SUBLETHAL EFFECTS OF CARBOFURAN AND METHIDATHION ON *REDUVIOLUS AMERICOFERUS* (CARAYON) (HEMIPTERA:NABIDAE)

by

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DEDICATION

I dedicate this dissertation

to my parents

and

to my wife, Leslie.

I thank each of you for your love,

support, and acceptance

through the years.

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INTRODUCTION

Many authors have noted reduced field populations of nabids following insecticide treatments. Dinkins et al. (1970) found that although field populations of predators in cotton had returned to pretreatment levels 2 wk after early-season insecticide treatment, late-season treatments kept predator populations low. They attributed this difference to reservoirs of predators which were present outside the test plots in early season, but not in late season. In explaining suppression of nabids until the 2nd cutting by stubble sprays of carbofuran, Weires and Radcliffe (1974) suggested that part of the effect might be due to reduced prey numbers, which would reduce retention of predators. Surgeoner and Ellis (1975) also attributed nabid population decline following carbofuran sprays to decreases in prey availability in addition to insecticidal mortality. Benedict (1975) suggested that early-season insecticide application did not significantly suppress nabid populations because of the very low numbers of individuals present in the field at the time of application and the relatively short residual period of carbofuran. Harper (1978) found that although methidathion reduced populations of *Reduviolus*(=*Nabis*) *alternatus* (Parshley) 2 days (d) after treatment, 9 d populations were not significantly different from the check.

Nabid population responses to carbofuran and methidathion may differ because of different toxicities and different residual periods. Martinez (1979) found a 24 h LC_{50} for adult *Reduviolus*(=*Nabis*) *americo-*

ferus (Carayon) for carbofuran of 19 ppm and for methidathion of 80 ppm. Fahey et al. (1970) reported that methidathion persisted on green alfalfa for 3 wk after treatment, while carbofuran disappeared by 2 wk.

Sublethal effects may also influence the dynamics of predator populations in alfalfa fields. Not only might adults and nymphs survive in areas of the field with reduced insecticide coverage, but adults might migrate into or out of fields and encounter sublethal residues of pesticides. In addition, as long as residues remained, newly hatched 1st instars could be exposed. Finally, the scavenging habits of nabids might result in ingestion of sublethal amounts of toxicant from insecticide-killed prey.

The primary objective of my research was to quantify sublethal effects of carbofuran (carbamate) and methidathion (organophosphate) on *R. americoferus*. These chemicals are used on alfalfa to control early-season *Hypera postica*. Because this nabid is a major predator in alfalfa, it would be useful to know whether there are significant changes in the biology of nabids which survive exposure to these insecticides.

Because the field situation is complicated by multiple factors -- all concentrations of insecticide from 0 to the maximum rate applied, many ages of nabids, and variations due to weather and varying prey populations -- I decided to approach the question of sublethal effects through a series of laboratory studies.

Section II

SUBLETHAL EFFECTS OF CARBOFURAN AND METHIDATHION ON ADULT *REDUVIOLUS AMERICOFERUS* (HEMIPTERA:NABIDAE)

SUBLETHAL EFFECTS ON ADULTS

INTRODUCTION

Moriarty (1969) pointed out that if insects are killed by exposure to a pesticide or other stressor, sublethal effects may be due in part to selection. Selective sublethal effects are apparent changes in the biology of survivors due solely to changes in population composition following selective mortality. Physiological sublethal effects are changes in the biology of survivors not due to changes in population composition. However, in some reports, even though insects died during the acute mortality period, the nature of the sublethal effect clearly indicates a physiological response. Duncan (1963) concluded that at LD_2 (lethal dose killing 2%) and LD_8 levels dieldrin decreased longevity and oviposition in *Aedes aegypti* by decreasing feeding. In the LD_8 group there was almost no feeding 13 days posttreatment --a physiological effect. Georghiou (1965) reported that *Musca domestica* treated with LD_0, LD_{10}, and LD_{30} levels of isolan showed decreases in feeding, oviposition rate, and total eggs laid. Because similar responses were observed at all 3 doses, the selective effect, if present, parallels the physiological effect. Zaghloul and Brown (1968) suggested that non-selecting doses of DDT on *Culex pipiens* increased resistance in a susceptible strain by hidden selection of ova. Thus selection may occur even at LD_0. Because physiological and selective effects are difficult to separate, both should be kept in mind as possible factors.

Sublethal effects on longevity vary with the stress and organism studied. Saini and Chiang (1966) found decreased longevity in male or female *Oncopeltis fasciatus* exposed to LE_{48} (lethal exposure) and LE_{80} durations at -35 °C. Zettler and LeCato (1974a, 1974b) reported decreased longevity in LD_{50} survivors of beetles exposed to malathion and dichlorvos. Kwan and Gatehouse (1978) described decreased longevity in male *Glossina morsitans* surviving for 48 h after LD_{15} and LD_{30} dieldrin or endosulfan treatment. Chrominski et al. (1982) observed decreased longevity in female *Melanoplus sanguinipes* exposed to 12 h, 24 h, or continuous ethylene. Hodjat (1971) observed decreased longevity in *Dysdercus fasciatus* due to crowding, starvation, or high sublethal doses of dieldrin. On the other hand, he described increased *Dysdercus* longevity following treatment with low sublethal doses of dieldrin. Similarly, Ball and Su (1979) found increased longevity in female *Diabrotica virgifera* exposed to LD_0 levels of carbofuran or carbaryl. In addition to changes in mean longevity, the form of the survivorship curve may change. Saini and Chiang (1966) found that *Oncopeltis* surviving -35 °C had an increased mortality rate followed by a decreased mortality rate compared to the control survivorship curve.

Some authors found no significant sublethal effects even though the dose was high. Exposing *Geocoris* spp. to residues of methyl parathion, carbaryl, and methomyl, Walker and Turnipseed (1976) found no significant differences in longevity, fecundity, or egg hatch. In field

tests the only significant reduction in longevity occurred in males surviving exposure to methomyl. Another sublethal effect reported by Lim and Lee (1982) was the tendency of female *Oxya japonica* to lose one or both hind legs after ingesting diflubenzuron. This injury apparently affected food consumption, and hence fecundity, and egg hatch.

Oviposition may also be negatively affected. Zaghloul and Brown (1968) found that non-selecting doses of DDT caused degeneration of some *Culex pipiens* ovaries, decreasing the proportion of females ovipositing. Moreover, the duration of oviposition may be decreased (Saini and Chiang 1966, Zettler and LeCato 1974a), or egg maturation and deposition may be delayed (Georghiou, 1965). Decreased oviposition rate has been observed by Loschiavo (1955)-- *Tribolium confusum* surviving LC_{20} DDT Georghiou (1965)-- *Musca domestica* surviving LD_0, LD_{10}, LD_{30} isolan Bariola and Lindquist (1970)-- *Anthonomis grandis* surviving continuous exposure to carbamates and Hodjat (1971)-- *Dysdercus fasciatus* fed boiled cotton seed. Increased oviposition rate has been documented for spider mites (Leigh and Wynholds 1980, Maggi and Leigh 1983), and planthoppers (Chelliah and Heinrichs 1980, Chelliah et al. 1980).

Chauthani and Adkisson (1966) observed a decrease in total eggs laid by *Heliothis* surviving treatment with LD_{14} to LD_{95} organophosphates and endrin. Hodjat (1971) reported decreases in *Dysdercus* egg production from high sublethal doses of DDT or dieldrin, crowding, feeding on boiled cotton seed, and starvation. Zettler and LeCato

(1974a, 1974b), and Lim and Lee (1982) also observed decreased fecundity in *Attagenus*, *Tribolium*, and *Oxya*.

However, Georghiou (1965) described an increase in total eggs laid by *Musca* surviving treatment with low doses of dieldrin. Likewise, Saini and Chiang (1966) discovered an increased fecundity for *Oncopeltis* surviving LE_{16} and LE_{48} exposures to -35 °C. This increase occurred even though female longevity remained the same or decreased. Although Hodjat (1971) observed increased egg production in *Dysdercus* from treatment with low doses of dieldrin and DDT, part of the increase may have been due to increased longevity. Johansson and Johansson (1972) reported increased egg production through the 27th day following exposure of *Tribolium confusum* to 0.01% sodium fluoride. Also, Zettler and LeCato (1974b) suggested an increased production of progeny in *Tribolium castaneum* surviving LD_{50} malathion when calculations were based only on living females. Finally, Ball and Su (1979) observed increased egg production in *Diabrotica virgifera* surviving LD_0 doses of carbofuran. Again increased fecundity was paralleled by increased longevity.

Although Hodjat (1971) observed increased fertility in *Dysdercus* exposed continuously to low amounts of DDT, he reported a decreased percentage of eggs hatching in females starved, crowded, fed boiled cotton seed, or exposed continuously to high sublethal doses of DDT. Similarly, Lim and Lee (1982) found decreased egg hatch in *Oxya* which had ingested diflubenzuron.

Reduviolus(=*Nabis*) *americoferus* (Carayon) is a dominant predator in alfalfa, a crop which is often treated with insecticides such as carbofuran or methidathion for alfalfa weevil. This research was intended to show whether there are any sublethal effects on adult *R. americoferus* in addition to the acute toxic effects.

MATERIALS AND METHODS

I collected nabids from alfalfa in Montgomery County, Virginia, with a 36 cm sweep net. To avoid excessive mechanical damage to the nabids, the sweep net was emptied into a sleeve cage after every 25-33 pendulum sweeps. The sleeve cages were held in a cold room (19 ± 2°C, 14 h photophase) for 1-6 days. To remove nabids from the sleeve cages I coaxed each nabid into an individual shell vial stoppered with polyurethane foam and sorted samples by species and instar.

In the cold room late instar *R. americoferus* were reared on alfalfa stems in 30 cm high cages made from 6 cm diam polybutylene tube. Each day teneral adults were transferred to individual 65 ml cages (made from 20 dram plastic snap cap vials) containing alfalfa. In order to accumulate enough nabids of the same physiological age, I held one-day-old adults at 6 ± 2°C. Before exposing them to insecticide I returned them to the cold room for one day. Assuming that young adults aged less than half a day while they were refrigerated, the physiological age of each individual at the beginning of the exposure period was 2 d as an adult at 19 ± 2°C.

At least a day before nabids were exposed I treated 3.7 cm Whatman 934AH Glass Microfibre Filters with 500µl aliquots of carbofuran or methidathion formulated in distilled water. I positioned each disk near the center of a 5 cm diam aluminum foil dish. I tried to insure uniform coverage of each disk by stirring the insecticide each time I withdrew an aliquot, and by applying each aliquot with a circular motion of the Eppendorf® pipette. Treatments based on 2 d mortalities were LC_5 carbofuran (0.005% vol Furadan® 4F per vol water), LC_{45} carbofuran (0.01% v/v), LC_5 methidathion (0.015% vol Supracide® 2E FL per vol water), LC_{25} methidathion (0.03% v/v), or control (distilled water). Preliminary work showed an adult LC_{50} for carbofuran of 0.010% v/v (95% fiducial limits 0.008 to 0.025) and an LC_{50} for methidathion of 0.037% v/v (f.l. 0.035 to 0.039). I placed the aluminum dishes in a covered cardboard box and dried the filters in a hood at room temperature for an hour and then in the cold room for a day.

I made individual exposure chambers by inverting 1 oz pea cups over the filters. For ventilation I had cut a 1 cm hole in the bottom of each pea cup and covered it with organdy. To restrict nabids to the filter paper, I dipped pea cups in Fluon® AD1. This created a surface too slick for the nabids to climb.

