

## EFFECTS OF *MESOCYCLOPS LONGISETUS* (COPEPODA: CYCLOPIDAE) ON MOSQUITOES THAT INHABIT TIRES: INFLUENCE OF LITTER TYPE, QUALITY, AND QUANTITY

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**ABSTRACT.** A 59-week study was conducted to evaluate the impact of adult *Mesocyclops longisetus* populations on larval mosquito species inhabiting tires. Greater than 90% reduction of number of 1st and 2nd instars was recorded by 4 wk with 90% reduction of number of 3rd and 4th instars after 7 wk. Reduced control was noted with the onset of cooler winter water temperature. Overall, a 52% reduction in the number of 1st and 2nd instars was achieved, and a 57% reduction was noted in number of 3rd- and 4th-instar mosquito larvae. Cooler temperatures resulted in a decline of adult *Mesocyclops*, which resulted in reduced larval control. Significantly greater numbers of *Mesocyclops* adults were collected in tires with either new litter or heavy amounts of litter regardless of litter type. Lastly, litter type, either oak leaves or pine needles, did not influence mosquito reduction or abundance of *Mesocyclops* populations.

### INTRODUCTION

Cyclopoids of the genus *Mesocyclops* are predators of mosquito larvae (Bonnet and Mukaida 1957, Riviere and Thirel 1981, Marten 1984) and some have been studied as potential biocontrol agents for mosquitoes in container habitats (Marten 1990a, 1990b; Schreiber et al. 1993; Tietze et al. 1994). In particular, *Mesocyclops* spp. have been evaluated especially in *Aedes aegypti* (Linn.) habitats (Riviere and Thirel 1981) in Tahiti, and against *Aedes albopictus* (Skuse) in the United States (Marten 1990a, 1990b). Integrated control with biorational larvicides appears to be the *modus operandi* for cyclopoid usage and control of pestiferous mosquito species inhabiting containers (Riviere et al. 1987, Marten 1990b, Tietze et al. 1994). This approach has been based on reports of delayed larval control due to a variety of operational and environmental variables including low copepod inoculation rates (Marten 1990b, Schreiber et al. 1993), water chemistry, food value of the organic matter within the container (Jennings et al. 1994), as well as amount and type of organic matter (Schreiber et al. 1993).

In this study, we compared the effectiveness of *Mesocyclops longisetus* (Thiebaud) in used automobile tires to control resident mosquito species for a 59-week period with 2 different litter types (oak and pine) 2 different litter amounts and age of the litter.

### MATERIALS AND METHODS

**Study site:** In late September 1993, 4 tire piles were established in a wooded area of Panama City,

FL (Airport Authority land), primarily vegetated with live oak (*Quercus virginiana* Mill.), slash pine (*Pinus elliottii* Englem.) with some sand pine (*Pinus clausa* Chapm. ex Englem.), and magnolia (*Magnolia grandiflora* Linn.). The understory was generally devoid of vegetation due to heavy amounts of pine straw. The vegetation present in this site consists of the dominant tree species in habitats throughout the Gulf of Northern Florida (Myers and Ewel 1990). This site was chosen because mosquito populations were limited to a few species, with *Ae. albopictus* making up the vast majority (Schreiber et al. 1993, Tietze et al. 1994). Each tire pile contained 32 used automobile tires naturally infested with mosquitoes. The 32 tires were stacked against the base of a tree and not against each other; they were all touching the ground and arranged so that water inside could be removed via a flap for examination. The flap consisted of 2 7.6-cm parallel cuts into one sidewall of a tire. The only source of water entering the tires during the study was rainwater. Thus, some tires ( $n = 9$ ) dried out sporadically during the study.

The tire piles were placed 300 m from each other and were more than 100 m from other potential *Ae. albopictus* developmental sites, namely discarded tires from the airport maintenance department. Adult *Ae. albopictus* has a limited dispersal range of no more than a few hundred meters (Hawley 1988).

