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ACIDIC FOG-INDUCED CHANGES IN HOST-PLANT SUITABILITY

Interactions of *Trichoplusia ni* and *Phaseolus lunatus*

JOHN T. TRUMBLE and J. DANIEL HARE

Department of Entomology
University of California
Riverside, California 92521

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Abstract—*Phaseolus lunatus* L. (Henderson Bush lima beans) were exposed to 2 hr acidic fogs with 2.5 : 1.0 (v/v) nitrogen–sulfur ratio typical of the west coast of the United States. Fogs with pH values of 2.0 ($P < 0.01$, t tests), 2.5 ($P < 0.05$), or 3.0 ($P < 0.01$) increased percent total nitrogen (dry weight) of foliage as compared to plants subjected to control fogs with a pH of 6.3–6.5. Fresh weight concentrations of soluble protein and certain free amino acid concentrations were increased by plant exposure to acidic fogs with a pH of 2.5 (t tests, $P < 0.05$). Concentrations of free amino acids considered essential for insect growth, as well as nonessential and total free amino acids were not significantly affected by any treatment ($P > 0.05$, t test). Water content (%) of foliage was not changed significantly ($P > 0.05$, t test) by exposure to any of the fogs. *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) larvae ate significantly more foliage and gained significantly more weight on plants treated with 3.0 pH fogs ($P < 0.01$, t test). Several potential explanations are offered for the lack of significant weight gain by larvae on plants in which soluble protein levels, free amino acid concentrations, or percent total nitrogen contents were enhanced by acidic fogs with a pH of 2.5 and 2.0. No larval feeding preference was detected for foliage exposed to acidic versus control fogs, and no significant differences were detected in percent survival of *T. ni* eggs exposed to acidic or control fogs. Some implications of acidic fogs for population dynamics of *T. ni* are discussed.

Key Words—Acidic fog, *Phaseolus lunatus*, *Trichoplusia ni*, cabbage looper, Lepidoptera, Noctuidae, nitrogen, free amino acid, soluble protein, plant-insect interactions, air pollution.

INTRODUCTION

Like the gaseous photochemical oxidants, acidic fogs can have beneficial as well as detrimental consequences for plants. Wet deposition of pollutants (rain, fog, dew) on foliar surfaces either can act as fertilizers or cause a sequence of cellular collapse resulting in necrotic lesions (Shriner, 1986). Previous reports noted deleterious effects such as lesion development, weathering of cuticular wax, foliar leaching, and modification of physiological responses (Cowling, 1982; Lintherst et al., 1982). These effects have been duplicated with short-term exposure to acidic fogs of pH 2.3 or lower under laboratory conditions (Granett and Taylor, 1981; Granett and Musselman, 1984). Because ambient fogs in the Los Angeles Basin exhibit an acidity of pH 1.69–3.0, significant foliar necrosis leading to yield losses in crop plants is not unusual (Hoffman, 1984).

Physiological changes in plants affected by air pollutants significantly alter the nutritional suitability of such plants for insect herbivores (Jones and Coleman, 1988). Substantial modifications in the form and content of nitrogen (e.g., both increases and decreases) have been reported following plant exposure to ozone and other pollutants (Chang, 1971; Mudd and Freeman, 1977). In tomatoes, ozone effects include an increase in free amino acids and soluble proteins (Trumble et al., 1987), which may be more readily assimilated by insects than the nitrogen bound in the structural components of cells. One objective of the study reported here was to determine if plants subjected to acidic deposition and damaged by acidic fogs develop different total nitrogen, soluble protein, and free amino acid profiles than plants exposed to nonacidic fogs.

The implications of increased concentrations of assimilable forms of nitrogen for insect herbivore development are considerable (Strong et al., 1984; White, 1984). Nitrogen availability dramatically affects key life processes such as growth rates, survival, and reproductive capacity (Onuf, 1978; Prestidge, 1982; Prestidge and McNeill, 1983). Because air pollutants in general, and acidic fogs in particular, often occur over broad geographic areas (Lee, 1982; Hoffman et al., 1985), the cumulative effects on herbivores at the population level may be of substantial importance (White, 1984). Thus, potential changes in plant physiology due to stress or direct injury from air pollutants may well have more serious consequences than are indicated when the plant system is examined in the absence of herbivores.