Since I needed sexes in approximately equal numbers for later pairing, in order to compensate for acute mortality I placed 2 of each sex on control disks, 4 of each sex on low concentration disks, and 8 of each sex on high concentration disks. To minimize time effects I added

individuals across treatments. After I had filled all 28 exposure chambers in a box, I replaced the cardboard lid, laid a stack of 4 paper towels on top, and moistened the towels with 7 ml tap water. I slipped a 76 by 30 by 20 cm polyethylene bag around the box, sealed the bag, and placed it in a 23 ± 2°C environmental chamber overnight. With this method I intended to maintain a reproducible 100% relative humidity during the exposure period. I began each exposure between 19:00 and 22:00 E.S.T., and standardized length of exposure time by removing each nabid 12 h after it had been introduced to an exposure chamber.

In removing nabids from the exposure chambers I avoided physically injuring them by using strips of paper to coax them into small shell vials. After adding to each vial 5^{+} vestigial-winged *Drosophila melanogaster* used throughout these experiments for food, I placed the vials in a 15 by 17 cm ZipLoc® plastic bag, which I slipped into a larger plastic bag containing a paper towel moistened with 1 ml water. I moved these recovery vials to a darkened environmental chamber (22 ±1 °C) for a day. At the end of this time I paired surviving males and females in cages by treatment.

Cages were 65 ml Crystal Plastic vials with a 2.5 cm ventilation hole covered with organdy and a 7 mm nabid entry opening sealed with a cork. For perching and oviposition sites, each cage contained a stem of alfalfa about 2 mm thick which projected downward through a polyurethane plug to another plastic vial half filled with water. For food I added 10^{+} *Drosophila*/nabid/d, and 2 d later (4 d after the start of the

experiment) I transferred the nabids to new cages with new potential mates from the same treatment. Cages were placed in a warm room (25 ± 5°C, 14L:10D). Every 4th d thereafter I transferred nabids to new cages with new potential mates. Because I did not know which nabids might contain parasites or be in reproductive diapause, this technique increased the possibility of successful mating.

For this study I defined mortality as the inability to return to a standing position. I checked for mortality 1) at the end of the 12 h exposure period after I removed nabids from the exposure chambers, 2) a day later at the end of the recovery period, and 3) each day thereafter until all had died. In the case of a nabid which appeared to be dead after exposure, but which was alive at the end of the recovery period, I defined the death date as the date after which the nabid was permanently unable to right.

I placed dead nabids in small vials for a day before freezing them because I thought that some parasites might emerge after the time of apparent death. In no case, however, did parasites emerge after nabid death. Later I recorded missing appendages, measured each pronotal width with an ocular micrometer, and dissected each nabid to count parasites or unlaid eggs. Five of the 284 nabids which I used had parasites and were deleted before data analysis.

Whenever I transferred nabids to new cages, the old cages with the alfalfa for oviposition were placed in the warm room until the eggs hatched. I recorded the number of eggs laid, the number which hatched

successfully, and alfalfa quality. I estimated the maximum oviposition rate for each female with the variable "maximum eggs laid per 4 day time interval." Because each alfalfa stem contained eggs from 4 days of egg-laying, this variable yielded a coarse measure of maximum reproductive potential for each female. By successful hatch I mean eggs from which nymphs had emerged and left. The few nymphs which had emerged but could not leave because they were held by legs or antennae were not included in the count. By alfalfa quality I refer to whether the alfalfa stem was alive, dying, or dead at the time of the count. If it died before the eggs had a chance to develop, I expected a reduced hatch.

I performed 5 sublethal experiments with adult nabids. In addition to the controls, experiments 1 and 2 included LC_5 and LC_{45} carbofuran; experiments 3 and 4 included LC_5 and LC_{25} methidathion, and LC_5 carbofuran; and experiment 5 included LC_5 carbofuran. To avoid missing cells in the data I analyzed experiments 1 and 2 together, experiments 3 and 4 together, and experiments 1-5 for control and LC_5 carbofuran only. In order to have a uniform definition of acute mortality for all stages, I chose 4 days post exposure as the end of the acute mortality period. Thus my analysis for sublethal effects included only individuals which survived beyond the 4th day post exposure.

I used SAS procedure GLM (SAS Institute 1982) to explore covariates and treatments. As variances of untransformed variables usually

increased with their means, count data were transformed with SQRT(X+1) and proportions with ARCSIN(SQRT(X)). I checked each covariate for similarity of slope before including it in analysis of covariance (ANCOVA) models. By a *possibly contaminated covariate* I mean a covariate, such as longevity, that may have been affected by treatment. When treatment was significant in ANCOVA, I used contrasts of the form "CONTROL vs OTHERS", "LC_5 vs LC_{HIGH}", or "LC_5 CARBOFURAN vs LC_5 METHIDATHION". To compare any two treatments I also used least-squares means. I report the error degrees of freedom (df) for ANCOVA and the sample size (n) for chi-square tests. I report probability values to indicate how close my test results were to my chosen significance level of 5%. Thus P=0.05 corresponds to a significant difference, values of P less than 0.05 correspond to increasingly significant differences, while values of P greater than 0.05 correspond to increasingly non-significant differences.

RESULTS

I discuss the effects on longevity separately by sex because the sexes responded differently to pesticide exposure. Males surviving exposure to LC_5 carbofuran did not live as long following exposure as did the controls ($P<0.05$, df=40) (Table 1); those surviving LC_5 and LC_{45} carbofuran showed no difference in longevity due to concentration level (P=0.4, df=26). Males surviving exposure to methidathion did not live as long following exposure as controls (P=0.001, df=46). LC_5

and LC_{25} methidathion treatments showed no difference in longevity due to concentration level (P=0.8, df=46), nor did LC_5 methidathion and LC_5 carbofuran (P=0.9, df=46).

Exposure to carbofuran did not influence the longevity of surviving females (P=0.4, df=34) (Table 1). In tests comparing the longevity of surviving control females with the longevity of females surviving exposure to LC_5 carbofuran, pronotal width was a highly significant covariate (P<0.001, df=57). Exposure to methidathion also did not influence the longevity of surviving females (P=0.6, df=57), and pronotal width was again a significant covariate (P<0.05, df=57). For both chemicals, an increase in pronotal width of 0.1 mm was related to an increase in post exposure longevity of 3-4 d ± 1 d (S.E.).

Significantly more methidathion-treated females were missing appendages than were control females (37% vs. 11%) (P<0.05, n=64). This was also observed for carbofuran-treated females at the time of death, but the difference was not significant (19% vs. 11%) (P=0.4, n=85). While as many males surviving exposure to carbofuran had missing appendages as did controls (P=0.9, n=60), males surviving methidathion showed a non-significant increase in numbers of individuals with missing appendages compared to controls (28% vs. 15%) (P=0.25, n=62). Considering both sexes together, carbofuran survivors showed a non-significant increase in missing appendages (P=0.5, n=145), while methidathion survivors showed a significant increase (P=0.013, n=127). In summary, exposure to methidathion showed greater numbers of individuals with missing appendages, especially in females.

Table 1. Longevity and fecundity of Reduviolus americoferus surviving exposure as young adults.

Treatment	Postexposure Longevity[1] (days)		Total Eggs[1] Laid
	Males[2]	Females	
Experiments 1-2			
control	17.1 ± 3.04 (8)	12.8 ± 2.82 (6)	71 ± 39.4 (6)
LC_5 carbofuran	11.3 ± 1.14 (13)	16.8 ± 1.46 (16)	130 ± 32.2 (16)
LC_{25} carbofuran	13.6 ± 1.45 (11)	17.4 ± 1.90 (19)	134 ± 32.0 (19)
Experiments 1-5			
control	24.7 ± 2.78a (26)	16.3 ± 1.55 (27)	80 ± 18.6 (27)
LC_5 carbofuran	13.9 ± 1.67b (23)	16.2 ± 0.78 (39)	93 ± 15.8 (39)
Experiments 3-4			
control	32.3 ± 3.59a (14)	17.9 ± 2.49 (14)	100 ± 30.2 (14)
LC_5 carbofuran	19.8 ± 5.68b (5)	15.1 ± 1.17 (14)	50 ± 14.2 (14)
LC_5 methidathion	19.8 ± 1.69b (15)	17.1 ± 2.18 (14)	83 ± 20.8 (14)
LC_{25} methidathion	19.4 ± 1.96b (21)	14.8 ± 1.29 (24)	59 ± 13.8 (24)

[1]Means ± S.E. (Sample size in parenthesis).
[2]Means followed by different letters were significantly different within groups of experiments. No letters indicate no difference. Comparisons were based on transformed Least-Squares Means. Experimental groups reflect the three sets of analyses used to avoid missing cells in the data. (I performed 5 sublethal experiments with adult nabids. In addition to the controls, experiments 1 and 2 included LC5 and LC45 carbofuran; experiments 3 and 4 included LC5 and LC25 methidathion, and LC5 carbofuran; and experiment 5 included LC5 carbofuran. To avoid missing cells in the data I analyzed experiments 1 and 2 together, experiments 3 and 4 together, and experiments 1-5 for control and LC5 carbofuran only.)

Neither exposure to carbofuran (P=0.35, n=84) nor exposure to methidathion (P=0.25, n=64) significantly affected the proportion of females laying eggs. There was no difference between total eggs laid by survivors of exposure to LC_5 carbofuran and controls (P=0.4, df=55) (Table 1). Although pronotal width was a highly significant covariate in ANCOVA to explain total eggs laid (P<0.01, df=55), when I added the possibly contaminated covariate, post exposure longevity (P<0.0001, df=54), to the model, pronotal width was non-significant (P=0.8). Nor was there any difference among treatments in the experiments which also included LC_{45} carbofuran (P=0.4, df=34). Total eggs laid by survivors of methidathion (LC_5, LC_{25}), LC_5 carbofuran and control treatments were similar (P=0.4, df=56). While pronotal width was a significant covariate (P<0.05), refrigeration was not significant.

Exposure to carbofuran did not affect the maximum number of eggs laid per 4 day time interval (P=0.3, df=58). While increased pronotal width (P<0.05) and increased refrigeration time (P<0.05) were related to increased maximum eggs laid, exposure to methidathion or LC_5 carbofuran did not affect maximum eggs laid by survivors (P=0.7, df=56).

Although pronotal width was a significant covariate in some models, no treatment significantly affected the time at which the 25th percentile or the 50th percentile egg was laid. When dissected following death, control females with larger pronotal widths contained higher numbers of eggs (P<0.05, df=28). However, neither exposure to carbofuran (P=0.2, df=34), methidathion (P=0.8, df=58), nor pronotal width (P=0.7)

significantly affected the number of eggs dissected from survivors. Neither exposure to carbofuran ($P=0.8$, $n=75$) nor exposure to methidathion ($P=0.25$, $n=50$) significantly affected the proportion of females laying viable eggs.

Pronotal width significantly influenced the total egg hatch of control females ($P<0.05$, $df=21$); those females which lived longer hatched more eggs ($P<0.001$). While pronotal width was a significant covariate ($P<0.01$), exposure to carbofuran did not influence the total egg hatch of survivors ($P=0.4$, $df=45$).

The results of experiments 3 and 4 are presented separately because of a significant interaction between treatment and experiment number ($P<0.01$, $df=56$) for these experiments when I examined the effects of methidathion on total egg hatch. In the 3rd experiment treatment was significant when both pronotal width and refrigeration were included as covariates ($P<0.05$, $df=25$). While females exposed to methidathion hatched a lower total number of eggs than control females ($P<0.01$), the means for LC_5, LC_{25} methidathion, and LC_5 carbofuran were similar. In experiment 4, treatment was not significant even when both pronotal width and refrigeration were included as covariates ($P=0.2$, $df=29$). In contrast to experiment 3, females exposed to methidathion tended to hatch a higher total number of eggs than control females ($P<0.1$). Again the means for LC_5, LC_{25} methidathion and for LC_5 carbofuran were similar. In both experiments increased pronotal width and increased refrigeration time appeared related to increased total egg hatch.