**Experimental design:** Each pile averaged 111 immature *Ae. albopictus* per tire on August 16, 1994, when treatments were applied. There were 4 treatment factors: *Mesocyclops* (yes or no) type of litter (oak leaves or pine needles), amount of litter (light or heavy), and age of litter (new or old), giving a total of 16 treatment combinations. Treatment combinations were randomly assigned to 2 tires within each tire pile and the tire placement was randomized. Oak leaves were obtained from live oak trees. Pine needle bundles were from slash pine trees. Both leaf types were collected from the im-

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mediate area of the study site with new leaves and needles taken from pruned or fallen trees. Preliminary investigations on litter fall helped to determine the "light" and "heavy" amounts experimentally placed into the tires. Light amounts of oak leaves were designated as 15 leaves, and light amounts of pine needles were determined to be 15 needle bundles (3 needles per bundle). Heavy amounts for oak leaves were 100 leaves, and 200 needle bundles were considered representative for slash pine. Old leaves and needles were from last year's leaf fall, taken primarily from mulch piles. Periodic removal, by hand, from tires of naturally occurring oak leaf and pine needle litter was conducted to maintain integrity of initial litter treatments.

The *M. longisetus* used in this study originated from John A. Mulrennan, Sr., Research Laboratory colonies initially received from the New Orleans Mosquito Control Board. *Mesocyclops longisetus* adults were inoculated into tires utilizing a hand pump sprayer that delivered an average of  $119 \pm 2.4$  individuals/tire (Hallmon et al. 1993).

Immature mosquitoes and *M. longisetus* adults were monitored from September 4, 1993, through September 27, 1994. All tires from each tire pile were sampled 15 times. Tires were sampled every 2–6 wk with the longer duration occurring during the winter months. All water and litter were removed from each tire and placed into a 19-liter plastic bucket and concentrated via a 1,000-ml plastic beaker with a screen mesh (24 per cm) located on 2 sides of the beaker 3 cm from the bottom. Individual beakers for treatment and controls were utilized. Following concentration, each tire sample was put into an individually marked plastic container and brought back to the laboratory for identification. Treatment combination, number of 1st and 2nd instars, and number of 3rd and 4th instars mosquito larvae were recorded. Lastly, the number of adult *M. longisetus* (adults based on size and presence of caudal filaments) was recorded. Aliquots of mosquito larvae from each tire were placed either in vials containing 70% ethanol for later species identification or examined directly under a stereomicroscope at the time of data recording. Following counting and identification, remaining mosquito larvae, pupae, and copepods were returned with water and litter to the respective tire. High and low temperatures were recorded by a maximum/minimum thermometer placed in one tire at each tire pile location.

**Statistical analysis:** Efficacy of different treatment combinations upon immature mosquito stages (i.e., 1st and 2nd instars and 3rd and 4th instars) were assessed by a split-plot-in-time analysis of variance, following square root transformation of the data to stabilize error variances and Student–Newman–Keuls mean separation test due to unequal sample sizes (SAS Institute 1990). In this study, whole-plot effects were: pile (or block) *Mesocyclops* treatment, individual litter variables, the

treatment by single litter–variable interactions, the 2-way litter–variable interactions, and the 3-way treatment by litter–variable interactions. Split-plot effects were the above effects with the additional effects of week (time) and pile by week interaction. Effects of high and low temperature on *M. longisetus* populations and effects of *M. longisetus* on individual mosquito stages were modeled utilizing curve fit option regression techniques (CA-Cricket Graph III, Computer Associates International 1992). The dependent variable was the mean number of *M. longisetus* adults, and the high and low water temperatures obtained from maximum/minimum thermometers placed in one tire within each tire pile were used as independent variables.

## RESULTS

A total of 2,157 mosquito larvae was sampled from tires. The dominant species was *Ae. albopictus* (88%). *Culex quinquefasciatus* Say, *Culex salinarius* Coq., and *Anopheles crucians* Wied. comprised 4.6, 3.0, and 3.1% of the larvae collected in our samples, respectively. Three other species were found during the study and comprised <2% of the total. They were *Aedes triseriatus* (Say), *Culex restuans* Theobald, and *Orthopodomyia signifera* (Coq.).

The impact of *M. longisetus* adults and leaf litter variables on developing 1st- and 2nd-instar mosquitoes varied in significance in the overall split-plot analyses (Table 1). The effect of pile (block) was statistically significant ( $P = 0.02$ ). Tires containing *Mesocyclops* ( $\bar{x} \pm SE$ ) had  $9.45 \pm 0.70$  1st and 2nd instars, whereas the control tires had  $24.43 \pm 1.01$  1st and 2nd instars ( $P = 0.0001$ ). Litter type (T) was statistically significant ( $P = 0.001$ ), with tires containing pine needles having  $17.46 \pm 1.02$  1st and 2nd instars and tires with oak litter having  $16.47 \pm 0.88$  1st and 2nd instars regardless of cyclops treatment.

