison of female to male ratios for teneral wet weight and cardenolide content of *O. fasciatus* reared on different seed diets.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Mean Teneral Wet Weight (mg)</th>
<th>Mean Cardenolide Content $^3$</th>
<th>Φ:♂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♀</td>
<td>♂</td>
<td>♀:♂</td>
</tr>
<tr>
<td>A. cordifolia</td>
<td>60.7</td>
<td>72.3$^1$</td>
<td>1.19</td>
</tr>
<tr>
<td>A. curassavica</td>
<td>53.2</td>
<td>64.5$^1$</td>
<td>1.21</td>
</tr>
<tr>
<td>Helianthus annus</td>
<td>45.9</td>
<td>55.6$^2$</td>
<td>1.21</td>
</tr>
</tbody>
</table>

$^1$ Data from Table XI

$^2$ Reared at 25°C; data from Table VIII

$^3$ μg per insect; data from Table VII

$^4$ NIL = no cardenolides detected
TABLE XIV: Correlations between mean cardenolide content and means of body weight, body size and larval duration in *O. fasciatus*.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Cardenolide content of insects vs. cardenolide content in diet</td>
<td>+0.789</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>B. Larval duration vs. cardenolide content (insects)</td>
<td>+0.264</td>
<td>p &gt; .50</td>
</tr>
<tr>
<td>C. Teneral wet weight vs. cardenolide content:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>males</td>
<td>-0.647</td>
<td>.10 &gt; p &gt; .05</td>
</tr>
<tr>
<td>females</td>
<td>-0.163</td>
<td>p &gt; .50</td>
</tr>
<tr>
<td>D. Adult dry weight vs. cardenolide content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>males</td>
<td>-0.482</td>
<td>.50 &gt; p &gt; .20</td>
</tr>
<tr>
<td>females</td>
<td>-0.172</td>
<td>p &gt; .50</td>
</tr>
<tr>
<td>E. Adult pronotum width vs. cardenolide content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>males</td>
<td>-0.288</td>
<td>.50 &gt; p &gt; .20</td>
</tr>
<tr>
<td>females</td>
<td>-0.405</td>
<td>.50 &gt; p &gt; .20</td>
</tr>
</tbody>
</table>

*r* = correlation coefficient
of cardenolide sequestration on the larval growth and development of O. fasciatus.
IV DISCUSSION

The ability of the lygaeid bugs *O. fasicatus* and *L. kalmii* to sequester and store cardenolides (cardiac glycosides) from their asclepiadaceous hosts has been well documented (Duffey and Scudder 1972, 1974); this ability is characteristic of many brightly coloured members of the Lygaeinae (Scudder and Duffey 1972, von Euw et al. 1971, Abushama and Ahmed 1976). Further, many species of *Asclepias*, and other natural hosts of North American Lygaeines have been shown to contain cardenolides (Roeske et al. 1976, Duffey and Scudder 1972). Together with the well known pharmacological effects of cardiac glycosides (Moe and Farah 1970, Hoch 1961, Thorp and Cobbin 1967), the hypothesis of a palatability spectrum based on plant-derived cardenolides in these insects appears to be well-founded. This thesis has demonstrated that a broad spectrum exists in the cardenolide contents of *O. fasicatus* and *L. kalmii* kalmii feeding on *Asclepias* in central California.

In terms of both values and variability, the data presented here for Californian species of *Asclepias* are largely in agreement with those of Roeske et al. (1976). As such, the comparisons between cardenolide content of plants and insects in this thesis, and between insects in this thesis and other reports (e.g. Brower et al. 1975, Roeske et al. 1976) are considered to be valid.

It should be realized that in collecting adult insects in the field the host plant experience of these insects in their larval stages remains uncertain. However, in this study the presence of soft-bodied
adults together with late instar larvae feeding on the same seed pod has been taken to indicate that the adults collected from such a site for analysis represent insects which developed exclusively on that food source.

The quantities of cardenolides in field-collected lygaeids were often much smaller than those predicted by extrapolating from the relationship between the quantity in the diet and the quantity sequestered in laboratory-reared bugs. Thus, there appear to be ecological parameters in the lygaeid-milkweed association which limit, or interfere with the insects' opportunity to sequester optimum quantities of cardenolides from their potential hosts.