Pronotal width did not influence the egg viability (percent successful hatch) of control females ($P=0.3$, $df=16$). Exposure to carbofuran did not influence egg viability ($P=0.18$, $df=51$). With egg viability from experiments 3 and 4 there was also an interaction between treatment and experiment number ($P<0.05$, $df=39$). In the 3rd experiment exposure to methidathion did not influence egg viability ($P=0.6$, $df=19$), but in the 4th experiment egg viability of females exposed to LC_5, LC_{25} methidathion and LC_5 carbofuran was higher than egg viability of control females ($P<0.05$, $df=22$).

DISCUSSION

In his review of sublethal effects Moriarty (1969) explained that the possible effect of selection can sometimes be removed by assuming that individuals which died would, had they lived, have had a similar response to the untreated group. However, his method of adjustment assumes that mortality and the response variables are independent. I suggest a more conservative approach. If the most extreme cases of the untreated response are deleted, the effects possibly due to selection are removed. In this situation by "most extreme" I refer to cases in the untreated group which contribute most to the difference in the means. For instance, if a LC_5 treatment is used, the "most extreme 5%" would be arbitrarily deleted for comparison.

Specifically, I observed reduced male longevity following exposure to either carbofuran or methidathion. By deleting the 2 longest-lived con-

trol males, I deleted 8% of the males used as controls for the carbofuran treatment. If selection had caused the reduced longevity, then after this deletion I would expect no difference in the means. In fact, I found that control male longevity decreased from 25 d to 22 d, still longer than the LC_5 carbofuran longevity of 14 d ($P=0.058$). The corresponding decrease from deleting 7% of the controls used with the methidathion treatment was 32 d to 31 d, significantly longer than the LC_5 methidathion longevity of 20 d ($P<0.01$). These are evidence of a physiological effect on both carbofuran and methidathion survivors. Even with selection removed as a possible factor, both chemicals reduced the longevity of males surviving exposure.

Moriarty (1969) noted that latent toxicity had been recorded only for Holometabola. The decrease in male nabid longevity which I observed provides an example of latent toxicity, as Moriarty defined it, for a carbamate and an organophosphate acting in Paurometabola.

The difference between male and female longevity response may be related to different mixed-function oxidase (MFO) levels during and after exposure. Benkie and Wilkinson (1971) showed that male house crickets had lower levels of microsomal epoxidase than females. In my experiments an increased rate of detoxication may have diminished the stress experienced by females. Averaging all treatments, at 2 days post exposure I found 19% mortality for males and 16% mortality for females. By the 4th day post exposure I found 26% mortality for males and 19% mortality for females. Because male mortality increased 7%

while female mortality increased 3%, males may have been degrading the insecticides less rapidly than females.

Increased pronotal width was related to increased female longevity, eggs laid and eggs hatched. Because longevity, egg production, and total egg hatch were highly correlated ($P<0.0001$, $n=122$), I suspect that part of the influence of pronotal width on egg production and total egg hatch came from longevity. Females which lived longer had more time to lay eggs and, assuming a relatively constant percent hatch, females which lived longer therefore produced more progeny. On the other hand, because large control females contained higher numbers of eggs than small control females when dissected after death, pronotal width may be directly related to egg production. Evans (1982a) showed that large, female *Podisus maculiventris* had a higher rate of egg production than did small females.

Pretreatment refrigeration had no measurable effect on acute mortality or posttreatment longevity of either males or females, nor, in general, did refrigeration affect total eggs laid. However, females which were refrigerated longer tended to lay an increased maximum number of eggs per 4-day count interval. From my data it appears that refrigeration can safely be used to adjust groups of nabids to a common physiological age.

Some mechanisms seem more likely than others for explaining reduced male longevity. Latent toxicity from internal insecticide residues would seem more likely if I had been working with chlorinated hydro-

carbons. Continued detrimental levels of hormones caused by enzyme induction would seem likely if environmental levels of toxicants had been demonstrated to induce enzymes. However, Terriere and Yu (1974) pointed out that levels of toxicants far in excess of the LD_{50} appear necessary to induce enzymes.

Irreversible structural changes which occurred during the acute mortality period could have affected the longevity of survivors. Roan and Hopkins (1961) cited evidence that either insecticides or stress may change hormonal levels. As long as these levels are abnormal, growth and repair mechanisms in the insect may function improperly. Thus abnormal subcellular components, cells, tissues, or organs may be formed as a result of disturbed hormonal levels. Once such structures are malformed, they may contribute either to continued inefficiency of the insect or to failure upon exposure to normal physical, chemical or biological stresses.

Section III

SUBLETHAL EFFECTS OF CARBOFURAN AND METHIDATHION ON SECOND INSTAR *REDUVIOLUS AMERICOFERUS* (HEMIPTERA:NABIDAE)

SUBLETHAL EFFECTS ON SECOND INSTARS

INTRODUCTION

Tanton and Khan (1978) described external abnormalities in both the larvae and adults of a chrysomelid beetle dosed topically with aminocarb or fenitrothion in the larval stage. They presented evidence that internal abnormalities of the alimentary canal and fat body of larvae may have directly affected longevity, fecundity, and fertility of the adults. They related increased developmental time of the treated larvae to poor food utilization and high excretion rates. In fact, increased developmental time of nymphs or larvae surviving exposure to chemicals has often been reported (Chauthani and Adkisson 1966, Katiyar and Lemonde 1972, Lawrence et al. 1973, Chrominski et al. 1982, Ross and Brown 1982, Stewart and Philogene 1983). However, decreased developmental time may also occur (Hodjat 1971, Chelliah and Heinrichs 1980, Chrominski et al. 1982). In addition, variation in developmental time may be affected. Abo-Elghar et al. (1972) reported that the range of the developmental period was usually decreased in *Spodoptera littoralis* exposed to insecticides. In contrast, Stewart and Philogene (1983) found increased variation in developmental time of *Manduca sexta* fed sublethal doses of fenitrothion.

Larval mortality may be increased (Ng and Ahmad 1980) by exposure to sublethal doses. However, Walker et al. (1974) found similar percentages of exposed and control *Geocoris* reaching adulthood. Longevi-

ty of adults developing from exposed nymphs may be decreased (Hodjat 1971, Abo-Elghar et al. 1972, Shour and Crowder 1980) or increased (Abo-Elghar et al. 1972).

Total eggs laid by adults which develop from surviving nymphs may be affected by the level of the toxicant. While Hodjat (1971) found a decrease in egg production with higher doses of dieldrin, he found an increase over the check with lower doses. Tanton and Khan (1978) reported a decrease in fecundity of adult *Paropsis* from treated larvae. However, Walker et al. (1974) found no differences in fecundity of adult *Geocoris* reared from nymphs exposed to LC_{20} to LC_{50} insecticides. Abo-Elghar et al. (1972) found increased pre-oviposition and oviposition periods and decreased percent egg hatch. Chelliah et al. (1980) discovered increased progeny production in adult *Nilaparvata lugens* exposed as 5th instars.

Sublethal exposure of *Reduviolus* nymphs in the field seems likely since nymphs may emerge to walk on alfalfa leaves having insecticide residues. Although surviving adults might disperse from sprayed fields, nymphs are restricted to these fields, which may also have diminished food supplies. Because a major portion of the field population is nymphal during the growing season, sublethal effects from nymphal exposure might significantly influence seasonal population dynamics. My primary objective was to quantify the sublethal effects on 2nd instar *Reduviolus*(=*Nabis*) *americoferus* (Carayon) caused by two insecticides used on alfalfa.

MATERIALS AND METHODS

To rear 2nd instar *R. americoferus* I placed groups of 10 pairs of field-collected adults in 30 cm high cages made from 6 cm diam polybutylene tube. Each cage contained a supply of vestigial-winged *Drosophila melanogaster* for food and an alfalfa stem for oviposition. After a week I transferred adults to new cages. As soon as the eggs began to hatch, I tapped each alfalfa stem daily over a pan rimmed with Fluon® AD1 to collect the first instars. I transferred these nymphs to 65 ml cages containing alfalfa stems and *Drosophila*, with 8-20 nymphs per cage. After rearing the nymphs for 4-5 days in a warm room (25 ± 5°C, 14 h photophase), I transferred them to a cold room (19 ± 2°C, 14 h photophase) for an additional 2-3 days before exposing them to insecticides.

Just before the beginning of the exposure I recorded for each 2nd instar the size of the abdomen in dorsal view relative to the head-thorax. In this rating system 1 = abdomen much smaller than the head plus thorax, 2 = abdomen smaller than the head plus thorax, 3 = abdomen equal to the head plus thorax, 4 = abdomen larger than the head plus thorax, 5 = abdomen much larger than the head plus thorax (Fig. 1). From preliminary work I suspected that 2nd instars with large abdomens were more mature than those with small abdomens.

Exposure was as described in the previous section except that: 1) I randomly selected 2nd instars shaken from each 65 ml cage; 2) I used forceps and paper disks in transferring nymphs into the exposure

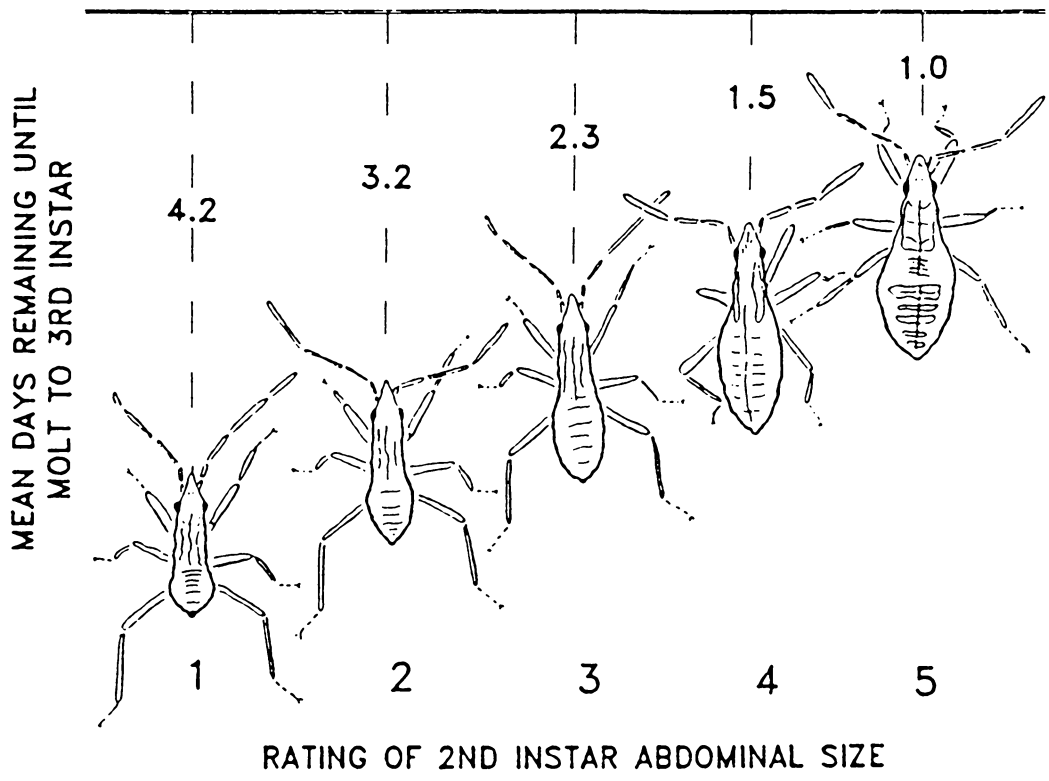

Figure 1: Rating of 2nd instar abdominal size relative to head plus thorax. Nymphs were rated just before exposure to insecticide-treated filter papers.

chambers; 3) I used forceps and moistened paper disks in transferring nymphs out of the exposure chambers.