The split-plot effects: week (W), week by treatment ( $W \times \text{Trt}$ ), and week by litter type ( $W \times T$ ) were highly significant statistically ( $P = 0.0001$ ,  $P = 0.0001$ , and  $P = 0.0007$ , respectively, in the number of 1st and 2nd instars collected). Additionally, the 3-way interactions: week by treatment by litter type ( $W \times \text{Trt} \times T$ ), week by litter age by litter type ( $W \times \text{Age} \times T$ ), and week by litter amount by litter type ( $W \times \text{Amt} \times T$ ) influenced the number of 1st and 2nd instars collected from tires. Lastly, the 4-way interaction: week by treatment by litter age by litter type ( $W \times \text{Trt} \times \text{Age} \times T$ ) was also significant ( $P = 0.04$ ).

The effect of litter type is illustrated in Fig. 1A. Through the 59-wk study, tires that had pine needles as their litter component had a greater number of 1st and 2nd instars ( $17.46 \pm 1.02$ ), than did tires with oak litter ( $16.47 \pm 0.88$ ). Despite these overall mean differences large and readily observable dif-

Table 1. Analysis of variance of *Mesocyclops longisetus* on 1st and 2nd instars of mosquitoes in tires from August 1993 through September 1994, Panama City, FL.<sup>1</sup>

| Source                         | df  | F value | P > F   |
|--------------------------------|-----|---------|---------|
| Pile                           | 3   | 3.71    | 0.0200  |
| Treatment (Trt)                | 1   | 239.07  | 0.0001  |
| Litter age (Age)               | 1   | 0.86    | 0.3587  |
| Litter amount (Amt)            | 1   | 1.09    | 0.3012  |
| Litter type (T)                | 1   | 12.51   | 0.0010  |
| Trt × Age                      | 1   | 0.04    | 0.8503  |
| Trt × Amt                      | 1   | 0.00    | 1.0000  |
| Trt × T                        | 1   | 0.00    | 1.0000  |
| Age × Amt                      | 1   | 0.03    | 0.8721  |
| Age × T                        | 1   | 0.18    | 0.6724  |
| Amt × T                        | 1   | 0.40    | 0.5279  |
| Trt × Age × Amt                | 1   | 0.55    | 0.4602  |
| Trt × Age × T                  | 1   | 2.37    | 0.1310  |
| Trt × Amt × T                  | 1   | 0.25    | 0.6188  |
| Age × Amt × T                  | 1   | 0.49    | 0.4885  |
| Trt × Age × Amt × T            | 1   | 0.65    | 0.4254  |
| Pile × Trt × Age × Amt × T     | 45  |         | Error a |
| Week (W)                       | 14  | 20.56   | 0.0001  |
| W × Pile                       | 42  |         | Error b |
| W × Trt                        | 14  | 18.15   | 0.0001  |
| W × Age                        | 14  | 1.62    | 0.0700  |
| W × Amt                        | 14  | 1.50    | 0.1051  |
| W × T                          | 14  | 2.72    | 0.0007  |
| W × Trt × Age                  | 14  | 0.87    | 0.5926  |
| W × Trt × Amt                  | 14  | 1.54    | 0.0921  |
| W × Trt × T                    | 14  | 3.79    | 0.0001  |
| W × Age × T                    | 14  | 2.69    | 0.0007  |
| W × Amt × T                    | 14  | 1.89    | 0.0246  |
| W × Age × Amt × T              | 14  | 0.73    | 0.7494  |
| W × Trt × Age × T              | 14  | 1.79    | 0.0364  |
| W × Trt × Age × Amt × T        | 13  | 0.88    | 0.5700  |
| W × Pile × Trt × Age × Amt × T | 618 |         | Error c |

<sup>1</sup> R<sup>2</sup> = 0.77; CV = 51.69.

ferences were only noted prior to *Mesocyclops* introduction and during the summer (weeks 40–50).