The ground-dwelling lygaeid *L. k. kalmii* overwinters in and near milkweed stands, feeding on seed on the ground, as well as the seeds of some non-asclepiadaceous plants, and dead insects (R.B. Root, pers. comm.). Among the winter hosts is the cardenolide-containing *Nerium oleander*, a ubiquitous cultivated plant in central California. In the early spring, before milkweeds have begun to sprout, *L. k. kalmii* reproduces on the seeds of other plants, such as the common groundsel *Senecio vulgaris*, which lacks cardenolides (K.E. Evans, pers. comm.). Although this lygaeid can successfully develop and reproduce on plants other than milkweed, milkweed seeds enhance larval development (R.B. Root, pers. comm.). *L. k. kalmii* colonizes most of the species of *Asclepias* in California, but remains at low densities until the seed pods develop late in the season. Because they have the dietary flexibility to develop in the absence of milkweed plants and seed, a portion of the
overall population of these bugs may not have the opportunity to sequester and store cardenolides.

The fact that L. k. kalmii is more efficient than O. fasciatus at sequestering cardenolides from plant material may have permitted this species to exploit a wider range of host species, including those which do not provide the insect with cardenolides. Since cardenolides may be retained in the dorso-lateral spaces of these insects for a period of weeks (experimental evidence undescribed in this thesis), a short exposure to a cardenolide-rich diet combined with efficient sequestration might allow this insect to build a considerable cardenolide store which is maintained while the insect exploits alternate host species.

However, the field data show that L. k. kalmii collected in the late spring on milkweed plants of several species, either contained small quantities of cardenolides, or lacked these compounds altogether. This finding suggests that these individuals either lost their store of cardenolides obtained before the winter, or are the offspring of overwintering adults which deposited eggs on non-asclepiadaceous hosts.

In contrast, the host plant range of O. fasciatus in temperate North America is restricted to members of the genus Asclepias (Ralph 1976). In California this lygaeid has been seen on five species of milkweed (A. fascicularis, A. erosa, A. eriocarpa, A. vestita vestita and A. californica greenei), and the laboratory-rearing experiments suggest that at least two other species (A. speciosa and A. cordifolia) will support larval development. O. fasciatus is known to colonize
A. speciosa in other parts of this insect's geographical range (Andre 1934).

O. fasciatus is a long distance migrant species, recolonizing milkweed stands in California every summer from its sites of overwintering in the south (perhaps Mexico). The host-plant associations of O. fasciatus at its sites of overwintering are unknown. If the adults which immigrate into California had developed on milkweed plants prior to their long dispersal flights, one would expect to find moderate quantities of cardenolides in these insects. However, the first arriving colonizers at all milkweed stands sampled contained small quantities of cardenolides. Further, comparison of thin-layer chromatographic profiles of cardenolides between the early colonizers and laboratory-reared insects suggest that these small quantities of cardenolides were accumulated on the plants on which the insects were collected, rather than on some overwintering host: the cardenolide profiles of insects were identical to profiles of bugs reared in the laboratory on the Californian species of Asclepias. This suggests that the overwintering hosts either lack cardenolides, or have very small quantities (cf. A. fascicularis).

Unlike populations of O. fasciatus in eastern North America, early colonizing O. fasciatus in California often arrive at milkweed stands before developing pods and seeds are available (Ralph 1976, K.E. Evans, unpubl. results, pers. observ.). Where this insect arrives in advance of the fruiting season of its host, it is able to reproduce and leave offspring which must develop on plant organs other than pods. However, it is only the seeds of the milkweed plant which support optimal growth and development of larvae (Ralph 1976), and populations of bugs only increase at a rapid rate when mature pods and seed are
present.

Determination of the cardenolide content of laboratory seed-reared *O. fasciatus* and bugs collected on the same hosts in the field shows that *O. fasciatus* can usually sequester moderate to large quantities of cardenolides from some of their host species during the fruiting season. However, the laboratory seed-reared insects often contain more cardenolide than insects collected in the field, suggesting that bugs in the natural environment may not feed exclusively on seed, even when it is available. Lygaeids obtain water by tapping the pod wall, leaves, and stems of milkweed plants and ingesting the milky sap. In so doing, they probably receive some nutrients in the sap, which may not contain the same concentrations of cardenolides as the seeds. Alternately, the thick pod wall of several species of *Asclepias* in California (particularly *A. erosa*, *A. v. vestita*, *A. californica greenei* and *A. speciosa*) may prevent lygaeids from feeding on the seeds until the earliest maturing pods begin to dehisce (Ralph 1976). Further, the period of time in which *O. fasciatus* can feed directly on seeds can be severely limited on milkweed species which have highly synchronous pod dehiscence and/or rapid seed dispersal from dehiscing pods. All of these factors probably limit the ability of both *O. fasciatus* and *L. k. kalmii* to sequester quantities of cardenolides within the physiological limits of the insects.