After a recovery period of a day I transferred nymphs to 65 ml cages and reared them individually until adulthood. For each individual I recorded days remaining until the molt into the 3rd instar, combined days in the 3rd-5th instars, and adult pronotal width. Before pairing I used refrigeration (6 ± 2°C) to adjust adults to a uniform physiological age of 2 days as adults at 19 ± 2°C. Adult longevity values based on a rearing temperature of 24 ± 4°C were adjusted by subtracting the number of days adults were refrigerated. To increase the probability of successful mating mates were rotated within treatments every 4th day. The number of eggs laid and hatched were determined as in the previous section.

Treatments based on mortalities within 2 d of treatment were LC_{15} carbofuran (0.0025% vol Furadan® 4F per vol water), LC_{35} carbofuran (0.005% v/v), LC_0 methidathion (0.005% vol Supracide® 2E FL per vol water), LC_5 methidathion (0.01% v/v), or control (distilled water). Preliminary tests showed a 2nd instar LC_{50} for carbofuran of 0.0058% v/v (95% fiducial limits 0.0042 to 0.0090) and a LC_{50} for methidathion of 0.019% v/v (f.l. 0.016 to 0.023). Seven sublethal experiments were performed using 2nd instar nabids. In addition to the controls, experiments 1 and 2 included LC_{15} and LC_{35} carbofuran; experiments 3 and 4 included LC_0 and LC_5 methidathion; experiments 5-7 included only controls and LC_{35} carbofuran.

There were several modifications of the method for experiments 5-7. First, I refrigerated nymphs for 7-8 days before exposure. Second, because all 65 ml cages were filled with nabids from earlier experiments, I reared these nymphs in 27 ml glass vials until the adult molt. Third, in order to test the effects of reduced food availability, I gave odd-numbered nymphs half as many *Drosophila* for food as I gave even-numbered nymphs. On alternate days odd-numbered nymphs were given 2 adult flies per 2nd instar nymph, 4 flies per 3rd instar nymph, 6 flies per 4th instar nymph, and 8 flies per 5th instar nymph. Finally, I refrigerated young adults as soon as they had molted, and paired them when I removed them from the refrigerator, during the 1st physiological day of adulthood. Because chemicals and methods varied, I analyzed separately experiments 1-2 ,3-4, and 5-7. However, when the response of a control variable was not affected by experiment number, I sometimes pooled the controls from experiments 1-4.

My analysis for sublethal effects included only nymphs surviving beyond the 4th day postexposure. In order to use sex as a classification variable, I analyzed "days remaining until the molt into the 3rd instar" using the 91% of the nymphs which reached adulthood because I could not sex nymphs. I used SAS procedure GLM (SAS Institute 1982) to examine the effects of exposure on the biology of survivors. I transformed count data with SQRT(X+1) and proportions with ARCSIN(SQRT(X)). For nymphal biology the only meaningful covariate was pretreatment abdominal size (relative to the head-thorax). For

biology of female adults reared from nymphs, abdominal size at the time of exposure was the only covariate because 2nd instar abdominal size and days in the refrigerator as young adults were highly correlated ($P<0.001$, $df=107$). For male adults reared from nymphs, although refrigeration of young adults was not as highly correlated with 2nd instar abdominal size, refrigeration was not a significant covariate in exploratory ANCOVA models. Furthermore, for both sexes refrigeration time could have been contaminated by treatment. Thus abdominal size at the time of exposure was the only covariate in these experiments.

When treatment was significant in ANCOVA or ANOVA, I used contrasts of the form "CONTROL vs OTHERS" or "LOW CONCENTRATION vs HIGH CONCENTRATION." Least-squares means and Tukey's studentized range test provided other tools for examining differences between means. I report the error degrees of freedom (df) for ANOVA and ANCOVA and the sample size (n) for chi-square tests.

RESULTS

When male and female responses differ, I will present results separately by sex. For both males and females abdominal size was a highly significant covariate for the variable "days remaining until the molt into the 3rd instar" ($P<0.0001$, $df=45$). Even though nymphs were 7-8 d old chronologically at the time of exposure, age was not a significant variable in exploratory models. However, individuals with large abdomens usually were near the molt to the 3rd instar.

For nymphs exposed to methidathion, females reached the 3rd instar more slowly (2.6 d) than males (2.2 d) ($P<0.05$, df=91). (These least-squares means differ slightly from those in Table 2 because when female and male developmental rates were compared in this analysis, means were adjusted to the mean abdominal size for both sexes). For female nymphs exposure to LC_{35} carbofuran significantly decreased days until the 3rd instar ($P<0.05$, df=38); LC_{15} carbofuran produced a non-significant decrease (Table 2). For male nymphs exposure to carbofuran had no significant effect on days till the 3rd instar ($P=0.16$, df=47). For male nymphs exposed to methidathion, treatment was not significant ($P=0.08$, df=45). Similarly, female nymphs exposed to methidathion were unaffected ($P=0.6$, df=45). However, for both males and females, with increased methidathion concentration there was a trend toward increased time until the 3rd instar. In brief, after adjusting for variations in pre-exposure abdominal size, LC_{35} carbofuran significantly decreased the time remaining until the molt into the 3rd instar for female nymphs, and male nymphs exposed to methidathion reached the 3rd instar more quickly than female nymphs.

Abdominal size ($P=0.7$) and treatment ($P=0.2$, df=38) did not affect the combined duration of the 3rd-5th instars of females exposed to carbofuran (Table 2). Neither abdominal size ($P=0.4$) nor treatment ($P=0.9$, df=45) influenced the duration of the 3rd-5th instar of females exposed to methidathion. For both chemicals, although male abdominal size was significant as a covariate ($P=0.001$), treatment was not signifi-

Table 2. Developmental time and adult longevity of Reduviolus americoferus surviving exposure as 2nd instars.

Treatment	Days remaining until molt to 3rd instar[1]		Duration of 3rd-5th instars[1] (days)		Adult longevity[1] (days)		
	Males[2]	Females[2]	Males[2]	Females[2]	Both sexes	Males	Females[2]
carbofuran							
control	1.8 ± 0.26a[3] (14)	2.6 ± 0.25a (12)	12.4 ± 0.021a (14)	12.0 ± 0.23a (12)	80 ± 6.2a (13)	100 ± 8.8a (8)	51 ± 5.2a (12)
LC_{15}	2.5 ± 0.27a (13)	2.4 ± 0.26ab (10)	12.6 ± 0.22a (13)	12.5 ± 0.25a (10)	56 ± 4.6b (23)	59 ± 6.9b (13)	50 ± 5.7a (10)
LC_{35}	2.1 ± 0.20a (24)	1.8 ± 0.19b (20)	12.8 ± 0.16a (24)	12.5 ± 0.18a (20)	67 ± 3.3a (44)	77 ± 5.1c (24)	54 ± 4.0a (20)
methidathion							
control	1.6 ± 0.23a (14)	2.7 ± 0.26a (12)	12.5 ± 0.24a (14)	12.0 ± 0.20a (12)	51 ± 5.8a (13)	55 ± 9.5 (6)	51 ± 5.4a (12)
LC_{0}	2.0 ± 0.25a (13)	2.8 ± 0.26a (12)	12.3 ± 0.26a (13)	12.0 ± 0.20a (12)	36 ± 4.2b (25)	41 ± 6.5ac (13)	29 ± 5.4b (12)
LC_{5}	2.3 ± 0.19b[4] (22)	3.0 ± 0.18a (25)	12.3 ± 0.19a (22)	12.0 ± 0.14a (25)	60 ± 3.0a (47)	62 ± 5.0ab (22)	55 ± 3.7a (25)

[1]Least-Squares Means ± S.E. (Sample size in parenthesis)
[2]Pooled checks from experiments 1-4. Least-Squares Means and Standard Errors of checks differ because different subsets of the data have different expected values for balanced designs.
[3]Least-Squares Means with different letters were significantly different when considered pairwise.
[4]Although the LSMEAN was higher for LC_5 males, treatment was not significant in the overall ANCOVA model.

cant (carbofuran, P=0.4, df=47; methidathion, P=0.8, df=45). Summarizing, for males increased pre-exposure abdominal size of 2nd instars was related to increased duration of the 3rd-5th instars; for females abdominal size was unimportant; for both sexes treatment had no effect.

Exposure of 2nd instars to carbofuran or methidathion had no effect on the pronotal width of the surviving adults. Pre-exposure abdominal size was unimportant as a covariate in explaining adult pronotal width.

Exposure of 2nd instars to carbofuran reduced the adult longevity of male survivors ($P<0.01$, df=42) (Table 2) to 70% of the control mean of 100 d. For both sexes, LC_{15} carbofuran reduced adult longevity compared with LC_{35} carbofuran ($P<0.05$, df=74). Males lived significantly longer than females ($P<0.0001$). Longevity of adults exposed to methidathion as 2nd instars was the same as control longevity. However, the LC_0 group had decreased longevity compared to the LC_5 group ($P<0.0001$, df=81). Adult male longevity in the LC_0 group was 65% of the LC_5 group, while adult female longevity in the LC_0 group was 55% of the LC_5 group. Again, males lived longer than females ($P<0.05$, df=81). In brief, while carbofuran reduced adult longevity when compared with controls, methidathion did not reduce adult longevity. For both chemicals longevity was reduced for the lower concentration (LC_{15} carbofuran and LC_0 methidathion) when compared with the higher concentration (LC_{35} carbofuran and LC_5 methidathion). Males lived longer than females. Exposure of nymphs to carbofuran or methidathion did not influence the number of adults with missing portions of appendages (P=0.3, n=233).

There was no apparent effect of exposure on the number of females laying eggs; over 95% of the surviving females laid eggs. Neither exposure to carbofuran nor abdominal size at the time of exposure affected total egg production of surviving females ($P=0.5$, $df=25$) (Table 3). Although total egg production by methidathion survivors was similar to the control value, total egg production by females exposed to LC_0 methidathion was significantly lower than that of LC_5 methidathion survivors ($P<0.01$, $df=45$).

The reduced longevity of females exposed to LC_0 methidathion compared to females exposed to LC_5 methidathion affected the time the 5th, 25th, and 50th percentile eggs were laid. Thus although LC_0 methidathion females appeared to lay eggs significantly earlier than LC_5 females ($P<0.01$, $df=39$), when longevity was introduced in the model as a highly significant ($P<0.0001$), contaminated covariate, treatment did not appear to influence the time eggs were laid ($P=0.4$, $df=38$). I conclude, in this case, that shifts in time of egg-laying are only a reflection of changes in longevity.

For the oviposition rate (eggs laid per female per adult days alive) from the two carbofuran experiments there was an interaction between treatment and experiment number. In the first experiment, exposure to carbofuran significantly increased the oviposition rate when compared with the control ($P<0.05$, $df=14$). In the second experiment, oviposition rate was not significantly influenced by treatment ($P=0.07$, $df=14$). In both experiments there was a trend of increased oviposition rate for the

Table 3. Fecundity, oviposition rate, and progeny production of Reduviolus americoferus surviving exposure as 2nd instars.