Through the majority of the study's duration the control tires had greater numbers of 1st and 2nd instars collected than did the *Mesocyclops*-treated tires. However, during the winter and beginning of spring (weeks 10–30), differences between the controls and treatment were minor or nonexistent (Fig. 1B). Thereafter, tires that contained *Mesocyclops* had generally less than 10 1st and 2nd instars collected/tire, whereas controls had about 25 1st and 2nd instars/tire. Thus, the change in magnitude of differences between the 2 treatments changed on a seasonal basis resulting in the week by treatment interaction being significant.

Regardless of overall treatment, litter age by litter type interaction over the course of the study did influence the number of developing 1st and 2nd instars collected from tires. Prior to inoculation with *Mesocyclops*, tires with new pine needles and old oak leaves contained more 1st and 2nd instars than did their counterparts. As the study progressed into fall and winter, differences between litter type coupled with litter age became difficult to separate.

This trend continued until the beginning of summer (week 43). During this time tires with pine needles, regardless of age, contained the most 1st and 2nd instars collected ( $61.88 \pm 32.22$ ), whereas tires with oak leaves contained  $37.53 \pm 25.09$  larvae. As summer progressed into fall differences between litter age and type again became difficult to distinguish.

Seasonal differences between treatments coupled with litter type were nonexistent from late fall (week 12) through the winter and spring (week 35). Separation of individual treatment combinations was not evident until onset of summer (week 40) with the greatest number of larvae collected from pine needle control tires ( $90.28 \pm 9.94$ ) as compared to the pine *Mesocyclops* tires ( $31.29 \pm 3.09$ ). As summer progressed to fall (week 50) and until the end of the study fewer 1st and 2nd instars were collected in tires with *Mesocyclops* regardless of litter type ( $3.85 \pm 0.05$ ). During the same interval differences between control tires by litter type also decreased in magnitude.

*Mesocyclops longisetus*, given a choice, prefers to prey upon 1st and 2nd instars; however, some