Before pods and seed are available, *O. fasciatus* feeds almost exclusively on the developing flower buds and open flowers (Brown et al. 1976, Evans, unpubl. results, pers. observ.). This study has shown that
for at least four species of *Asclepias* in California, the umbels (buds and flowers) contain relatively large quantities of cardenolides, yet in most samples, the bugs did not sequester proportional quantities of cardenolides from these sources. Ralph (1976) reported that *O. fasciatus* feeding on flowers of *A. syriaca* did not ingest material from the ovaries, but instead fed primarily on the nectar, which may lack cardenolides entirely. Thus, the mode of feeding of *O. fasciatus* might make the availability of seed of the host plant critical for the sequestration of cardenolides.

*L. k. kalmii* has been shown to sequester greater quantities of cardenolides from the umbels of milkweeds than *O. fasciatus*. Whether this is a reflection of the higher sequestration efficiency of *L. k. kalmii*, or a more efficient mode of feeding than *O. fasciatus* requires more detailed investigation.

As previously indicated, *L. k. kalmii* was seen on all but one of the nine species of *Asclepias* examined (*A. solanoana* being the exception), and was represented by at least some individuals at almost every milkweed stand visited. The largest numbers of bugs were seen at stands of *A. fascicularis*, *A. cordifolia* and *A. californica greenei* which bore mature seed pods. On the other hand, *O. fasciatus* is a more selective herbivore, in terms of the numbers of host species utilized.

In the milkweed-rich Fresno area, large populations of *O. fasciatus* occur on four broad-leaf species (*A. v. vesita, A. erosa, A. eriocarpa, and A. californica greenei*) as well as the narrow-leaved
A. fascicularis. All of these host species appeared to be colonized to more or less the same extent. In the more northerly collecting area (Napa valley and adjacent Yolo, Solano and Contra Costa counties), the only milkweed colonized by O. fasciatus is A. fascicularis. Large stands of A. cordifolia, A. speciosa, and A. eriocarpa were not colonized during the summer of 1976, even though many of these plants bore large numbers of pods and often grew within a few kilometers of A. fascicularis stands which were infested with O. fasciatus. Furthermore, field observations indicated that even the smallest, most isolated milkweed stands in the Fresno area were colonized by some bugs, suggesting that the broad-leaf species in the north bay area were not simply overlooked by immigrating O. fasciatus on the basis of their population size or habitat.

The failure to find O. fasciatus on A. cordifolia at any time at localities studied is particularly mysterious. In the larval rearing experiments described in this thesis, seeds of A. cordifolia supported larval growth and development of O. fasciatus equal to that of most other species of Asclepias, suggesting that there is no nutritional barrier preventing O. fasciatus from exploiting this milkweed species. Perhaps A. cordifolia lacks some visual or olfactory cues which O. fasciatus may use in orientation to its hosts (Pantle and Feir 1976). One might even conclude that O. fasciatus in this region does not utilize an optimum foraging strategy, for it does not colonize host species which are capable of supporting populations of bugs. On the other hand, laboratory rearing of O. fasciatus at 25°C indicated that
larval development was more rapid on *A. fascicularis* compared to other milkweed species tested, which would suggest a shorter generation time of *O. fasciatus* feeding on this host in the natural environment.

*A. fascicularis* stands in the Napa Valley support large reproducing populations of *O. fasciatus* (K.E. Evans, unpubl. results).

The analysis of plant and insect material described in this thesis has shown that these insects contain relatively small quantities of cardenolides in their bodies, reflective of the cardenolide content of the host plant. Therefore, it is suggested that *O. fasciatus* in this part of its geographical range does not maximize its opportunities to sequester large quantities of cardenolides, for it does not colonize the species of *Asclepias* which are richer in these secondary plant substances.

52 per cent of the field-collected lygaeids in this study were found to contain less than 50 μg of cardenolide per insect. While this sample of lygaeids in California is not a random one, it is a representative one, based on the occurrence of *O. fasciatus* and *L.k. kalmii* on the different milkweed species observed: a large proportion of this 52 per cent was *O. fasciatus* from *A. fascicularis*. This plant is the most widespread species of milkweed in California, and although it probably does not constitute the largest species in the state in terms of biomass, it certainly occurs in high densities in many different habitats and plant communities. It has been suggested that the abundance and distribution of *A. fascicularis* could account in part for the high proportion (47 per cent) of monarch butterflies collected in California
with only small to immeasurable quantities of cardenolides in a study of wild monarchs by Brower and Moffitt (1974).

