THE RESISTANT TRITONYMPHAL INSTAR AND ITS IMPLICATIONS IN THE POPULATION DYNAMICS OF *NAIADACARUS ARBORICOLA* FASHING (ACARINA : ACARIDAE) [1]

BY

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INTRODUCTION

Although most mites of the family Acaridae inhabit terrestrial situations, members of the subfamily Naiadacarinae are aquatic (FASHING, 1974). One species, *Naiadacarus arboricola* Fashing, is restricted to water-filled treeholes in eastern North America. The life cycle of *N. arboricola* consists of the following instars : larva, protonymph, facultative deutonymph, tritonymph and adult. The facultative deutonymph (hypopus) is a dispersal stage and occurs in the life cycle only during May and June (FASHING, 1976), but other instars are generally present in the treeholes throughout the year.

While studying the biology of this species, it was noted that during middle to late summer and also in winter the tritonymphal instar predominates in the populations (ca 40-60 %). At the same time, the combined larval and protonymphal instars comprise only 10-20 % of the populations. This seemed a rather high proportion of tritonymphal to other instars since the tritonymphal stadium occupies only 6-9 % of the mean life expectancy of a mite under adequate environmental conditions.

However, environmental conditions in the treeholes vary over the year. Dead leaves, the primary food of *N. arboricola*, usually enter the treehole only once a year during the autumn leaf drop, but decreased activity due to low temperatures does not allow much of the food to be utilized until the following spring (FASHING, 1975a). A period of rapid reproduction ensues as the water temperatures increases in the spring, and by mid-summer the leaf supply is usually exhausted. Thus during middle to late summer food is scarce, and during winter mite populations are subjected to adverse low temperatures. The build-up of tritonymphs coincides with these periods of adverse conditions.

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A similar phenomenon has been observed in three other species of acarid mites from distinctly different habitats. ZYROMSKA-RUDZKA (1964) found a predominance of tritonymphs in laboratory cultures of *Carpoglyphus lactus* (L.) when the food supply became inadequate in declining cultures. She attributed this to an observed slowdown in the rate of development of immatures when reared on inadequate food sources, and speculated that an increased resistance of the tritonymph to unfavorable conditions would also contribute to a predominance of tritonymphs. SOROKIN (1948) (cited in ZYROMSKA-RUDZKA, 1964) found a dominance of tritonymphs in autumn field populations of *Tyrophagus longior* (Gerv.), which he attributed to adverse low temperatures. Since members of the genus *Tyrophagus* have no hypopial stage, SOROKIN thought the tritonymph might be especially adapted to withstand adverse conditions as does the hypopus in many other species. DOSSE and SCHNEIDER (1957) studied the ecology of *Calvolia transversostriata* (Oudemans) (= *Czenspinkia lordi* Nesbitt) and found that it overwinters only in the tritonymphal instar. By the end of August, development of immatures no longer continued past the tritonymphal stage. When brought into the laboratory, these overwintering tritonymphs could not be induced to molt until the following spring. They concluded that *C. transversostriata* enters an obligatory diapause in the autumn.

It appears that the observed predominance of tritonymphs in populations of some species of acarids during the periods of adverse conditions could be due to one or more of the following factors : higher resistance to adverse conditions in the tritonymph than in other instars ; a slowdown in development of immatures ; and a tritonymphal diapause. To gain insight into the causes of tritonymphal accumulations in *N. arboricola*, laboratory studies were conducted. Also, populations in the field were sampled throughout the year and their morph-structure, i.e., the percentage of each instar present in the population, was determined.

METHODS

Stock cultures

N. arboricola was collected from water-filled treeholes at the University of Kansas Sunflower Entomological Reserve and cultured in the laboratory. Mites from these cultures were used to determine the tolerance of the various instars to adverse conditions. Hypopi were not tested since they are not present in populations of *N. arboricola* during times of environmental stress (FASHING, 1976).