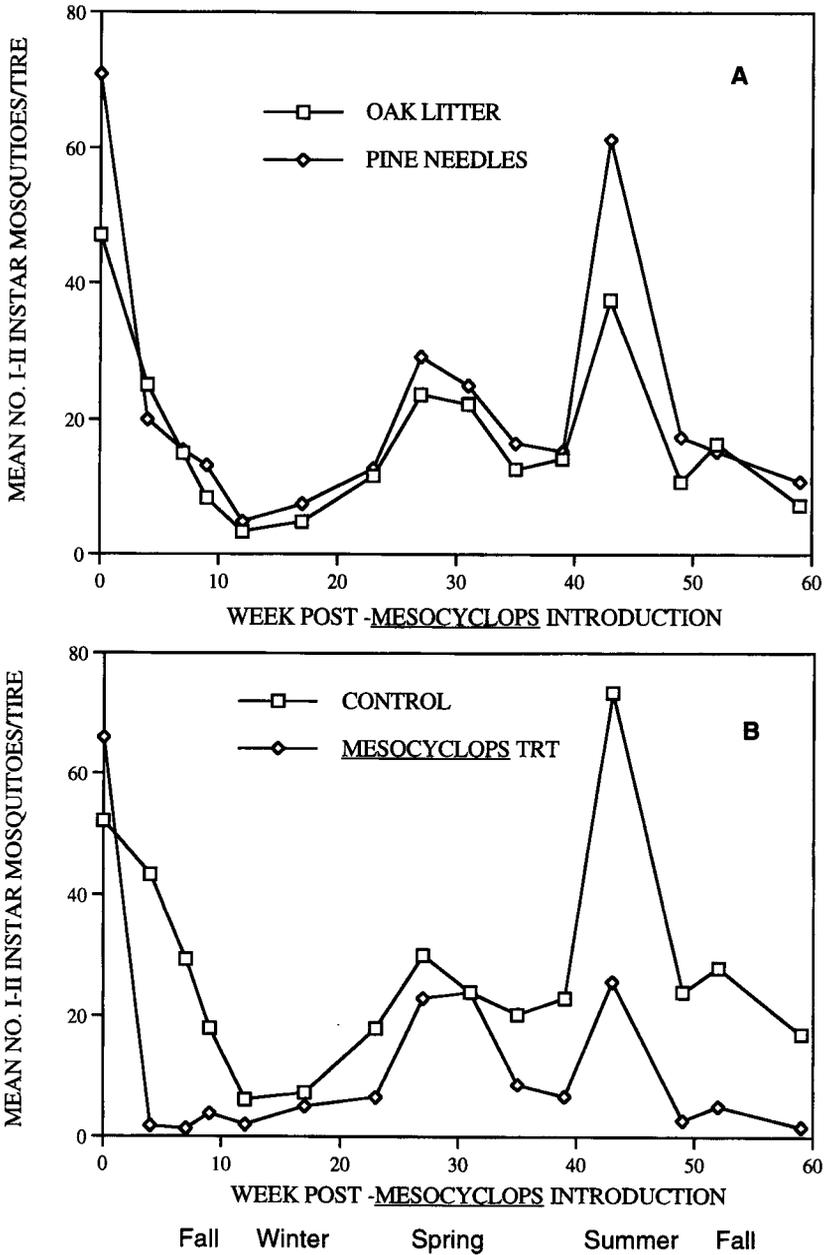


Fig. 1. Mean 1st- and 2nd-instar mosquitoes from tires after introduction of *Mesocyclops longisetus*. A. Number of 1st- and 2nd-instar mosquitoes in oak litter and pine needles. B. Number of 1st and 2nd instars in tires containing *Mesocyclops* and controls from September 1993 to September 1994, Panama City, FL.

predation on 3rd and 4th instars does occur. The 2 whole-plot effects that influenced number of 3rd and 4th instars in tires were treatment (with control tires having  $4.40 \pm 2.15$  larvae/tire and *Mesocyclops* tires  $2.56 \pm 1.71$  larvae/tire,  $P = 0.0001$ ) and interaction between litter age by litter type ( $P = 0.04$ ). Differences were not readily observable with respect to larvae from tires with oak litter by age, with new having  $3.54 \pm 2.03$  larvae and old having

$3.37 \pm 2.08$  larvae. The differences most evident were those with pine needle litter and age combinations, with new needles having fewer 3rd and 4th instars collected ( $3.28 \pm 2.19$ ) than old needles ( $3.68 \pm 2.27$ ).

As with the analyses of 1st and 2nd instars, many split-plot effects for the later instars were also statistically significant. The following split-plot effects were all highly significant: week ( $P = 0.0001$ ),

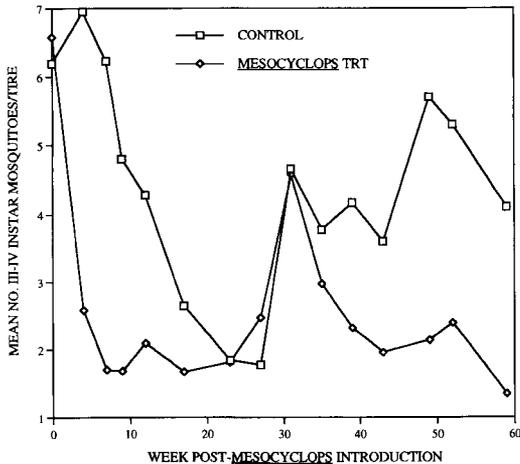


Fig. 2. Mean number of 3rd- and 4th-instar mosquitoes from tires after introduction of *Mesocyclops longisetus*. A. Mean number of 3rd and 4th instars per tire in *Mesocyclops*-treated tires vs. controls. B. Mean number of 3rd and 4th instars per tire in different litter amounts in tires from September 1993 to September 1994, Panama City, FL.

week by treatment ( $P = 0.0001$ ), week by litter amount ( $P = 0.024$ ), and week by litter age by litter type ( $P = 0.008$ ).

The week by treatment interaction is depicted in Fig. 2. Observable differences in the number of 3rd and 4th instars in tires (less in *Mesocyclops*-treated tires than controls) were evident much of the year until midwinter and most of the spring months. During this time period no differences between treatments were readily apparent. At the onset of spring (week 30) through the end of the study, observable differences occurred in the number of 3rd and 4th instars collected between tires with *Mesocyclops*. Lastly, the abundance of 3rd and 4th instars collected from tires with light or heavy amounts of litter, regardless of litter type or treatment, was significant. However, the only real differences were noted during the 4th week (heavy amounts had about 45.0 3rd and 4th instars/tire compared to light amounts, which had 7.0/tire). Thereafter no differences were apparent for the rest of the study.