At present, little research has focused on the effects of acidic fogs on the nutritional suitability of plants for insect herbivores. Although several studies have, in some cases, documented an increase in food consumption or preference for foliage exposed to ozone or SO_2 (Hughes et al., 1981; Jeffords and Endress, 1984; Endress and Post, 1985; Trumble et al., 1987; Coleman and Jones, 1988; Jones and Coleman, 1988), these are by no means the only responses noted

(Jones and Coleman 1988). The impact on herbivores of foliar deposition of nitrates associated with high-nitrogen acidic fogs and potential damage or stresses associated with acidic fogs have not been reported. Therefore, a second objective of our research was to document the potential influence of acidic fogs on some key factors influencing herbivore population dynamics.

METHODS AND MATERIALS

The Plant–Insect System. Henderson bush lima beans, *Phaseolus lunatus* L., used in the experiments were germinated in the greenhouse in UC Soil Mixture (Matkin and Chandler, 1957) and fertilized twice weekly with one-half strength Hoagland's nutrient solution (Downs and Hellmers, 1975). Thus, all plants were provided with adequate nitrogen, similar to what would occur in an agricultural setting. Although populations of nitrogen-fixing symbiotes were not surveyed, the provision of adequate nitrogen and the use of washed seed and sterilized soil probably minimized the potential for differential impact due to symbionts between treatments. All plants within a test were from the same seedlot, the same age (4–5 weeks old), and had the primary and first trifoliate leaves fully expanded.

For the nitrogen studies, 10 pairs of plants within each pH level of fog were further standardized by CO_2 assimilation rates using a Li-Cor 6000 photosynthesis measurement system (Li-Cor Inc., Lincoln, Nebraska). Nondestructive measurements were recorded 20 hr pretreatment in the laboratory under a metal halide lamp providing a minimum of 1000 $\mu E/sec \cdot m^2$. After sampling the primary leaves of ca. 40 plants, 10 pairs of plants were identified that differed in CO_2 assimilation by less than 0.003 mg $CO_2/sec \cdot m^{-2}$. One of each pair of plants with comparable CO_2 assimilation rates was exposed to a control fog, and the other to an acidic fog. Standardizing test plants by CO_2 assimilation rates is important, because even plants from the same seedlot grown concurrently can differ significantly in photosynthetic and physiological activity (Trumble et al., 1985), and CO_2 assimilation rates are proportionally related to percent nitrogen and soluble protein levels in plants (Hesketh et al., 1983; and references therein).

Cabbage looper, *Trichoplusia ni* (Hübner), eggs and larvae were obtained from a laboratory colony reared on artificial diet (Shorey and Hale, 1965). The colony was maintained at a photoperiod of 12 : 12 hr light–dark and a temperature of 21 $\pm$ 1°C. Although larvae fed on an artificial diet may not respond to stimuli exactly the same in all situations as foliage-fed larvae, this approach did provide standardized experimental organisms with regard to age, stress, and nutritional backgrounds.

Simulated Acidic Fogs. Simulated acidic fogs were prepared by adjusting

distilled water to pH 2.0, 2.5, and 3.0 with reagent-grade nitric and sulfuric acid mixed at a 2.5:1 (v/v) ratio. This mixture is typical of the high-nitrate, low-sulfate fogs in California, but lacks other ionic components of ambient moisture (Waldman et al., 1982). Control fogs consisted of distilled water with a pH of 6.3–6.5. The simulated fogs were created within a 1-m^2 chamber using a fogging apparatus designed by Musselman et al. (1985), which produces a droplet size averaging 20 μm in diameter. Test plants were fogged for 2 hr and then placed outdoors to dry for at least 1–2 hr.

Total Nitrogen, Soluble Protein, and Free Amino Acid Analyses. Ten pairs of plants were analyzed for each pH level of the acidic fog. After air drying, the primary leaves and an upper trifoliate leaf were excised, combined, and then divided into aliquots for analyses of total nitrogen, soluble proteins, and free amino acids. Samples were weighed immediately after collection, frozen in liquid nitrogen, and stored in an ultracold freezer (−65°C). Enough leaf material was available on each plant to allow two samples of each of the variables to be analyzed. Potential long-term changes in plant chemistry were not addressed by this study.