Tolerance to freezing

Forty individuals of a given instar were placed in a small stender dish with water and a piece of leaf and preconditioned at 8° C for one hour. The dish was then placed in a freezer for 24 hours, after which the ice was thawed and the percent survival of mites recorded. Several replicates were run for each instar.

Tolerance to high temperature

Larvae, protonymps, and tritonymphs in the prequiescence condition were isolated from the stock culture and allowed to molt. Immediately after ecdysis, 20 mites of each instar were placed individually in rearing cells provisioned with green ash leaf (*Fraxinus pennsylvanica* Marsh) and water using the methods of FASHING (1975b). Twenty larvae collected within 12 hours after larviposition were also placed in such cells. The mites were maintained at 30 ± 1°C, a temperature known to be detrimental to development in *N. arboricala* (FASHING, 1975b), and observed daily until death.

Tolerance to starvation

The same procedures were followed as for high temperature, except that mites were maintained without food at 25 ± 1° C, which is probably near the optimal temperature for *N. aboricola* (FASHING, 1975b).

Duration of immature stages under unfavorable conditions

N. arboricola was reared on several different food sources ranging from adequate to inadequate for development of the immatures, and the durations of the immature stages noted. The durations of the immature stages at a low temperature (15° C) was also investigated. These rearing experiments were carried out jointly as part of this study and as part of a study of intrinsic rates of increase in *N. arboricola* under various conditions. The latter study gives the methods and results in detail (FASHING, 1975b).

Field observations

Samples were collected from treeholes at the University of Kansas Sunflower Entomological Reserve at intervals throughout a 13-month period. The morph-structure of three selected populations (treeholes) was determined by slide-mounting a random sample of mites (average 205 individuals) from each population and determining the instar of each mite. The various instars occur randomly within the contagious distribution of *N. arboricola* (Fashing, 1975a), therefore such a sample should contain a true representation of the morph-structure of a population. During each collection, the temperature within the treehole was recorded as well as the abundance of leaves and water.

RESULTS AND DISCUSSION

N. aboricola subjected to freezing temperatures for 24 hours demonstrate a differential survival among instars (Fig. 1). The tritonymphs are by far the most tolerant of cold, with an average of 61.6 % survival. An average of only 37 % of the protonymphs, 37.7 % of the females, 10 % of the males and 10 % of the larvae survived after 24 hours freezing.

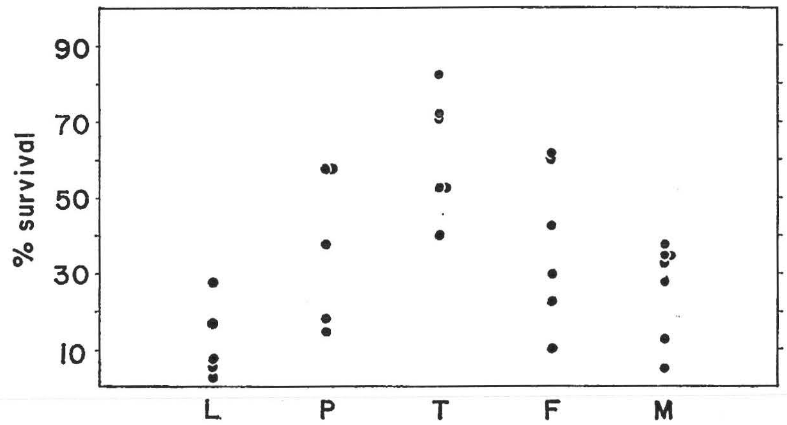

FIG. 1. — Survival of instars of *N. arboricola* after 24 hours freezing. Each point represents the percent survival of 40 mites. L = larva, P = protonymph, T = tritonymph, F = female, M = male.