Treatment	Total eggs laid.[1]	Oviposition rate.[1]	Total progeny production.[1]
control[2]	422 ± 73.1 (12)	7.5 ± 0.89 (12)	241 ± 51.1 (12)
carbofuran			
LC_{15}	477 ± 58.7 (11)	9.0 ± 0.69 (11)	259 ± 39.5 (11)
LC_{35}	400 ± 36.8 (20)	7.2 ± 0.46 (20)	228 ± 30.5 (20)
methidathion			
LC_{0}	220 ± 28.6 (12)	7.7 ± 0.43 (11)	96 ± 21.0 (12)
LC_{5}	406 ± 31.1 (24)	7.2 ± 0.47 (24)	178 ± 32.1 (24)

[1]Means ± S.E. (Sample size in parenthesis)
[2]Pooled controls from experiments 1-4.

LC_{15} carbofuran group compared with the control, but decreased oviposition rate for the LC_{35} carbofuran group compared with the LC_{15} group. In the second experiment this reduction was significant ($P<0.05$, df=14). Thus I have evidence that LC_{15} carbofuran tends to stimulate an increased oviposition rate, while LC_{35} carbofuran tends to decrease oviposition rate compared to LC_{15} carbofuran (Table 3). Although not significant ($P>0.05$, df=32), I found the same trends in maximum eggs laid in any 4-day count interval.

Exposure to LC_0 or LC_5 methidathion did not influence oviposition rate ($P=0.7$, df=38) (Table 3). Similarly, second instar abdominal size at the time of exposure had no influence on oviposition rate ($P=0.8$, df=43). Although not quite significant, increased pronotal width (possibly contaminated by treatment) was related to increased oviposition rate ($P=0.054$, df=38).

Exposure to carbofuran did not affect the number of females which laid only non-viable eggs. Although there was a tendency for more females exposed to LC_5 methidathion to lay eggs which did not hatch when compared with control females (21% vs 6%), this was not significant ($P=0.1$, n=55).

Neither abdominal size of nymphs nor exposure to either insecticide influenced the total progeny production (total successful egg hatch) of surviving females ($P=0.4$, df=28, 36) (Table 3). Likewise, exposure had no effect on egg viability (percent hatch) ($P=0.5$, df=28, 35).

In experiments 5-7 I found no direct influence of the food supply of the nymphs on any of the response variables. Although the data suggested a reduction in total eggs laid by adults from nymphs fed half as much, this was not significant ($P=0.1$, $df=25$). However, I found a significant interaction of feeding rate and exposure to LC_{35} carbofuran. Nymphs exposed to LC_{35} carbofuran and fed half as much hatched a lower percentage of eggs than exposed nymphs fed more *Drosophila* ($P<0.05$, $df=22$). The trend in the unexposed groups was opposite. Unexposed nymphs fed half as much tended to hatch a larger percentage of eggs than unexposed nymphs fed more *Drosophila* ($P<0.1$).

DISCUSSION

While the relationship between 2nd instar abdominal size and days until the 3rd instar was simple (larger abdomens corresponded to more mature nymphs), the effect of 2nd instar abdominal size on the duration of the 3rd-5th instars of males was less clear. Benke et al. (1972) demonstrated large variations in microsomal oxidase activity within instars of *Gromphadorhina portentosa,* with lowest activity at ecdysis. Because increased abdominal size of 2nd instar males was related to increased duration of the 3rd-5th instars, I might hypothesize that large abdominal size corresponded to male nymphs with decreased enzyme activity. In that event the stresses of the experiment might have had a greater impact on males almost ready to molt. However, Benke et al.

(1972) found similar variations in nymphal enzyme levels in both sexes of *C. portentosa*. Because female nabid nymphs showed no similar effect, this explanation seems unlikely.

Reexamination of my data showed that nymphs with larger abdomens survived better than nymphs with smaller abdomens ($P<0.01$, $n=306$). This suggests that stored energy and fluids may have been more important factors for my experiments than variations in mixed-function oxidase activity. Nymphs with large abdomens may have survived exposure better because they had more stored energy available to cope with stress and more fluid available for excretion. Tanton and Khan (1978) found up to 360-fold increases in excretion rate of *Paropsis* 2nd instars exposed to fenitrothion or aminocarb compared to controls.

Although not always significant, insecticides were observed to affect the rate of development to the 3rd instar. First, carbofuran tended to accelerate development, especially in females. Second, methidathion tended to slow development, especially in males. Even though treatment did not significantly affect the rate of development of females exposed to methidathion, these females developed more slowly than the males. My conclusions are 1) that the two insecticides appeared to have opposite effects on 2nd instar developmental rate (although this may have been influenced by different LC values) and 2) that sex influenced the response even in 2nd instars.

Treatment did not significantly affect the rate of development of the 3rd-5th instars. Because increased 2nd instar abdominal size of males

was related to increased duration of the 3rd-5th instars, while female abdominal size was unimportant, I see further evidence of sex differences. However, the nature of these differences is unclear.

I may speculate that carbofuran or methidathion did not interfere with the food-gathering and food-assimilating abilities of the nabids because exposure of 2nd instars to these chemicals had no effect on adult pronotal width. If there had been decreases in these abilities, I would have expected either decreased pronotal width or compensation through increased developmental time.

Both carbofuran and methidathion influenced adult longevity. However, while carbofuran reduced the longevity of adults compared to the control, methidathion reduced the longevity only of the LC_0 group compared to the LC_5 group. As I suggested earlier (Section II), structural damage which occurred during the period of intoxication might have longterm effects. In the case of nymphs exposed as 2nd instars, even though 4 molts occurred before adulthood, internal structural abnormalities could have been carried through into the adults. It is interesting that exposed adults showed an increase in the frequency of missing portions of appendages while adults from exposed nymphs showed no increase.

Most of the observed effects of 2nd instar exposure on the egg-laying of adult females were a direct result of changes in longevity. An exception was the increased oviposition rate for LC_{15} carbofuran, but a decreased rate for LC_{35} carbofuran compared to LC_{15} carbofuran.

This pattern is reminiscent of Hodjat's (1971) finding that total eggs laid increased in *Dysdercus* adults which developed from nymphs surviving low doses of dieldrin, while total eggs laid decreased in adults from nymphs surviving high doses.

I performed experiments 5-7 to answer the question of whether reduced food availability changes the effect of sublethal doses on nabid nymphs. Because none of the response variables (including pronotal width) showed any influence due to feeding rate alone, I suspect that individuals fed half as much still had enough food for normal development. If, as Evans (1982b) suggests, success of nymphs in acquiring food influences adult body size, I would expect reduced pronotal widths in poorly fed nymphs. Nevertheless, the finding that nymphs exposed to LC_{35} carbofuran and fed half as much produced a lower proportion of viable eggs than exposed nymphs fed more during the nymphal period suggests that food availability may interact with pesticide exposure to influence the biology of survivors.

Section IV

SUBLETHAL EFFECTS OF CARBOFURAN AND METHIDATHION ON FLICKING IN MALE *REDUVIOLUS AMERICOFERUS* (HEMIPTERA:NABIDAE)

SUBLETHAL EFFECTS ON FLICKING

INTRODUCTION

Ekblom (1926) described the structure and playing of the sound organ of male *Nabis flavo-marginatus* Scholz and other Swedish species. However, because he could hear no sound, he speculated that the sound was "too weak or too fine to be perceived by the human ear." Leston (1957) in his analysis of stridulation in Hemiptera concluded that the so-called "stridulatory" organs of Nabidae are non-stridulating, i.e. non-sound-producing. I propose the term *flicking* to describe this behavior in male nabids. Flicking, as I define the term, is a rhythmic, repeated movement of the hind leg of a male nabid, in which the tibial spines are usually brushed along a comb on the same side of the bent abdomen tip as the moving leg. I prefer the term *flicking* to *stridulating* or *playing* for the following reasons: 1) I find no evidence that sound is produced (Leston 1957); 2) I feel that the word *flicking* suggests the rapid motion of the leg during each stroke, whatever the functions of the behavior; 3) I believe that the usual function of the stroke is to flick pheromone droplets from the tibial spines into the air because I have observed waves of mist-like droplets leaving the hind tibia. Since I have observed no response to this material, its status as a pheromone has not been substantiated. Nevertheless, because I believe that this fluid will prove to be a pheromone, I will use the term *pheromone* throughout this paper.

In flicking, the strokes are repeated too rapidly to count. However, a photograph of a male *Reduviolus*(=*Nabis*) *americoferus* (Carayon) shows at least 9 waves of pheromone beside the hind tibia. I infer that these 9 waves correspond to a series of 9 strokes of the hind leg. A typical nabid male performs a series of strokes, pauses briefly, and then performs another quick series of strokes. At times, however, males may not flick for long periods of time. Instead they may be perched motionless, or preening, or walking, or attempting flight.

Flicking movements occasionally are much slower than normal. At such times flicking might be confused with cleaning. However, in flicking, the tip of the abdomen is bent, and with each stroke the hind tibia appears to accelerate, then abruptly halt, while in cleaning, the abdomen is usually not bent, and the tibial movements are irregular rather than rhythmic. Although I have observed males flicking on more than 1500 occasions, I have never seen females flicking. Thus I suspect that the stridulating females reported by Hendrick (page 66, 1967) may have been preening.

While males often flick on one side for long periods of time, they may also change sides. One male, which I observed flicking nightly for almost a month, had no left hind tibia. When he bent the tip of his abdomen toward the left, he flicked his left hind femoral stump with the same rhythm he used when flicking with his complete right leg. Thus, despite the absence of left hind tibial spines, I would still label this behavior as flicking.

In some flicking, the tibia, although present, did not touch the tip of the abdomen. A male, which flicked normally on one side, did not touch the tibia to the abdomen when flicking on the other side. In this case, perhaps flicking served to fan pheromone rather than fling it.

Nabids sometimes bent the tip of the abdomen without flicking. Most often this appeared to represent a nabid which was preparing to flick, but which was disturbed. Several times, however, I observed nabid males dragging the bent tip of the abdomen along an alfalfa or cage surface. In these cases I think that they may have been marking the surfaces with pheromone. Although nabids also bent the tip of their abdomens when defecating, the behavior was different from that involved in pheromone dispersal.

Several authors have documented increases in insect activity after sublethal exposures to insecticides. Hodjat (1971) observed increased numbers of *Dysdercus fasciatus* males fluttering a week or more after treatment with dieldrin or DDT. Irving and Wyatt (1973) found that treatment of arenas with pirimicarb doubled the scale-stabbing activity of female *Encarsia formosa*. Kwan and Gatehouse (1978) inferred that mutual disturbance became an important mortality factor after *Glossina morsitans* males and females were treated with endosulfan.

On the other hand, Floyd and Crowder (1981) showed that wing-fanning responses of male *Pectinophora gossypiella* to gossyplure were decreased in survivors of 4 day LD_{50} and LD_{80} dosages of permethrin; however, these males mated as often as controls.

With this background my objective was to document some of the factors which influenced flicking in male nabids. Specifically, I wanted to know whether sublethal exposures to carbofuran or methidathion influenced flicking in surviving *R. americoferus*.

MATERIALS AND METHODS

I observed adult male *R. americoferus* daily for evidence of flicking. Observations were made of nabids in 65 ml cages between 1800 and 2400 h with cages held about 20 cm from a 60 W light bulb and 10 cm from the observer's eye. I observed each nabid for about 10 s for evidence of flicking. For males exposed as 2nd instars (Section III), if there were no signs of flicking, I reexamined the cage for another 10 s after examining all the other cages in a box. For each male I recorded 1) flicking, 2) flicking only on reexamination, 3) not flicking during either examination, but with bent abdomen tip for at least part of one of the intervals. Every 4th d nabids were transferred to new cages with new mates. From the flicking data I created an index of flicking where 0.0 = no flicking observed on any evening during the 4-day time interval, and 1.0 = flicking observed on each of the 4 evenings. I created a mean flicking index over the lifespan of each nabid by summing the number of evenings each male was observed flicking and dividing by the number of evenings each male was observed. Because I recorded these codes for nabids from different treatments until all individuals had died, I were able to examine the effect of exposure to insecticides

on the frequency (of occurrence) of flicking. Data from experiments on adults exposed to insecticides (Section II) were limited to one 10-s examination each evening of males exposed to LC_5 carbofuran, LC_5 methidathion, LC_{25} methidathion, or checks.