The present work has shown that the lygaeid bugs *O. fasciatus* and *L. k. kalmii* in central California contain varied quantities of cardenolides. The role of cardenolides in these milkweed-feeding lygaeids remains largely in question. It must be emphasized that only when the natural predators of these insects are known will we be able to test the effectiveness of cardenolides as a potential chemical defence agent in these insects. No incidents of predation of lygaeids were seen during the course of field work during this study. Sauer and Feir (1972) studied predation of *O. fasciatus* in a stand of *A. syriaca* in Missouri over a period of five months and reported that the only predators of *O. fasciatus* observed were insect predators, e.g. brown lacewing larvae (*Hemerobiidae*). In the present study, several anthropod predators were observed frequently on milkweed plants, including mantids (*Stagomantis californica* Rehn & Heb.), assassin bugs (*Zelus* sp.), ambush bugs (*Phymata erosa* L.), crab spiders (*Misumena* sp.) and jumping spiders (*Phidippus* sp.). Individuals of the former three were taken into the laboratory and offered adult, or second and third instar *O. fasciatus*, but none of the predators could be induced to attack the lygaeids. Mantids collected on *A. erosa* plants were assayed for cardenolides, but were found to lack these compounds, suggesting that their prey items consisted solely of pollinators of *Asclepias*. Pollinators such as the honey bee, *Apis mellifera* L. were frequently caught by crab spiders hiding in milkweed umbels.
Gelperin (1968) reported that the mantids *Paratanodera sinensis* Saussure and *Mantis religiosa* L. would attack adult *O. fasciatus*, but subsequently reject the lygaeid as a prey item, possibly on the basis of olfactory cues. Mantids learned to reject *O. fasciatus* on sight alone, but inhibition of the strike response was dependant on the nutritional state of the mantid.

No incidence of predation of lygaeids by vertebrates were observed in this field study, or in the study of Sauer and Feir (1972). However, gut analyses of birds and amphibians (R.T. Mitchell, unpublished results) indicate that large numbers of *L. kalmii* are eaten by vertebrates, in particular the sage grouse *Centrocercus urophasianus* Bonn., the scaled quail *Callipepla squamata* Vigors, and the roadrunner *Geococcyx californianus* Lesson. It was not known whether any of these insects contained cardenolides or not. Because it is not known what level of cardenolide content in the Californian lygaeids is required to cause an emetic or distressful response in a vertebrate predator, the full implication of the cardenolide variation cannot yet be established.

Perhaps the best documented bioassay of *Asclepias* cardenolides and cardenolides stored by an insect feeding on *Asclepias* is the blue jay emetic dose$_{50}$ test of Brower et al. (1968).

In examining the emetic response of the blue jay (*Cyanocitta cristata*) to the monarch butterfly (*D. plexippus*) reared on the neotropical milkweed *A. curassavica*, Brower et al. (1975) reported that one emetic dose (ED$_{50}$) required between 68 and 84 μg equivalents of digitoxin for an 85 g bird. Additional calculations from the data of Brower and
Glazier (1975) indicate that for the abdomen of such a butterfly, a single ED$_{50}$ consists of from 44 to 54 µg of cardenolide whereas a single ED$_{50}$ from the cardenolides stored in the wings of the same insect requires from 232 to 238 µg.

It has been established that owing to physiological barriers in vertebrates polar cardenolides tend to have lower emetic potencies than do non-polar ones, when administered via the oral route (see Duffey 1976 for a complete discussion). This relationship is largely because of the more rapid absorption of lipophilic cardenolides across the gut, and their more effective protein binding in the bloodstream (White and Gisvold 1952, Parsons and Summers 1971).

Preliminary studies (Parsons 1965, Reichstein et al., 1968) have shown that the principle cardenolides stored by the monarch reared on A. curassavica are the relatively non-polar chemicals calotropin and calactin (Figure 1), which could account for the relatively high emetic potential of the whole butterfly (68-84 µg per ED$_{50}$) compared to that of the wings (232-238 µg per ED$_{50}$).