At 30°C, *N. arboricola* is unable to molt and simply remains at the same instar until death. Survivorship curves for the various instars maintained at 30 °C indicate that tritonymphs also tolerate high temperature best (Fig. 2a). The number of days at 30°C required to kill 50 % of each instar follows : tritonymph, 67 days ; female, 57 days ; protonymph, 48 days ; male, 43 days ; and larva, 16 days.

Under conditions of starvation, no molting occurred in any instar. Survivorship curves (Fig. 2b) and the number of days without food required for 50 % mortality of a given instar demonstrate the extreme tolerance of tritonymphs to starvation : Tritonymph, 84 days ; protonymph, 49 days ; male, 35 days ; female, 33 days ; and larva, 6 days.

In summary, adverse conditions produce a differential mortality among the instars of *N. arboricola*. The tritonymphal instar is most tolerant and the protonymphal instar the next most. The larvae and males are most susceptible to adverse conditions.

As with other acarid mites, the total duration of immature stages in *N. arboricola* is prolonged by an inadequate food source (FASHING, 1975b). Such a prolongation can be due to poor nutrition in insects (BAUMBERGER, 1919), and the cause is undoubtedly the same for mites. ZYROMSKA-RUDZKA (1964) stated that a prolongation of immature life coupled with prior cessation of oviposition accounted for the build-up of tritonymphs she observed in declining cultures of *Carpoglyphys lactus*. Her results could be more easily explained, however, if molting were controlled by nutrition and the nutritional threshold for molting were lower in larvae and protonymphs than in tritonymphs. The larvae and protonymphs would slowly develop to the tritonymphal stage and then be unable to molt, thus causing an accumulation of tritonymphs in the population. Preliminary evidence indicates that this might be the case in *N. arboricola*. When *N. arboricola* is reared on a sub-adequate food source, many individuals develop no farther than the tritonymphal stage (FASHING, 1975b). In fact, on three inadequate food sources, an average of only 27 % of the mites that reached the tritonymphal stage progressed to adulthood. Some of the remaining tritonymphs died, but others were still alive when the experiment was terminated after approximately 14 weeks.

A similar differential temperature for molting appears to be a factor in the predominance of tritonymphs in winter populations of *N. arboricola*. *N. arboricola* reared at 15°C developed to the tritonymphal stage but did not transform to the imago (FASHING, 1975b). After an average of 84 days in the tritonymphal stage, they were placed at 25°C and ecdysis occurred. When placed at 25°C, however, the tritonymphs took an average of 12.25 days before ecdysis ; approximately twice as long as the total tritonymphal stadium in populations reared at a continuous 25°C. Therefore, it is possible a tritonymphal diapause could be involved in this differential molting response, but more research is needed to substantiate this.

It appears that both inadequate food and low temperatures allow molting in larvae and protonymphs, but not in tritonymphs. Thus, during periods of adverse conditions a build-up of tritonymphs results.

Even when *N. arboricola* is reared on the same food source there is a certain amount of variation in the duration of the larval, protonymphal and tritonymphal stag es. The coefficient of variation was determined for the duration of each immature stage in several rearing experiments (FASHING, 1975a, 1975b), (Table 1). Since the rearing experiments were conducted on differen t foods and/or at different temperatures, there is a high degree of variability among coefficients of variations within one immature stage (columns of Table 1). Therefore a Wilcoxon matched-pairs signed-ranks test (SIEGEL, 1956) was used to test for differences between coefficients of variation of the immature stages. The protonymphal stadium was found to have a significantly greater amount of variability than the larval stadium ($p < .01$) ; and tritonymphal stadium was