Although feeding on *Drosophila* did not interrupt nabid flicking (I often observed males feeding and flicking simultaneously), on a few evenings I found decreases in flicking that were apparently due to too many *Drosophila* in the cages. When there were so many *Drosophila* that they were crawling on the nabids, the nabids spent more time scraping them off and trying to move to a less disturbed environment than they did flicking. This situation occurred rarely.

Another factor that may have played a role was change in light intensity. One male about 2 wk in adult age stopped flicking when his cage was placed about 15 cm from a 60 W incandescent light. Whenever his cage was moved above the circle of light into semi-darkness, he recommenced flicking. This mini-experiment was repeated 15 times in about 5 minutes. On the other hand, a 4-wk old male started flicking when light from an overhead bank of fluorescent lights was blocked. He stopped flicking when the lights were exposed (repeated 3 times). However, two males over 6 weeks old continued flicking when only 8 cm from the 60 W light. Thus although light may have had some influence, its effect may have changed with nabid age.

Usually copulation and flicking seemed to be mutually exclusive. However, one 2-wk old male was observed flicking while *in copula* with

a female. Males that were unable to retract their aedeagus after mating continued flicking at apparently normal frequencies until death weeks later.

To examine the influence of time of day on flicking in the laboratory, on one day I observed a group of 10 isolated males, each of which was at least 11 weeks in adult age. For each male I recorded 1) whether it was flicking when observed under standard conditions hourly from 1100 to 2300 h, and 2) how many seconds each nabid was flicking when observed with the cage undisturbed on the shelf for two 60-s observations, ca. 1000, 1400, and 1800 h. As individual leg strokes occurred too quickly to count, I turned off the stopwatch whenever pauses greater than one second occurred.

Mean flicking indexes were based on a mean male longevity of 63 days as an adult; thus they approximated a continuous distribution even though they were derived from discrete data. I analyzed flicking indexes with procedures FREQ, GLM, and UNIVARIATE of SAS (SAS Institute, 1982). I checked for equality of variances using the variance ratio and F_{max} tests with tables in Rohlf and Sokal (1969).

RESULTS

Adults reared from 2nd instars exposed to insecticides

Within each treatment the mean flicking index was bimodal. Specifically, after maturity most males (88%) were observed flicking on most evenings; the remainder were observed flicking rarely, if at all.

Neither treatment ($P=0.2$, $n=85$), nor second instar abdominal size ($P=0.7$), nor young adult refrigeration ($P=0.17$) seemed to affect the proportion of males flicking with normal vs subnormal indexes.

Analyses of the data from all males revealed no significant influence due to carbofuran ($P=0.8$, $df=47$), methidathion ($P=0.5$, $df=46$), or the potential covariates, 2nd instar abdominal size and young adult refrigeration. Although the residuals from these analyses were bimodal, the variances among treatments were similar.

When only those males which were regularly observed flicking were considered (ca. 88% of the population), decreases in the mean flicking index were found due to exposure to carbofuran ($P<0.05$, $df=41$), or methidathion ($P<0.01$, $df=39$) (Table 4). No covariates were significant. In all treatments flicking was observed rarely in young males and frequently in males older than 10 d (Fig. 2).

For males exposed to carbofuran there was a significant increase in the number of times the bent tip of the abdomen was observed when males were not flicking ($P<0.05$, $n=1188$), while for males exposed to methidathion there was no difference from the controls ($P=0.4$, $n=1107$).

For 10 older males observed hourly in the insectary from 1100 to 2300 h on one day, time of day had no apparent effect on the number flicking. At every hour, 7-9 males were flicking when observed under standard conditions. However, only 5-8 males were flicking when observed a minute at a time undisturbed on the shelf at 1000, 1400, and 1800 h. These males spent a mean of 14 s of each minute flicking.

Table 4. Mean flicking indexes of Reduviolus americoferus surviving exposure as 2nd instars.

Treatment	All surviving males[2] (including subnormal flickers)	Most (88%) surviving males[2] (excluding subnormal flickers)
check	0.75 ± 0.072 (14)	0.88 ± 0.023 (11)
carbofuran		
LC_{15}	0.74 ± 0.056 (12)	0.82 ± 0.022 (10)
LC_{35}	0.78 ± 0.032 (24)	0.81 ± 0.017 (23)
methidathion		
LC_{0}	0.66 ± 0.051 (13)	0.70 ± 0.043 (12)
LC_{5}	0.67 ± 0.048 (22)	0.74 ± 0.024 (19)

[1]For each male the flicking index was the number of evenings observed flicking divided by the total evenings observed.
The mean flicking index was the mean of flicking indexes within each treatment.
[2]Mean flicking index (1.0 = every male flicking every evening observed) ± S.E.
(Sample size in parenthesis)

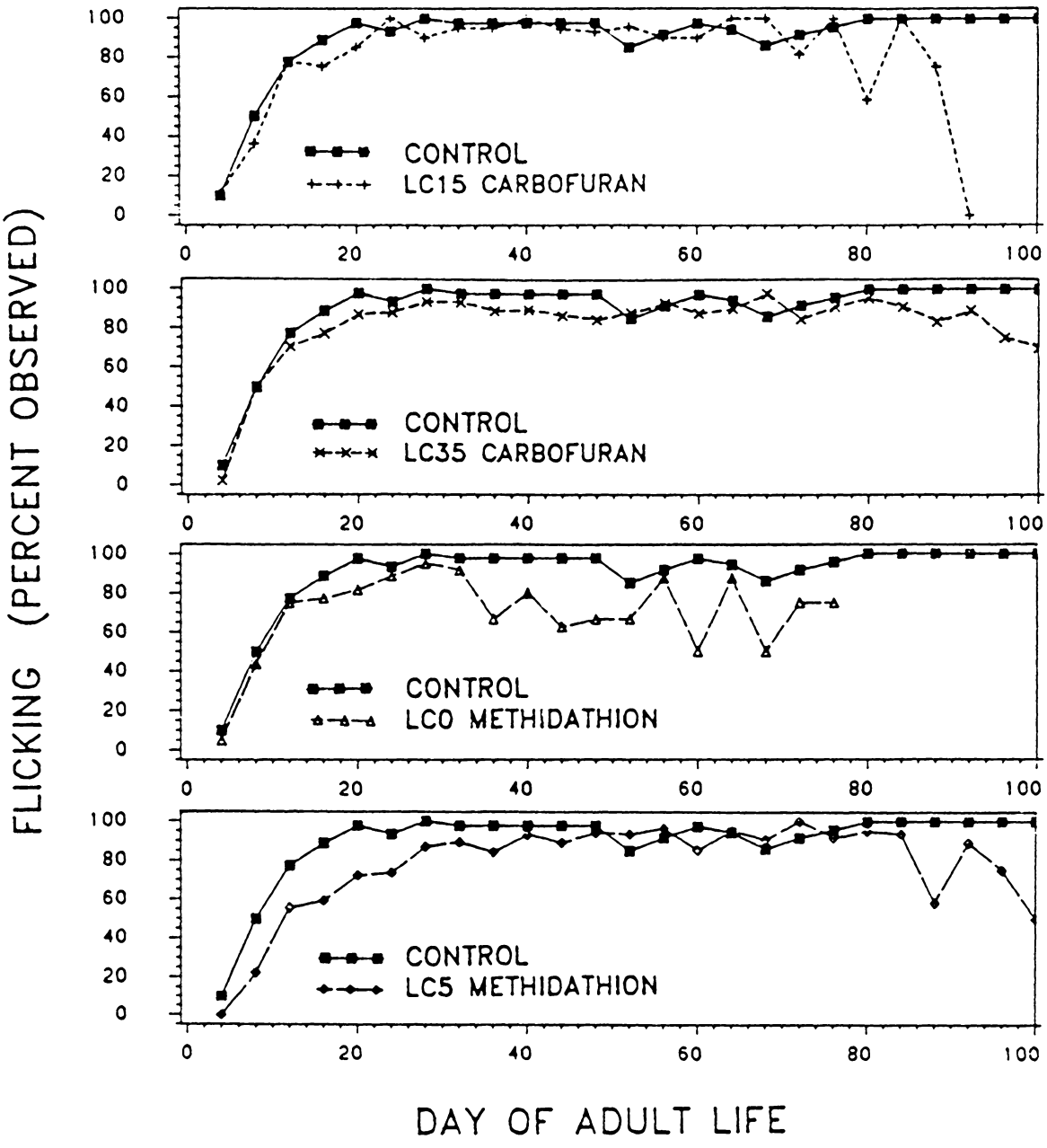

Figure 2: Percentage of males flicking as a function of adult age (survivors of exposure as 2nd instars). Only the 88% of the male population with normal mean flicking indexes were included.

Time of day did not significantly affect the number of seconds spent flicking.

Young adults exposed to insecticides

For males exposed to insecticides as young adults (Section II) both the mean flicking indexes and the residuals from the analyses were unimodal within each treatment. In one experiment, exposure to methidathion significantly decreased flicking ($P<0.01$, $df=17$). LC_5 carbofuran decreased flicking, but not significantly ($P=0.4$). Increased pretreatment refrigeration decreased flicking ($P<0.01$). For another experiment, although refrigeration was almost significant as a covariate ($P=0.055$, $df=18$), treatment was non-significant ($P=0.9$).

DISCUSSION

When I considered all the males reared from 2nd instar nymphs, I found no significant influence of exposure on the mean flicking index. However, when I considered only the 88% of males with mean flicking indexes above 0.42 (this value divided the upper from the lower mode in all treatments), I found that exposure to either insecticide significantly decreased the mean flicking index. Until I understand what caused some males to flick infrequently, I cannot conclude that insecticides did, or did not, affect flicking. Although chi-square values indi-

cated that neither treatment nor refrigeration caused some adults to flick infrequently, I would need more data to exclude these factors.

When I considered all males exposed to carbofuran as 2nd instar nymphs, I found a significant increase in the number of times the bent tip of the abdomen was observed, even though the insect was not observed flicking. Methidathion appeared to have no effect. If this increase had been due to an enhanced sensitivity of exposed males to disturbance, I would have expected a decrease in the mean flick index for the same males. Because I observed no decrease in mean flick index when all males were considered, another explanation is needed.

Time of day (from 1100 to 2300 h) influenced neither the number of older males flicking nor the amount of time each spent flicking; however, younger males or males in the field may behave differently. It is interesting that even though undisturbed males flicked a mean of only 14 s per minute, they were usually observed flicking during the standard, two 10-s examinations. This suggests either that males distribute their flicking through time effectively to be noticeable, or that vibrations or other features of the standard examination procedure tend to stimulate flicking. Whereas adults from exposed nymphs had bimodal mean flicking indexes, adults exposed as 2-d old adults had unimodal mean flicking indexes. Because 2-d old adults were reared from field-collected, late instar nymphs, it seems possible that some abnormal, laboratory-reared nymphs may have survived to adulthood due to reduced selective pressure in the laboratory.

Both experiments for males exposed as 2-d old adults showed that increased pretreatment refrigeration was related to decreased posttreatment flicking; however, exposure to insecticides decreased flicking in only one experiment.

In conclusion, it appears that exposure to carbofuran or methidathion decreased the mean flick index of male nabids that flicked normally. However, with the current data, I do not know whether this decrease is biologically significant. Until the biological significance of flicking is better understood, I cannot predict the effect of changes in flicking on the population dynamics of nabids.