It is conceivable that the lower emeticity of the cardenolides in the wings of the monarch butterflies may be owing to the presence of a larger proportion of polar cardenolides in these organs in comparison to the cardenolide compliment of the emetically-potent abdomen. Thus the monarch may partition its cardenolides in a fashion similar to O. fasciatus which preferentially sequesters more polar compounds in the dorso-lateral space fluid, while more lipophilic cardenolides occur in the fat body (Duffey, unpubl. results).
The relationship between emetic activity and polarity of Asclepias cardenolides may be considerably more complex though. Roeske et al. (1976) reared D. plexippus in the laboratory on the leaves of A. curassavica and the African asclepiad Gomphocarpus sp., and found the cardenolides stored by the butterflies reared on A. curassavica to be 2.6 times more emetically potent (per mg of cardenolide) than those stored by the monarchs reared on Gomphocarpus. However, the cardenolide profiles of these insects show that the cardenolides stored by the butterflies from the different plants occur over the same polarity range, with roughly the same proportion of polar to non-polar compounds. Therefore, their results indicate that the cardenolides sequestered by the butterfly from the different plants differ qualitatively in terms of their emetic potencies, irrespective of their polarity. Calotropin and its stereoisomer calactin account for almost 65 per cent of the cardenolides stored by the butterflies reared on A. curassavica, whereas these compounds were found to be absent in monarchs reared on Gomphocarpus (Roeske et al. 1976).

In an earlier study, Brower and Moffitt (1974) reported a causal relationship between cardenolide content of wild monarch butterflies and their emetic potency. However, butterflies collected in California were 4.6 to 6.5 times more emetic over the same range of cardenolide content as butterflies taken in Massachusetts. As in the rearing study, the cardenolide profiles of the Californian and Massachusetts monarchs covered the same polarity range, with approximately equal proportions of polar to non-polar compounds in each group.
(Roeske et al., 1976). Again the higher potencies per milligram of cardenolide in the Californian monarchs reflect qualitative differences in the cardenolides stored by the two groups of butterflies. Calotropin and calactatin were absent from the butterflies collected in Massachusetts, while some of the butterflies in the California sample contained a large proportion of these compounds amongst their cardenolide stores.

Similarly, the African grasshopper *Poekilocerus bufonius* stores large quantities of calotropin and calactatin, when reared on the milkweeds *A. curassavica* and *Calotropis procera* (von Euw et al., 1967); this insect has been shown to cause emesis in white mice and the European jay (*Garrulus glandarius* L.) (Rothschild 1966). While largely circumstantial, this evidence along with the different emetic potencies of the separate body parts of *D. plexippus* as reported by Brower and Glazier (1975), suggest that the presence of calotropin and calactatin may largely account for the emetic response seen in the blue jay. Rothschild et al. (1975 and unpubl. res.) reared *D. plexippus* on their 'Oxford strain' of *A. curassavica*, which lacks calactatin entirely. This compound was also absent from the butterflies, which failed to induce emesis in pigeons (*Columba livia* Gm.).

Examination of whole body extracts of field collected *O. fasciatus* and *L. k. kalmii* (not described in this thesis) shows that all of the cardenolides stored in these insects are more polar than calotropin or calactatin. Should these more polar cardenolides derived from the Californian species of *Asclepias* by lygaeids be found to have similar pharmacological properties to the cardenolide compliment
in the wings of the monarch butterfly reared on A. curassavica (Brower and Glazier 1975), it would seem possible that few, if any of the individual lygaeids collected in this study contained enough cardenolide of the required type to elicit an emetic response from a predator with a sensitivity similar to the blue jay. Roughly 50 per cent of the field-caught milkweed bugs examined were found to contain less than 50 μg of cardenolide per insect. However, these insects could increase their emetic potential by way of their gregarious behaviour; a predator might ingest several individuals at one feeding, with the total cardenolide pool of several insects eliciting an emetic response (Pough et al. 1973). On the other hand, the number of insects needed to condition the predator to avoid the species as a prey item might outweigh the advantage of such gregariousness.

Even if the adults of O. fasciatus and L. k. kalmii with greater than 100 μg per insect are found to have some chemical protection from predators, the data presented in this thesis suggest that the effectiveness of cardenolide sequestration as a chemical defense strategy may vary over the geographical range of these species, and may vary in the course of a season at a particular location.