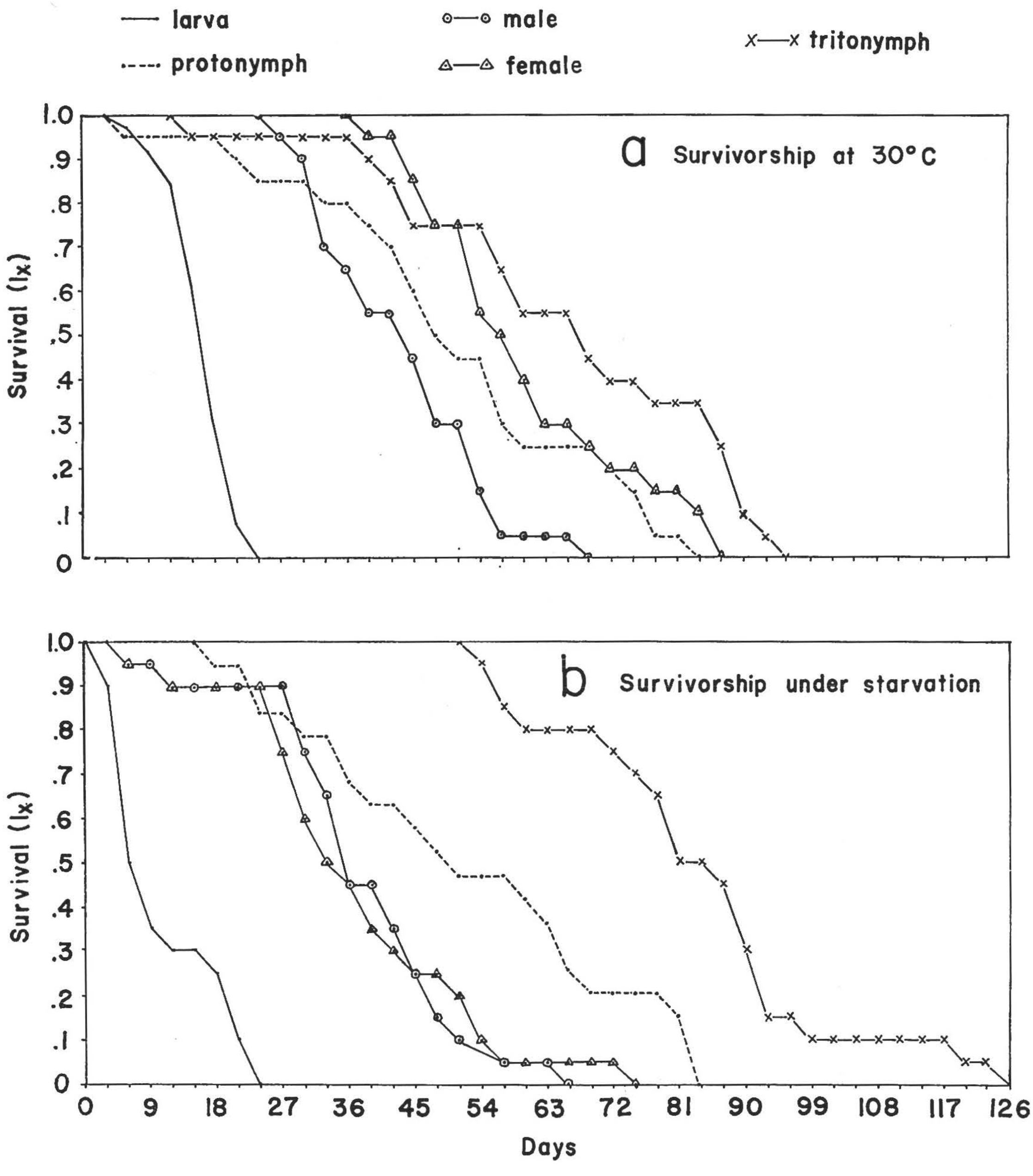

FIG. 2. — Survivorship (l_x) curves for the instars of *N. arboricola* reared at (a) 30°C on green ash leaves, and (b) at 25°C under starvation.

even more variable than the protonymphal stadium ($p. < .01$). The tritonymph is, therefore, genetically the most flexible immature instar in regard to length of duration of its stage, as well as the most tolerant to adverse environmental conditions.