Section V

BIOLOGY OF *REDUVIOLUS AMERICOFERUS* (HEMIPTERA:NABIDAE)

BIOLOGY

INTRODUCTION

Reduviolus(=*Nabis*) *americoferus* (Carayon) is usually the most common arthropod predator found in alfalfa in Virginia. Recently Braman et al. (1984) reported the temperature-developmental rates of *R. americoferus* and *Reduviolus*(=*Nabis*) *roseipennis* (Reuter). Their results suggest that below ca. 24 °C males developed more slowly than females while above 24 °C males developed more rapidly.

In a study of feeding rates, Propp (1982) observed that fifth instar *R. americoferus* consumed a maximum of about twice as many *Spodoptera* larvae as 4th instars consumed. In addition, he noted a reduced consumption rate before the molt. Sloderbeck and Yeargan (1983) found that 3rd instar *R. americoferus* consumed about twice as many *Plathypena* eggs or 1st instars in 24 h as 1st instar *R. americoferus*, while 5th instars consumed about 20 times as many. However, they suggested that the 3rd instar *R. americoferus* consumption rates may have been low because nymphs were approaching the molt to the 4th instar. For *R. roseipennis* they found 5-fold and ca. 20-fold increases for 3rd and 5th instar consumption, respectively, compared to 1st instars. Donahoe and Pitre (1977) found similar increases for *R. roseipennis* feeding on 1st instar *Heliothis zea* with 4-fold and 20-fold increases for 3rd and 5th instar over the daily consumption rates for 1st instar. Mundinger (1922) mentioned that adult nabids will reach for a second prey item

before the first is completely consumed. Donahoe and Pitre (1977) found that *R. roseipennis* females consumed more prey than males.

Braman et al. (1984) found a pre-oviposition period for *R. americoferus* of 8-10 days at 24°C. Perkins and Watson (1972) noted peak oviposition rates for *Reduviolus*(=*Nabis*) *alternatus* (Parshley) when females were about 12-13 d old. Although Taylor (1949) found caged female *R. alternatus* laying 90-200 eggs each (mean 127 eggs) with a mean oviposition period of 20 days, Perkins and Watson (1972) reported 17-595 eggs (mean 281) with a mean oviposition period of 31 days at 28°C. Similarly, Hormchan et al. (1976) quantified aspects of the biology of *Tropiconabis capsiformis* (Germar).

Parasites may play an important role in nabid population dynamics. Werner and Butler (1957), Clancy and Pierce (1966), Morrill (1969), and Hendrick and Stern (1970) presented parasitization rates of nabids by *Leucostoma simplex* (Fallen). Morrill (1969) suggested that holding *R. alternatus* at 5°C for 4-5 hr stimulated emergence of *L. simplex*. Muesebeck (1963) reported *Wesmaelia pendula* Foerster from a nabid in New Jersey. Hendrick and Stern (1970) found *W. pendula* parasitizing less than 1% of *Reduviolus* in California. Stoner (1973) and Stoner et al. (1975) described *W. pendula* as a common parasite of male *R. alternatus*, *R. americoferus*, and *Tropiconabis capsiformis* near Tucson, Arizona, but a rare parasite of female nabids. Although Benedict (1975) did not find *W. pendula* in studies of *Reduviolus* near Davis, California, he found mymarids parasitizing nabid eggs at rates of 26%-70%. Also,

Mundinger (1922) described a trombidiid mite parasitizing a 2nd instar nabid.

Two publications suggest that evening may be the most efficient time for collecting nabids with a sweepnet. Benedict (1975) collected peak numbers of nabid nymphs after 2 P.M. and peak numbers of adults after 8 P.M. in early August in California. Donahoe and Pitre (1977) found peak searching activity of adult *R. roseipennis* around the time of sunset. In studies of sublethal effects of insecticides on *R. americoferus* (Sections II, III), I made extensive observations on the biology of untreated nabids.

MATERIALS AND METHODS

I collected nabids with a 36 cm sweep net in Montgomery Co., Virginia, alfalfa fields. Nabids were held in a sleeve cage in a cold room (19 ± 2°C) with other insects and alfalfa tips from the sweep samples for 1-7 d. Nabids were coaxed into 1/2 dram vials to be sorted by species and sex.

Late instar *R. americoferus* were placed in 30 cm high cages made from 6 cm Diam polybutylene tube. Each cage contained vestigial-winged *Drosophila melanogaster* for food and alfalfa stems for perching sites. A day after the molt to the adult stage, young adults were placed in the refrigerator (6 °C) in 65 ml cages until enough had been accumulated for an experiment. Adults were first paired at a physiological age of 4 d (17-23 °C). I also used refrigeration to adjust young

adults lab-reared from the egg to a common physiological age before pairing.

Pupae of parasites which emerged were held at room temperature (20-25 °C) in 1/2 dram vials placed in a 65 ml vial together with a 1/2 dram vial filled with water. To maintain a high relative humidity while allowing some air movement, the 65 ml vial was covered with a piece of paper for a loosely fitting lid. Some parasites which emerged from the nabids were maintained in the cold room in 65 ml cages with a drop of honey and water (1:1) for food and an alfalfa cutting for perching sites.

Individual adult females were transferred every 4th day to new 65 ml cages with fresh alfalfa cuttings with 2 mm Diam stems and paired with a different male whenever one was available. Older males were often maintained alone in cages because males usually outlived females,

In experiments which began with 2nd instars, nymphal development through the 3rd, 4th, and 5th instars was estimated by recording the instar of each nymph daily. Because the temperature in the rearing room decreased about 1 °C in winter, rate of development also decreased.

In one set of experiments odd-numbered nymphs were fed half as many *Drosophila* every other day as even-numbered nymphs. Daily consumption of *Drosophila* by nabid nymphs was estimated by recording the decrease in the number of living flies 24 hours later and correcting for natural fly mortality. Dead flies were left in the rearing vials until nabids matured.

Adult survivorship was determined by recording the day of death compared to the day each individual molted to the adult. Adjustments to adult longevity were made by subtracting days spent under refrigeration from the total days alive as an adult. Age-specific fecundity was estimated from the number of eggs laid during each 4-d period on an alfalfa cutting.

RESULTS AND DISCUSSION

Nabid species and abundance in local Montgomery Co., Virginia, alfalfa fields based on 1800 adults collected mid-June to early November were *R. americoferus* (90%), *R. roseipennis* (8%), *Nabis rufusculus* Reuter (1%), *Tropiconabis capsiformis* (<1%), and *Nabicula subcoleoptrata* (Kirby) (<1%).

Information on nymphal and adult biology is summarized in Table 5 . Greater estimates of adult longevity, total eggs laid, and eggs laid per day alive of lab-reared compared to field-reared nabids may be due, in part, to an unlimited laboratory food supply during the nymphal stages. However, because these rearings were sequential rather than concurrent, other factors such as improved handling techniques may have been important. Likewise, it is unclear whether the decreases when 2nd instars were refrigerated 7-8 days were due to refrigeration or to other factors. What is clear is that I have obtained varied estimates of nabid biological parameters. Similarly, whereas Hormchan et al. (1976) recorded a mean of 105 ± 36 (S.D.) eggs laid for 20 *T. capsiformis* fe-

males, a *T. capsiformis* female which I collected laid 355 eggs. Thus my laboratory studies may reflect laboratory technique as much as field longevity and fecundity.

Analysis of covariance of 3rd-5th instar feeding rates showed that female nymphs tended to consume more *Drosophila* than male nymphs ($P=0.051$, $df=218$), and that consumption decreased with increasing age within instar ($P<0.0001$). Although nymphs fed half as much consumed fewer *Drosophila* per day than nymphs fed at normal rates, consumption rates were low enough that all nymphs had abundant food. Consumption of *Drosophila* between nabid instars increased by a factor of 1.25 compared to the 2.11 factor suggested by the results of Sloderbeck and Yeargan (1983). This low factor may have resulted from nymphs feeding on dead *Drosophila* in the rearing vials.

Adult mortality curves for field-collected and lab-reared nabids show that although field-collected nabids did not live as long as lab-reared nabids, in both cases males lived longer than females (Fig. 3). Although female survival appeared higher in the early days of adulthood, male survival was higher for most of the lifespan.

Age-specific fecundity for field-collected and lab-reared nabids reflects higher mean fecundity for lab-reared nabids (Fig. 4). For both groups oviposition peaked around 18-22 d after the molt to adult.

Both *Leucostoma simplex* (Diptera:Tachinidae) and *Wesmaelia pendula* (Hymenoptera:Braconidae) were found as parasites of *R. americoferus*, although *L. simplex* seemed more common. *W. pendula* may be more

Table 5. Biological information on field-collected and laboratory-reared Reduviolus americoferus.

	Field collected as late instars[1]	Lab reared from eggs[1]	Lab reared: refrigerated[1,2]	Lab reared: all[1]
MALES		(14)[3]	(11)	(25)
3rd instar (days)	...	3.7 ± 0.29	3.3 ± 0.19	3.5 ± 0.18
4th instar (days)	...	3.5 ± 0.14	4.5 ± 0.16	3.9 ± 0.14
5th instar (days)	...	5.3 ± 0.13	7.1 ± 0.21	6.1 ± 0.22
3rd-5th instars (days)	...	12.5 ± 0.25	14.8 ± 0.26	13.5 ± 0.30
	(27)			
Pronotal width (mm)	1.50 ± 0.013	1.54 ± 0.018	1.61 ± 0.020	1.57 ± 0.015
Refrigeration[4] (days)	4.6 ± 0.62	2.4 ± 0.44	6.0 ± 1.27	4.0 ± 0.7
Adult longevity(days)	26 ± 2.8	80 ± 9.2	47 ± 11.3	66 ± 7.8
FEMALES		(12)	(21)	(33)
3rd instar (days)	...	3.2 ± 0.18	3.6 ± 0.16	3.5 ± 0.12
4th instar (days)	...	3.7 ± 0.14	4.2 ± 0.15	4.0 ± 0.12
5th instar (days)	...	5.1 ± 0.15	6.5 ± 0.11	6.0 ± 0.15
3rd-5th instars (days)	...	12.0 ± 0.17	14.3 ± 0.24	13.5 ± 0.25
	(28)			
Pronotal width (mm)	1.64 ± 0.014	1.67 ± 0.016	1.68 ± 0.015	1.68 ± 0.011
Refrigeration[4] (days)	4.3 ± 0.47	2.2 ± 0.56	7.5 ± 0.81	5.6 ± 0.71
Adult longevity(days)	18 ± 1.6	51 ± 6.4	43 ± 4.4	46 ± 3.6
Total eggs laid	78 ± 18.1	422 ± 73.1	263 ± 33.6	323 ± 36.5
Eggs laid per day alive	3.3 ± 0.64	7.5 ± 0.89	5.8 ± 0.50	6.5 ± 0.48

[1]Means ± S.E.
[2]Early 2nd instars in this column were refrigerated (6 °C) 7-8 days before experiments began.
[3]Sample size
[4]Refrigeration as young adults before pairing