All litter age by litter type combinations behaved similarly with respect to the number of 3rd and 4th instars (oscillating up and down) collected through the study period. However, no single litter age by type combination consistently yielded more 3rd and 4th instars than the other combinations for any particular length of time of the study. However, some generalizations can be inferred: tires that had either old oak litter ( $17.96 \pm 0.02$ ) or new pine needles ( $22.87 \pm 0.13$ ) had the greater number of 3rd and 4th instars collected than did tires new oak litter ( $17.15 \pm 0.16$ ) or old pine needle ( $21.97 \pm 0.13$ ) over the entire study.

**Effects on *Mesocyclops* populations:** The different litter variables and their subsequent interactions on the collected number of *Mesocyclops* adults had a number of significant effects. The whole-plot effects (pile, litter age, and litter amount) were statistically significant ( $P = 0.0005$ ,  $P = 0.0002$ ,  $P = 0.002$ , respectively). Greater numbers of *Mesocyclops* adults were collected in new litter, regardless of amount or type ( $50.94 \pm 2.00$ ), than in old litter ( $33.84 \pm 2.00$ ). Greater numbers of *Mesocyclops* adults were collected in heavy amounts of litter ( $49.21 \pm 4.90$ ) than in light amounts of litter ( $35.92 \pm 0.60$ ) through the entire study. Additionally, split-plot effects of week (W), week by litter amount (W  $\times$  Amt), and week by litter type (W  $\times$  T) on *Mesocyclops* abundance were statistically significant ( $P = 0.0001$ ,  $P = 0.02$ ,  $P = 0.01$ , respectively).

A greater mean number of adult *Mesocyclops* was collected from tires with heavy amounts of litter ( $58.15 \pm 5.14$ ) vs. light amounts ( $36.66 \pm 3.57$ ) during the initial part of the study. No differences were noted during late winter and spring (weeks 23–35). Observable differences returned again by summer (week 43), between heavy amounts ( $43.24 \pm 1.25$ ) compared to light amounts ( $30.96 \pm 1.04$ ). Thus, the difference between litter amounts in the latter period was less than before the winter interval.

Observable differences in mean number of adult *Mesocyclops* collected from tires with respect to oak litter and pine needles occurred regardless of litter age or amount. During the first 10 wk of the study, no discernible trend was apparent in the number of adults collected in the 2 litter types. At the onset of winter more adults were collected in tires with oak litter ( $48.82 \pm 1.02$ ) than in tires with pine needles ( $46.18 \pm 0.91$ ). Through the spring months (weeks 20–30) no observable differences in adult *Mesocyclops* abundance were evident (both about 20 per tire). Once summer arrived, however, through the end of the study, *Mesocyclops* adults were collected in greater numbers from tires containing pine litter (about 55.0/tire) compared to tires containing oak litter (about 34.0/tire).

As the previous paragraphs indicated, a decline in the number of *Mesocyclops* adults collected was noted with the onset of winter and subsequently cooler water temperatures. This relationship between mean minimum temperature and abundance of adult *M. longisetus* in tires is expressed mathematically and graphically in Fig. 3. The regression model illustrated that when mean minimum water temperatures were between 7 and 17°C, numbers of adult *M. longisetus* were relatively constant. A sharp decline in abundance of *M. longisetus* was noted once the mean minimum temperature went below 5°C. Minimum temperatures that are, on average, below this range resulted in a dramatic decrease in numbers of *M. longisetus* adults, whereas higher temperatures resulted in an increase.

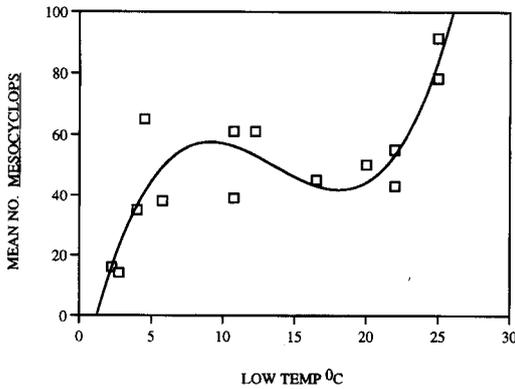


Fig. 3. Regression line ( $y = 0.04x^3 + 1.76x^2 + 21.39x - 23.60$ ,  $r^2 = 0.77$ ) of the mean number of adult *Mesocyclops longisetus* collected from tires at different mean minimum water temperatures from September 1993 to September 1994, Panama City, FL.