A further complication to understanding the effectiveness of cardenolides stored by lygaeids is the variation of sensitivities to cardenolides among potential vertebrate predators. The blue jay (C. cristata) and European jay (G. glandarius) are considered to be species which are sensitive to cardenolides, the European hedgehog (Erinaceus europaeus L.) and laboratory rat are not, and the Japanese quail
(Coturnix japonicus Tem.) can tolerate quantities of digitoxin up to 50 times greater than the human lethal dose (Rothschild and Kellett 1972). Duffey (1970) found that O. fasciatus reared in the laboratory on seeds of A. syriaca (the insects contained a mean of 284 μg of cardenolide per insect) were completely palatable to both frogs (Rana pipiens Schreber) and toads (Bufo boreas Baird & Girard). Thus in considering the position of milkweed-feeding lygaeids in mimicry complexes one can only repeat the statement of Rettenmeyer (1970), who wrote:

"... instead of referring to an insect as a Batesian mimic, it would be more nearly accurate to state that under the specified conditions the insect, or some part of its population in a certain locality has a Batesian relationship to a specific predator."

It may not be valid to assess plant-derived cardenolides as a defensive strategy of lygaeids strictly on the basis of their emetic potential to a vertebrate. It is entirely possible that predators could become conditioned to avoid brightly coloured lygaeids and other milkweed-feeding insects on the basis of gastric distress (i.e. nausea) or delayed toxicosis, neither of which need be outwardly visible to an experimentor (cf. emesis).

Cardiac glycosides are capable of causing direct irritation of gastric tissue (Gold et al. 1950), and have been shown to cause strong contraction of vertebrate smooth muscle in isolated preparations (Haustein et al. 1966, Godfraind and Godfraind-de Becker 1961, Shigei et al. 1963). Rowe et al. (1970) studied the toxicity of the milkweed A. brachystephana Englem in sheep, and reported that symptoms of sub-lethal doses of cardenolide-containing plant material (from which the animal
fully recovered), included diarrhea and laboured breathing.

While difficult to quantify, effects of this nature might possibly lead to the conditioned aversion of predator to cardenolide-containing insects. Czaplicki et al. (1975) demonstrated the theoretical viability of the concept of olfactory mimicry. In an artificial situation, garter snakes (Thamnophis sp.) were conditioned to reject edible prey items that bore an olfactory resemblance to worms after having experienced a delayed toxicosis following their ingestion of the worms. Laboratory rats and quail can also learn to associate gustatory and visual cues with delayed toxicosis, leading to their subsequent rejection of food and water which contains these cues (Wilcoxon et al. 1974).

To date, the only pharmacological investigations using cardenolides from Asclepias-feeding insects are those of Brower and co-workers using the blue jay emetic dose_{50} bioassay (Brower et al. 1968). A pilot study of the toxicity of 'lygaeinin' (tentative name for a compound isolated by the author from both O. fasciatus and L. k. kalmii, sequestered from several Californian species of Asclepias) using the embryonic chick heart bioassay of Lehman and Paff (1942), indicated that this compound was as potent as commercially obtained digitoxin (undercribed experiment). However, the intravenous emetic potency of calactin in the cat is three times greater than that of digitoxin (Parsons and Summers 1971). Clearly, all of the pharmacological effects of Asclepias cardenolides and those obtained by insects from milkweed plants are in need of investigation before the ecological role of the
compounds in the insects will be understood.

One pharmacological effect of cardenolides which may have been greatly underestimated is their bitter taste. Most cardenolides (Brower and Glazier 1975 cite T. Reichstein), like other plant glycosides (Kingsbury 1964) are bitter to the human taste. Some birds have been shown to have well developed taste receptors (Kitchell et al. 1959, Duncan 1960, Yang & Kare 1968, Wenzel 1973) and have shown an aversion to bitter compounds (Wenzel 1973); such a response has been used to investigate learning ability in some species (e.g. Alcock 1971; see Brower and Glazier for several other references). Brower and Glazier (1975) have rightly adopted this argument to suggest that some birds could use the bitter taste of cardenolides to discriminate between emetic and non-emetic monarch butterflies. Their findings that the wings of the monarch contain the largest concentrations of cardenolides, but the least emetic among those in the body, support the concept of an anti-predator strategy based on the taste of cardenolides. Similarly, the highest concentrations of cardenolides in the lygaeid bugs O. fasciatus and L. k. kalmii are found in the fluid of the dorso-lateral spaces (Duffey and Scudder 1974): when roughly handled, the fluid from these spaces is released as discrete droplets onto the cuticle from the dorso-lateral edge of the metathorax and abdominal segments (Scudder and Duffey 1972; Graham and Staddon 1974).