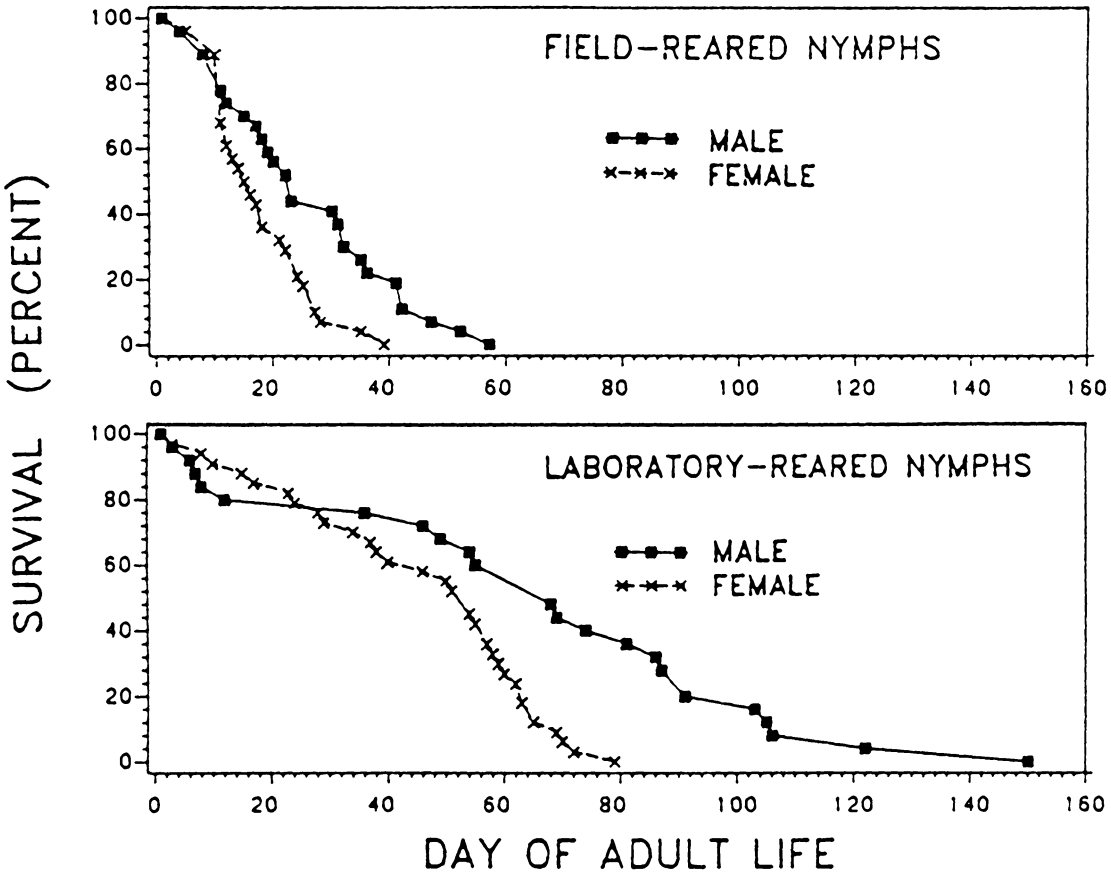

Figure 3: Survival of field-collected and laboratory-reared *R. americoferus*. Apparent differences may reflect changes in laboratory technique or time of year.

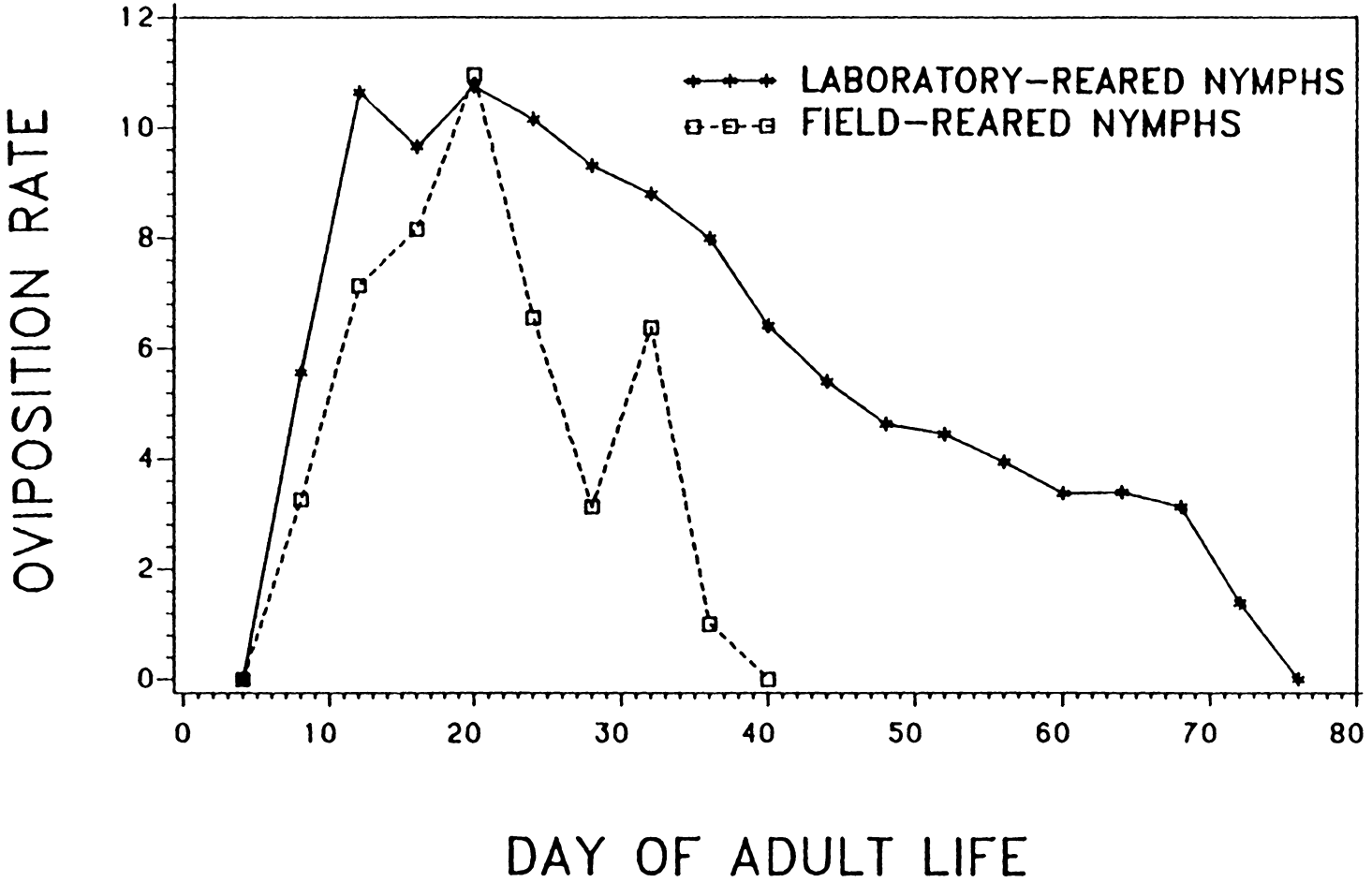

Figure 4: Age-specific fecundity of field-collected and laboratory-reared *R. americoferus*. Laboratory technique or time of year may have influenced any apparent differences.

abundant than the 4% parasitization rate based on rearing suggests, since, in addition to rearing the parasites, Stoner (1973) based parasitizaton rates of nabids by *W. pendula* on trophamnion cells in the host. Besides indicating that *W. pendula* may be relatively common, my study provided some data on *W. pendula* adult longevity. At the end of 7 weeks of adulthood at 19 ± 2 °C all 8 *W. pendula* adults were still alive, at 9 weeks 5 remained alive, and at 11 weeks 1 was alive.

One incidental observation of nabid behavior in the laboratory has implications for behavior in the field. Well fed nabid adults would walk slowly on a small paint brush while being transferred from cage to cage, whereas hungry nabids were quick to fly. This suggests that adults may disperse quickly from fields with inadequate numbers of prey. If this is true, nymphs remaining in the field after a cutting may be more important than adults in influencing the growth of pest populations. Another behavioral note is that when disturbed, nymphs at times remained motionless with legs drawn in as though dead. Such 'freezing' behavior was indistinguishable from death until the nymph was observed alive at a later time.

The effect of time of day on sweep-net collection efficiency was checked only once, on 2 Sept. 1982, but a highly significant ($P<0.0001$, $df=23$) difference was observed between samples taken in mid-afternoon and about sunset. The evening samples had almost 2 times as many adults and 3 times as many nymphs as the afternoon samples. Although one sample date provides minimal evidence, the trend is the same as

that suggested by Benedict (1975) and Donahoe and Pitre (1977) and suggests a diurnal pattern of changes in searching activity or habitat preference.

Section VI

SUMMARY AND RECOMMENDATIONS

SUMMARY

Sublethal concentrations of carbofuran or methidathion changed the biology of *R. americoferus*. Both chemicals decreased the adult longevity of males exposed as young adults. More methidathion-treated females were missing appendages than the control females. Nabid females exposed to LC_{35} carbofuran as 2nd instar nymphs reached the 3rd instar more quickly than control nymphs. Also, nabids exposed to carbofuran as nymphs had reduced adult longevity.

The finding that male longevity was reduced to ca. 60 percent of the control value, while female longevity was hardly affected, suggests that we need to quantify the importance of male nabids in field population biology. If a combination of high parasitization rates of male nabids and reduced male longevity results in an increase in female infertility, then sublethal effects may be important in the field. However, if there is an abundant pool of potent males, then sublethal effects on population dynamics may be minimal.

Given the importance of prey availability to predator growth, I recommend additional research on the influence of nymphal feeding rates on the expression of sublethal effects. I expect that nymphs surviving field applications of pesticides would develop in an environment with little food. Effects due to reduced prey consumption may be as significant as direct sublethal effects. In order to make predictions of the effects of pesticide treatments on predators in the field there is a need

to quantify interactions of lethal and sublethal effects, prey availability, and parasites with predator population dynamics.

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SUBLETHAL EFFECTS OF CARBOFURAN AND METHIDATHION ON REDUVIOLUS AMERICOFERUS (CARAYON) (HEMIPTERA:NABIDAE)

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(ABSTRACT)

The objective was to quantify sublethal effects of two insecticides on the common damsel bug, a major predator in alfalfa. Groups of 2-day-old adult nabids were exposed for 12 h to filter paper treated with LC_5 or LC_{45} carbofuran, or LC_5 or LC_{25} methidathion. Males which survived the 4-day acute mortality period lived only 60 percent as long as control males, with low and high concentrations of insecticides producing similar decreases in longevity. More females which survived acute exposure to methidathion were missing portions of appendages at the time of death than in the controls. Although larger pronotal width was related to increased female longevity, to increased egg production, and to increased progeny production, exposure to insecticide had no consistently positive or negative effect. Pretreatment refrigeration affected neither postexposure longevity nor total eggs laid by females.

Second instar nabids were exposed for 12 h on filter paper treated with LC_{15} or LC_{35} carbofuran, or LC_0 or LC_5 methidathion. Female nymphs which survived exposure to LC_{35} carbofuran reached

the 3rd instar more quickly than control nymphs, whereas methidathion tended to slow development to the 3rd instar. Neither insecticide significantly affected the duration of the 3rd-5th instars. While carbofuran reduced the longevity of adults compared to controls, methidathion only reduced the longevity of the LC_0 group compared to the LC_5 group. Although LC_{15} carbofuran increased egg production per day alive, carbofuran did not affect total egg production.

In laboratory studies male nabids were observed dispersing mistlike droplets (probably pheromone) by rapid movement of a hind leg, or by *flicking*, a term proposed here. Exposure to carbofuran or methidathion appeared to decrease the frequency of flicking in surviving nabids; however, the decreases were not always significant.

Oviposition rates of control females averaged 3-4 eggs/female/d for adults from field-collected nymphs and 6 eggs/female/d for adults from insectary-reared nymphs with peaks around 18-22 d after the final molt. A tachinid, *Leucostoma simplex* (Fallen), was the most common nabid parasite reared (parasitization rates of up to 40%), while the braconid, *Wesmaelia pendula* Foerster, parasitized 0-4% of *R. americoferus*.