## DISCUSSION

Classical biological control agents regulate pest populations of interest rather than eliminating them as do current chemical control procedures. *Mesocyclops longisetus* introduced into tires to reduce larval mosquitoes works in this fashion during the majority of the year. However, our results showed that *M. longisetus* populations failed to control the target mosquito population during the winter months when minimum water temperatures dropped below 10°C. In our study, this was from about week 17 to week 35 (January through April). In spring, both the mosquito and copepod populations increased in size and it was not until week 39 (May) that *Mesocyclops* populations had recovered enough to have an impact on developing 1st- and 2nd-, and 3rd- and 4th-instar mosquito populations (71% and 68.5% reduction as compared with the controls, respectively). Thus, low water temperature is an important component factor diminishing the efficacy and survival of *M. longisetus*. Decline of *Mesocyclops* efficacy following the winter season needs to be factored into any integrated management strategy (i.e., reapplication of *Mesocyclops* to target containers in the spring) to reduce container-inhabiting mosquitoes in more temperate regions. Conversely, regions that do not reach these low temperatures may achieve lasting and continual control of mosquitoes without augmentation releases.

The type, amount, and age of litter in tires did influence control efficacy of copepods on different mosquito stages based on statistical significance; however, only litter type had a large enough effect to be biologically meaningful (tires with oak litter had a mean of 16.5 larvae, whereas tires with pine needles had 17.5 larvae) for 1st and 2nd instars. The effect of litter type, however, was not evident in the later mosquito stages ( $P = 0.72$ ). The other

litter variables measured, although statistically significant, did not appear to have any real biological consequence due to the overriding effect of abiotic factors (i.e., minimum water temperatures; confounded in this study with weeks) with differences in the mean number of 1st and 2nd or 3rd and 4th instars between and among various treatment combinations. The effect of litter type associated with abundance of 1st and 2nd mosquito instars may be a result of ovipositional preferences by gravid *Ae. albopictus* due to water chemistry via leached organics from their resident leaves (i.e., tannins, lignins, and terpenoids). Furthermore, the significance of pile effect with respect to the number of 1st and 2nd instars may in part be explained by some ovipositional preference by *Ae. albopictus* in our tire piles. Indeed, the pile effect was not significant with respect to 3rd and 4th instars.

Water chemistry has been shown to be an important factor in oviposition and development for a number of mosquito species (Petersen and Chapman 1969, Bradshaw and Holzapfel 1986, Walker and Merritt 1988, Bentley and Day 1989, Walker et al. 1991). Other studies suggested that leached chemicals have an indirect reducing effect by limiting the number of microorganisms available for consumption of developing mosquitoes (Walker et al. 1988, 1991; Sota 1993). Similarly, the effects of the biological and physiochemical attributes of containers for copepod production have been investigated (Jennings et al. 1994). Those authors found that low levels of food for the developing copepods were pivotal in survivorship of *Mesocyclops aspericornis* (Daday), along with pH and salinity. Our study did not examine these factors; rather, we investigated the broad categories such as litter type, amount, and age.

The rate of 119 *M. longisetus* per tire appears to be an adequate number for field application. At this rate, reduction in larval mosquitoes was achieved relatively quickly and was of long duration. Winter temperatures will reduce control in much of the United States, so applications of *M. longisetus* in spring may be required once the low-temperature theoretical threshold of 10°C is exceeded, or an application of *Bacillus thuringiensis* var. *israelensis* (*B.t.i*) to allow for copepod populations to recover may be prudent.

Our findings showed that litter type, amount of litter, and litter age were not important in the efficacy and growth of *M. longisetus* populations, as was first hypothesized in earlier studies (Schreiber et al. 1993, Tietze et al. 1994). Thus, these dominant litter types in northern Florida should be of no concern to mosquito control workers when applying *M. longisetus* in the field for reduction of container-inhabiting mosquitoes. Rather, the abiotic factor, water temperature, is of pivotal importance in achieving season-long control with this copepod.

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