The advantage of having the highest concentrations of bitter-tasting cardenolides in both the wings of the monarch and the secreted space fluid of the lygaeid is that the predator can sample the insect without the former sacrificing its life.
Other vertebrates may be highly sensitive to the bitter taste of cardiac glycosides. In the veterinary literature, most authors suggest that plant material of *Asclepias* may lose its disagreeable flavor when dry, while retaining its toxicity (Hulbert and Oehme 1965, Vail 1942). The reason for this observation could be that the availability of the bitter cardenolides to the taste receptors is lessened when in the dry state.

It is conceivable that cardenolides in lygaeid bugs, secreted in highly concentrated droplets onto the cuticle, could give rise to an extremely rapid response by a predator taking the insect into its mouth. Although no quantitative studies have been undertaken, small quantities of cardenolides, such as those found in many of the Californian lygaeids, may be enough to elicit an effective response from a vertebrate predator, even though the quantity and quality of those cardenolides is far below that necessary to cause emesis.

If plant derived cardenolides are not being employed by lygaeid bugs as an anti-predator strategy, then what strategies might these warningly-coloured insects rely upon to avoid predation? Like most Hemiptera, *O. fasciatus* and *L. k. kalmii* possess meta-thoracic scent glands in the adult stage (Games and Staddon 1973). These glands, which excrete volatile alkyl compounds (e.g. hexanols) could provide the basis for unpalatability in certain responsive vertebrate predators. Duffey (1970) found that among several grades of vertebrate predators, none responded to the cardenolides of *O. fasciatus* when reared on seeds of *A. syriaca*, whereas some predators (turtles and chickens) avoided this
insect because of its volatile scent gland secretions. Similar results have been shown for anolis lizards presented with sunflower-reared \textit{O. fasciatus} (Weber 1975). It is conceivable then, that some predators in the natural environment could reject \textit{O. fasciatus} and \textit{L. k. kalmii} because of these secretions, irrespective of the cardenolide content of the insects.

Graham and Staddon (1974) have demonstrated the presence of a histamine analogue in the dorso-lateral space fluid, and other body fluids of adult and larvae of \textit{O. fasciatus}. This compound was shown to produce strong contractions in vertebrate smooth muscle, and as no such activity was found in the sunflower seeds on which the insects were reared (excluding the possibility of cardenolides producing the effects), the authors suggested that this previously undescribed compound may be synthesized by the bug (Graham and Staddon 1974). Histamine has also been found in the defensive secretions of the grasshopper \textit{P. bufonius}, which like \textit{O. fasciatus}, sequesters cardenolides from its milkweed host (von Euw et al. 1967). Perhaps there is a synergistic effect of these compounds, i.e. cardenolides present in the secretions enhance the contractile effects of histamine on vertebrate smooth muscle. The presence of an effective compound like histamine could offer one explanation as to why an insect such as \textit{O. fasciatus} can afford to be an inconsistent storer of cardenolides, as shown in this thesis, while maintaining its conspicuous coloration and behaviour.

Another possibility is that many of the populations of \textit{O. fasciatus} and \textit{L. k. kalmii}, mimic their conspecifics which contain
pharmacologically effective quantities of cardenolides. One problem of
this hypothesis though, is that it requires selection pressure from a
migratory predator, one which could sample insects from many geographi-
cally distinct populations. Otherwise, a predator might never come into
contact with a population of lygaeids which contain significant quantities
of cardenolides. While this type of mimetic system seems appropriate for
the migrating monarch butterfly (Pough et al. 1973, Brower and Moffitt
1974), its status as an anti-predator strategy in lygaeids requires a
better knowledge of the potential predators of these bugs.

A further possibility is that predators capable of visual dis-
"crimination may generalize their avoidance to all prey items with contrast-
ing combinations of white, yellow, orange, red and black. Phyrphorcid
bugs of the genus Dysdercus Guerin Meneville share a body size, shape and
coloration similar to many lygaeines, and the geographical ranges of these
two groups largely overlap (van Doesburg 1968). Although Dysdercus species
do not sequester cardenolides (Duffey and Scudder 1972) they do secrete
volatile ketones and aldehydes (e.g. hex-2-en-1-al, Calam and Scott, 1969)
which may effectively deter predators (Parsons 1940). Sexton (1964) repor-
ted that the lizard Anolis carolinensis Voigt rejected all multi-coloured
prey items. In a laboratory situation, the common fence lizard Sceloporus
occidentalis Baird & Girard avoids adult O. fasciatus on sight alone,
but will accept mealworm larvae (Tenebrio molitor L.) under the same
conditions (undescribed experiment). Feir and Suen (1971) also indicated
that the fence lizards will not accept O. fasciatus as a food item. In
fact, most insect herbivores feeding on milkweed are brightly coloured,
suggesting that they participate in some form of mimicry complex (Price 1975). However, recent evidence strongly suggests that such a mimicry complex could not be based on plant-derived cardenolides, as few members of the milkweed insect fauna store significant quantities of these compounds (Isman et al. 1977).