TABLE I. — Coefficients of variation for the duration of the larval, protonymphal and tritonymphal stadia of *N. arboricola* reared under various conditions. Differences between adjacent column means indicated by** = $p < .01$; Wilcoxon Matched-pairs Signed-ranks test.

rearing conditions	sex	Coefficient of Variation				
		larva		protonymph		tritonymph
Green Ash 15°C	♂	10.30		28.12		—
	♀	11.18		16.64		—
Green Ash 20°C	♂	26.41		49.58		62.13
	♀	26.66		36.52		56.56
Green Ash 25°C	♂	29.44		31.86		69.21
	♀	20.41		48.45		67.48
Red Oak 25°C	♂	38.24		26.16		59.52
	♀	28.64		54.33		59.86
Bitternut Hickory 25°C	♂	17.66		28.39		64.61
	♀	14.76		49.75		71.94
Sycamore 25° C	♂	16.97		29.96		34.21
	♀	26.67		20.94		28.40
American Elm 25°C	♂	16.01		16.67		57.46
	♀	11.37		92.01		72.41
Agar + leaves 25°C	♂	18.25		20.80		29.78
	♀	20.76		25.96		84.39
	mean	20.86	**	36.01	**	58.43

Field observations support the above conclusions. A predominance of tritonymphs is found in populations in early spring. Fig. 3a illustrates a typical morph-structure of a sample taken at this time ; approximately 66 % of the population is in the tritonymphal stage, 23 % adult stage, and only 11 % on the combined larval and protonymphal stages. When such a sample is subjected to the warm temperatures of the laboratory, the immatures enter quiescence and molt almost simultaneously (Fig. 3b). In fact, 80 % of the immatures entered quiescence within the first three days in this case. Thus a population of almost totally adults (89 %) was produced, and of these 74 % were newly emerged adults.

A build-up of tritonymphs also occurs in response to a shortage of food. The morph-structure for a population inadvertently left for almost two years in a gallon jar with only its original supply of leaves is given in Fig. 4a. As would be expected from the starvation tolerance tests, no larvae survived. Tritonymphs made up 79 % of the population, adults 23 %, and protonymphs 7 %.

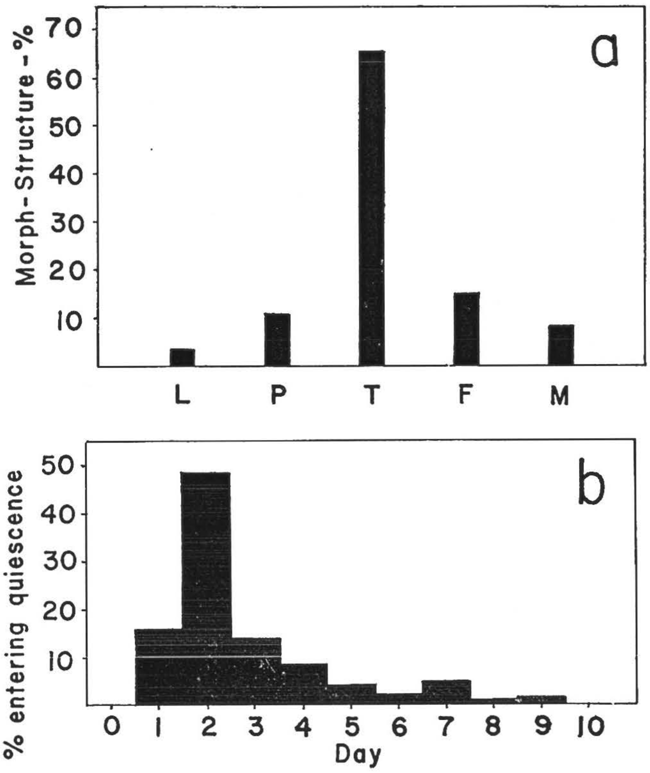

FIG. 3. — (a) Morph-structure of a population of *N. arboricola* collected from a treehole in March. L = larva, P = protonymph, T = tritonymph, F = female, M = male. (b) Number of days required for immatures from 3a to enter quiescence after being placed at laboratory temperature (23°C).