Total nitrogen was analyzed using the micro-Kjeldahl technique (McKenzie and Wallace, 1954). The technique was modified by replacing the mercuric oxide catalyst with copper sulfate, and by utilizing bromocresol green in place of methylene blue as an indicator. Percent water content of the foliage was determined by subtracting the dry weight (oven-dried at 70°C for 72 hr) from the fresh weight, dividing by the fresh weight, and multiplying by 100.

The technique of Jones et al. (1988) was used to quantify soluble protein. Frozen samples (0.4–0.6 g fresh weight) were ground and extracted with 10 ml 0.1 M NaOH for 30 min at room temperature. Leaf tissue was removed by centrifugation (11,500*g* for 10 min) and the decanted supernatant was brought to 10 ml with 0.1 M NaOH. Protein concentration in the NaOH solution was measured with the Bradford (1976) reagent using ribulose 1,5-biphosphate carboxylase (RuBPase) (Sigma Chemical Co., St. Louis, Missouri) as the standard. Samples were diluted when necessary to avoid deviations from linearity due to NaOH at high protein concentrations. Duplicate readings were made on each extraction. Values are reported as milligrams RuBPase equivalent protein per gram fresh weight of foliage.

Free amino acids were extracted using procedures modified from Hare (1983). Twenty amino acids, including a nonprotein amino acid (gamma aminobutyric acid) and two secondary amino acids (hydroxyproline and proline) were quantified by reverse-phase HPLC on a Beckman model 332 liquid chromatograph with a fluorescence detector after precolumn *o*-phthaladehyde (OPA) derivatization using methods described by Cooper et al. (1984). A Beckman 3-μm Ultrasphere-XL ODS column 4.6 mm ID × 70 mm in length was used to improve resolution. Prior to injection, 200 μl of 1.3 M sodium phosphate (pH

3.5) was added to reduce the pH of the sample in order to improve column life (Sista, 1986). Quantities of each amino acid were calculated from their peak areas in relation to the peak area and known quantity of the appropriate internal standard (Cooper et al., 1984).

Data on nitrogen and water contents of treated and control plants were analyzed with paired and unpaired *t* tests. Paired *t* tests were included because CO_2 assimilation rates are proportionally related to percent nitrogen and soluble protein levels in plants (Hesketh et al., 1983). Unpaired *t* tests were presented for comparative purposes because no clear relationships between either free amino acid or water content and CO_2 assimilation rates have been established. Free amino acid analyses were performed on each amino acid individually, all combined, and grouped by the "essential" vs. "nonessential" criterion of Taylor and Medici (1966).

Influence of Acidic Fogs on Insect Developmental Parameters. In order to document the direct effects of acidic fogs on survival of eggs, caged moths were allowed to oviposit for 8 hr on 4- to 5-week-old *P. lunatus*. Plants with 20 eggs developing to the point of head capsule visibility were assigned randomly to control fogs or acidic fogs with a pH of 2.0 or 3.0. A control accompanied each pH level of fog because these tests were not concurrent. Six replicates of 20 eggs were tested at each pH level. Plants with eggs were exposed to control and acidic fogs for 2 hr and then held at 26.7 ± 1° C, 65 ± 5% relative humidity and a photoperiod of 16:8 hr light–dark until eclosion. Eggs were monitored at 24-hr intervals for at least five days. Comparisons between treatment and control groups were made with unpaired *t* tests.

Food consumption by *T. ni* larvae on plants exposed to fogs with pH values of 2.0, 2.5, and 3.0 was quantified by individually caging a neonate larva on foliage of 30 treated and 30 control plants (one larva per plant) ca. 2 hr after fogging. All larvae were treated concurrently on plant material prior to transfer to cages on the test plants. Plants and larvae were randomly selected for each treatment. A control fog was conducted simultaneously for fogs with pH values of 2.0 and 2.5 + 3.0 because logistics precluded running all acidic fogs concurrently. Plants and larvae were maintained at room temperature, which ranged from 20 to 26°C. Larvae were held until any leaf in the test began to senesce (12–13 days, at which time larvae were in the third instar). All larvae then were removed and the test was concluded. The test was terminated at this time because attempts to relocate the larvae to undamaged leaves were unsuccessful, and substantial mortality resulted. Immediately after larvae were removed, test and control leaves were photocopied and the leaf area with and without excision of feeding damage was measured on a Li-Cor 3000 leaf area meter. Differences between the measurements provided the leaf area consumed per larva. To account for potential differences in specific leaf weight at 7 and 14 days post-treatment, 20 plants were treated with 2.0 pH acidic fog as described earlier

and compared for specific leaf weight (fresh weight) with 20 control plants treated with a pH 6.3–6.5 fog (10 per sample date). Six to eight 22-mm-diameter cores (No. 14 cork borer) were taken from each expanded primary leaf (at least 70% of the total leaf available) and the fresh weight measured. This information was necessary to calculate differences in consumption rate based on leaf area if specific leaf weight varied between treatments.