Finally, three observations suggest that cardenolide sequestration is not an important anti-predator strategy among lygaeid bugs in central California. Firstly, if predators are able to exert a selection pressure on lygaeids to sequester large quantities of cardenolides, then we would expect this pressure to be operating most effectively when the density of insects is low. For lygaeids, this period would be in the late spring and early summer, when immigrating adults first colonize milkweed stands. However, as the data in this thesis have shown, lygaeids sampled in May and June either lacked cardenolides or contained small quantities of cardenolides, only acquiring larger quantities later in the season when the reproductive structures of Asclepias are well developed.

Secondly, predators do not appear to hinder population growth of O. fasciatus on stands of A. fascicularis in the north bay area, even though these insects have been shown by the present work to contain relatively small quantities of cardenolides. Once established, O. fasciatus populations on A. fascicularis increase in number exponentially (K.E. Evans, unpubl. results). This finding is consistent with population growth of O. fasciatus in the midwestern United States, where it most frequently colonizes A. syriaca (Brown et al. 1976).
Thirdly, there does not appear to be a physiological or metabolic cost involved in the sequestration and storage of cardenolides by O. fasciatus and L. k. kalmii, (which would be outweighed by the advantages of protection from predation). No measurable differences in the amount or rate of growth of larval and adult O. fasciatus were detected when this insect was reared on the seeds of eight different species of Asclepias, all of which are potential natural host plants of this insect. The fact that cardenolide content of the food does not influence the amount or rate of growth of O. fasciatus is in agreement with the data obtained by Erickson (1973). Erickson (1973) compared the larval development and growth of the monarch butterfly D. plexippus on four different species of Asclepias on which this insect is known to feed, and failed to show significant differences.

Thus, the data presented in this thesis provide strong evidence against the possibility that the sequestration and storage of cardenolides has an adverse effect upon growth and development of O. fasciatus, even though O. fasciatus in this study stored up to seven times as much cardenolide by weight as the monarch butterfly. Further, the range of cardenolide content in the field collected bugs falls well within that of the laboratory reared insects. The largest quantity of cardenolides detected in a field collected lygaeid was 372 μg, whereas laboratory-reared O. fasciatus have been known to contain as much as 800 μg of cardenolide. Therefore, this finding is considered to be valid for lygaeids feeding on Asclepias in the natural environment.

As suggested by Feeny (1976), the toxic compounds of early
successional herbs (e.g. cardenolides in *Asclepias*) may act as a 'qualitative' barrier to generalist insect herbivores while having little or no effect on the growth and fitness of highly adapted herbivore species. The inability of the present study or the findings of Erickson (1973) to demonstrate a detrimental effect of cardenolides on the growth and development of the milkweed-feeding insects *O. fasciatus* and *D. plexippus* support this hypothesis.

If then, cardenolides are not stored by lygaeids as an anti-predator strategy, and these compounds do not affect the growth and development of the insects, then how does one explain the presence of these compounds in the lygaeids? One possible explanation is that cardenolide sequestration is or was used by these lygaeids or closely related species in previous predator-prey interactions over evolutionary time, or at the present day, in other areas of the species' geographical range.

Most Lygaeines are tropical insects; few species have successfully invaded temperate regions of the world. In the tropical environment, where several species of brightly-coloured lygaeids occur sympatrically, these insects may depend on plant-derived cardenolides as a defensive strategy, as they could be subject to predation from a wider variety of predators than in a temperate habitat (e.g. central California). Alternately *O. fasciatus* and *L. k. kalmii* may have descended from ancestral species which benefited from cardenolide storage as an anti-predator device. Such a physiological system, which does not interfere with the normal development of the insect, could conceivably
exist in present day lygaeids as an artifact of a previous predator-prey interaction, long after the predator evolved different feeding habits which did not include lygaeids.

In conclusion, the role of cardenolides in *O. fasciatus* and *L. k. kalmii* feeding on milkweeds in central California remains in question, although the colouration and behaviour of these species strongly suggest that they benefit from warning colouration and some form of mimicry which can condition predators to avoid these insects. Cardenolide sequestration does not appear to be an important organizing factor in the feeding and population ecology of these lygaeid species in this part of their geographical range.
REFERENCES


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