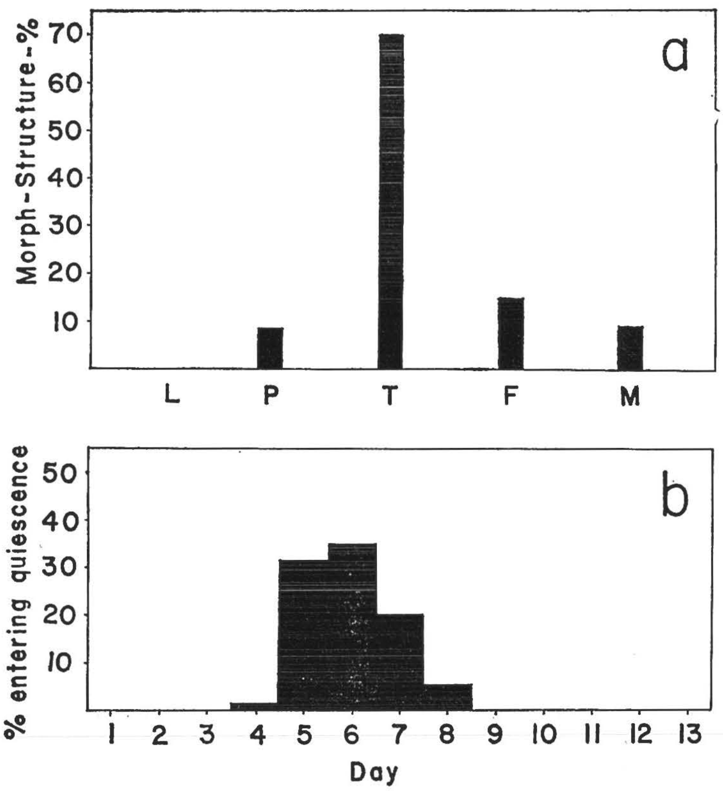

FIG. 4. — (a) Morph-structure of a population of *N. arboricola* from a culture to which no additional food had been added for almost two years. L = larva, P = protonymph, T = tritonymph, F = female, M = male. (b) Number of days required for immatures from 4a to enter quiescence after being provided with fresh food.

When the immatures were placed on a fresh food source, virtually all molted simultaneously (Fig. 4b) to produce an almost totally adult population (93 %). Again, most of this population (75 %) consisted of freshly emerged adults.

Changes in the morph-structure in a single treehole over a 13-month period illustrate the build-up of tritonymphs in the field due to adverse conditions (Fig. 5). The temperature of the treehole water at intervals during this period is also shown. In fall, the morph-structure consisted of 44 % tritonymphs and 44 % adults, with the remaining 12 % primarily protonymphs. By March, however, differential mortality due to cold temperatures had increased the percentage of tritonymphs to 65, and also slightly increased the percentage of protonymphs. Although not specifically shown in Fig. 5, the largest mortality was observed in adult males as would be