Larval weight gain on foliage treated with acidic (pH 2.0 or 3.0) or control fogs was measured by individually caging 15 neonate larvae per treatment on plants in the same fashion as the food consumption test. Plants and larvae were randomly selected for each treatment. When any leaf was ca. 90% eaten or began to senesce (as evidenced by onset of yellowing), the surviving larvae (third instars) were removed and weighed. Data were analyzed by unpaired *t* test.

Preference of *T. ni* larvae for foliage exposed to acidic fogs or control fogs was evaluated using a leaf disk bioassay technique. After random selection for treatment, plants were exposed to acidic fogs with a pH of 2.0 or 3.0 (nonconcurrently), or control fogs, and allowed to dry for 3 hr before 22-mm-diameter leaf disks (No. 14 cork borer) were cut from expanded primary leaves. Two control and two test disks from individual plants (total 20 plants per treatment) were placed on moistened filter paper at alternating cardinal points in 8 × 2-cm Petri dishes. Two larvae reared to third instar on artificial diet then were placed centrally in the Petri dish and allowed to feed for 23–24 hr. This test was replicated 20 times for each acidic fog and control fog tested. The remaining leaf area of the disks was measured with a Li-Cor 3000 leaf area meter. All Petri dish experimental units contained feeding damage. Differences in leaf area consumed were analyzed with a paired *t* test (treated versus control disks within a Petri dish) to remove variability in feeding rates between larvae.

RESULTS AND DISCUSSION

Total Nitrogen, Soluble Protein, and Free Amino Acid Analyses. The total nitrogen content of *P. lunatus* exposed to acidic fogs was substantially altered as compared to control plants (Table 1). Plants exposed to fogs with increasing acidity had significantly higher nitrogen content as compared to their respective controls ($P \leq 0.1, 0.05, 0.01$, for fogs of pH 3.0, 2.5, 2.0, respectively). This increase was not linear, suggesting a nonuniform uptake. Presumably at least some of the additional nitrogen was deposited by the acidic fog in the form of nitrate and was not converted on the leaf surface to a form usable by the plant. However, considerable evidence is available, which suggests that growth of plants exposed to acidic fogs may be enhanced by the increased availability of nitrogen and sulfur (Ferenbaugh, 1976; Irving, 1979; Shriner, 1986). The sig-

TABLE 1. TOTAL NITROGEN, SOLUBLE PROTEIN AND WATER CONTENT IN *Phaseolus lunatus* EXPOSED TO FOGS OF VARYING ACIDITY[a]

	pH of test fog					
Source[b]	2.0		2.5		3.0	
Percent total nitrogen (dry wt)		*df*, *t* value		*df*, *t* value		*df*, *t* value
Control plants	2.01	18, 4.257**	2.39	18, 2.488*	3.89	18, 1.904[c]
Test plants	2.68		2.63		4.64	
mg/g frest wt of RuBPase equivalent protein						
Control plants	10.64	16, −1.046	8.57	18, 3.522**	9.63	16, 0.248
Test plants	9.41		12.75		9.89	
Percent water content						
Control plants	86.8	18, 1.766	84.0	18, −0.525	87.4	18, 1.000
Test plants	85.8		84.3		87.9	

[a] Analysis by unpaired and paired *t* tests, results were similar with both tests, so only the unpaired values were reported. Values presented in percent were subjected to arcsine square root transformation prior to analysis. Samples from paired comparisons based on 9–10 pairs of plants exhibiting simlar photosynthetic rates (± 0.003 mg $CO_2/sec/m^{-2}$). Plant material was collected approximately 4 hr after fogging. **significance at $P < 0.01$ level, *$P < 0.05$.
[b] Control plants fogged with distilled water with a pH of 6.3–6.5.
[c] Significant at $P < 0.073$ level.