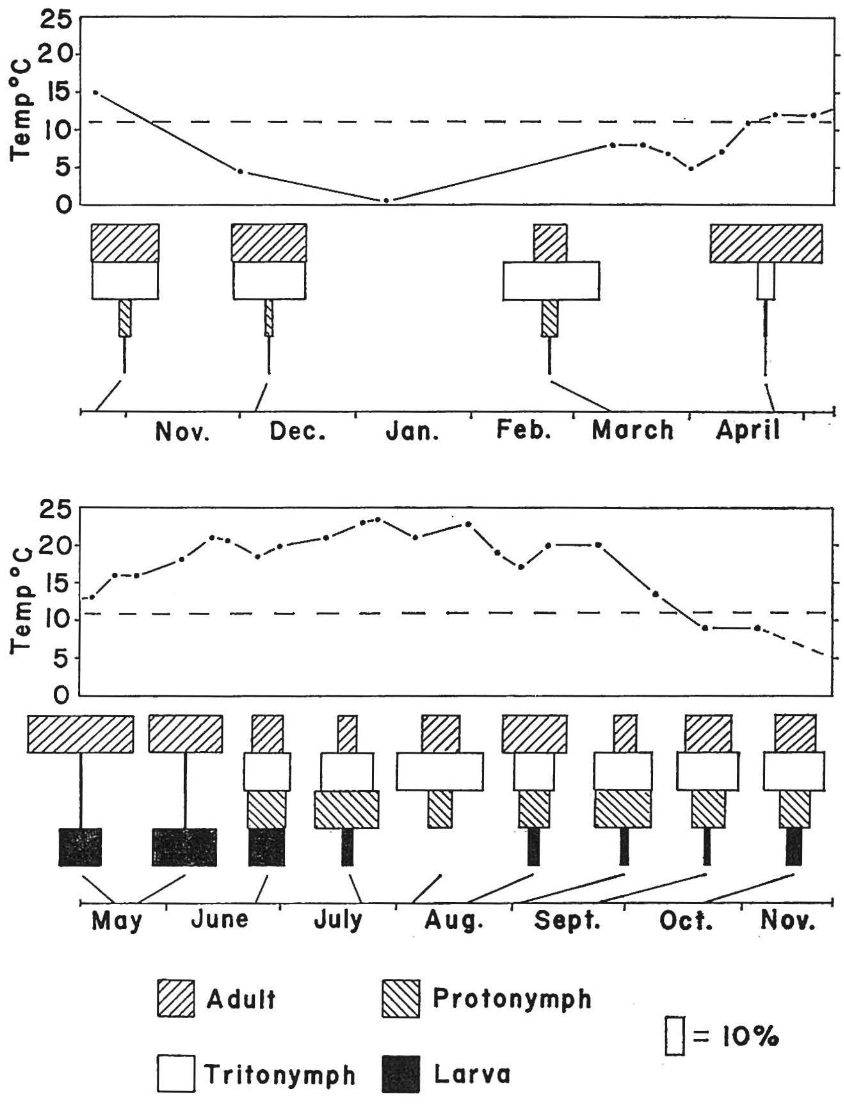

FIG. 5. — Morph-structure of a population of *N. arboricola* in the field taken at varying intervals throughout a thirteen month period, and the temperature within the treehole over this period. The broken line at 11°C refers to a baseline below which immatures of *N. arboricola* do not develop.

expected from their susceptibility to cold (Fig. 1). Larvae declined from 3.3 % to 1.27 % over the winter. In April, simultaneous molting of immatures created an almost totally adult population. By May the population was actively reproducing and consisted primarily of adults and larvae ; and in late June there was an abundance of all instars in the treeholes. By late July the treehole organisms had exhausted the food supply and larviposition by mites ceased. During early August, no larvae were found in the treehole, and the immatures again acculumated at the tritonymphal stage. Additional food then entered the treehole in mid-August, probably due to some premature leaf-drop, and the tritonymphs again molted to adults. The great differences observed in the morph-structure after the molt in the spring were not observed in late summer since high temperatures of late summer caused rapid development of all stages. By fall, cooling temperatures again caused an accumulation of immatures at the tritonymphal stage.

The importance of the " tritonymphal bottleneck " in the life history of *N. arboricola* during adverse conditions is evident if viewed in relation to population dynamics. The intrinsic rate of increase (r_m) of a population depends on its age distribution. Therefore the instar(s) which spans the duration of an adverse condition will determine the rate of population increase after the adverse condition has subsided (Birch, 1948). Thus the optimal strategy for maximal population increase is to endure adverse conditions as an adult, since reproduction can resume immediately upon cessation of an adverse condition. However, if the reproductive potential of an adult is decreased by exposure to an adverse condition or simply by aging during the interval, the optimal strategy would be to utilize the penultimate instar to endure adverse conditions. Upon ecdysis, such a population would consist of young adults which have the highest potential rate of increase.

The latter strategy is that taken by *N. arboricola*, although it is not known whether adverse conditions actually lower the reproductive efficiency of adult mites. This might be inferred, however, from the fact that the penultimate instar (tritonymph) is especially adapted to withstand adverse conditions and immatures accumulate at this stage when subjected to such conditions. This strategy seems rational when one considers the water-filled treehole habitat to which *N. arboricola* has adapted. Most of the organisms in the treehole community are saprophagus, feeding on decaying organic matter or microorganisms associated with decay (Fashing, 1975a). The main source of energy for these organisms is dead leaves which usually enter the treehole only once a year during the autumn leaf drop. Decreased acitivity due to lower autumn temperatures does not allow the treehole organisms to utilize much of the food supply until the following spring. In *N. arboricola* nearly all immatures accumulate at the tritonymphal instar duing fall and winter and ecdyse almost simultaneously in the spring when the weather warms, thus forming an almost totally adult population. Composed primarily of young adults, the mite population has its highest reproductive potential at such times. Thus it can rapidly take advantage of the abundant food supply and favorably compete with the other saprophagus organisms in the treehole.

A similar sequence of events is induced by the low availability of food during the late summer. Immatures accumulate at the tritonymphal stage, and if new food falls into the treehole, mass ecdysis forms an almost totally adult population. However, in most cases, the new food (leaves) falls into the treehole rather late in the year, and low temperature limits the ability of the mites to take advantage of it. Therefore both lack of food and low temperature usually act together to produce the build-up of tritonymphs.

Conclusions

The tritonymphal stage of *N. arboricola* phases the population in response to cooling temperatures and/or lack of food, resulting in a population of young, highly reproductive adults when adequate conditions return to the treehole. Such a population, through its high reproductive potential, can best compete with other treehole organisms for the available food supply.

Abstract

The penultimate instar (tritonymph) of *Naiadacarus arboricola,* a saprophagus mite restricted to water-filled treeholes, is the most tolerant of all instars to adverse environmental conditions when tested in the laboratory (i.e., freezing, high temperatures, starvation). Also, when reared on inadequate food sources or at low temperature, development usually proceeds only to the tritonymphal stage.

Field observations on natural populations indicate that during a dearth of food and also during winter the tritonymphal instar predominates. When adequate environmental conditions return, the tritonymphs transform to adults almost simultaneously.

The tritonymphal stage thus phases the population in response to cooling temperatures and/or lack of food, resulting (by synchronous molting) in a population of young, highly reproductive adults when adequate conditions return to the treehole. Such a population, through its high reproductive potential, can best compete with other saprophagus organisms for the available food supply.

Zusammenfassung

Naiadacarus arboricola ist eine saprophytische Milbe die sich auf wassergefülte Baumhöhlen beschränkt. Das vorletzte der fünf Stadien der Milbe (Tritonymphe) ist das wiederstandsfähigste gegen Frieren, Hitze, oder Aushungerung in Laboruntersuchungen. Wenn die Milbe nicht gut gefüttert oder auf niedrigen Temperaturen gehalten wird, geht die Entwicklung nicht weiter als zum Tritonymphenstadium.

Im Winter oder in Perioden von Futtermangel bestehen natürliche Milbenvölker in Baumhöhlen auch größtenteils aus Tritonymphen. Im Frühling und wenn genügend Laub in den Baumhöhlen ist, verwandeln sich die Tritonymphen schnell zu Erwachsene. In diesem Zustand können die Milben sich paaren und vervielfältigen. Die unmittelbare Vervielfältigungsfähigkeit ist ein wichtiger Vorteil in den Futterwettbewerb zwischen den verschiedenen saprophytischen Bewohnern der Baumhöhle.

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