nificant ($P < 0.01$) increase in soluble protein concentrations for plants exposed to fogs with a pH of 2.5 suggests that some of the nitrate nitrogen responsible for the increase in total nitrogen was assimilated even within the 2–3 hr following fogging. If the increase in soluble protein was simply a response to stress, as has been demonstrated for other environmental factors (Beckerson and Hofstra, 1979; White, 1984), then foliage exposed to the 2.0 fogs also should have developed elevated concentrations. Fogs with pH values of 3.0 or below (i.e., more acidic) are well within the range shown to cause both visible and physiological damage to legumes (Shriner, 1986).

Although significant differences in concentrations of specific amino acids were observed in *P. lunatus* subjected to acidic fogs with a pH of 2.5 as compared to plant material from control fogs, no significant differences in total free amino acids, insect-essential or nonessential amino acid concentrations were evident (Table 2). Interestingly, all of the specific amino acids that showed significant changes in concentration actually increased in concentration. This is in contrast to the impact of another air pollutant, ozone, on the tomato system, where concentrations of certain amino acids were reduced (Mudd and Freeman, 1977; Trumble et al., 1987). No significant differences were observed for specific or total amino acid concentrations between plants exposed to control fogs

TABLE 2. INFLUENCE OF ACIDIC FOG OF pH 2.5 ON FREE AMINO ACID CONCENTRATIONS IN *Phaseolus lunatus*

	Fresh weight (μg/g)		Paired t test[a]		Unpaired t test[b]	
Amino acid	Control	Acidic fog	P	t	P	t
Aspartic acid	210.50	299.55	0.01	3.07	0.03	2.75
Glutamic Acid	397.26	473.14	0.03	2.80	0.10	1.75
Glutamine	19.50	43.39	0.03	2.80	0.03	2.46
Threonine	38.35	47.83	ns[c]	1.82	0.05	2.12
Valine	21.91	32.90	0.01	3.80	0.01	3.91
Essential AA	910.48	742.66	ns	−1.44	ns	0.84
Nonessental AA	2487.06	2715.68	ns	0.96	ns	0.72
Total AA[d]	3525.50	3575.40	ns	0.17	ns	0.12

[a] Samples for paired comparisons from 10 pairs of plants exhibiting initially similar photosynthetic rates ($\pm$0.003 mg CO_2/sec/m^{-2}). Photosynthesis data collected 24 hr pretreatment under metal halide light providing a minimum of 1,000 μE/sec/m^{-2}. Control plants fogged with distilled water with a pH of 6.3–6.5.

[b] Unpaired t test, $df = 18$.

[c] ns = not significant at the $P < 0.10$ level.

[d] Includes the nonprotein amino acid, gamma-amino butyric acid.

or acidic fogs with a pH of 2.0 or 3.0 and are not reported. Thus, the extensive quantitative changes in amino acid levels associated with physiological effects of other air pollutants (e.g., ozone) (Menzel, 1971; Craker and Starbuck, 1972) did not occur consistently (i.e., at all pH levels) in our study.

Influence of Acidic Fogs on Insect Developmental Parameters. There were no significant differences in specific leaf weight between treatments at either 7 (means $\pm$ SD = 6.7 $\pm$ 0.5 mg/cm^2 for controls and 7.0 $\pm$ 0.6 mg/cm^2 for pH 2.0; $P > 0.05$, t test) or 14 days posttreatment (means $\pm$ SD = 7.2 $\pm$ 0.5 mg/cm^2 for controls and 7.1 $\pm$ 0.5 mg/cm^2 for pH 2.0; $P > 0.05$, t test), so consumption has been reported as leaf area consumed.

Exposure of host foliage to the low and moderately acidic fogs chosen (pH 3.0 and 2.5) had a significant impact on larval feeding and weight gain (Table 3). *T. ni* larvae ate significantly more leaf area ($P < 0.05$, t test) and gained significantly more weight ($P < 0.01$, t test) on plants subjected to fogs of pH 3.0 than on plants exposed to control fogs.

Although a trend for greater average leaf area eaten per larvae was observed for larvae on plants exposed to fogs of pH 2.0 and 2.5, the magnitudes of the differences were less than those at pH 3.0 and were not significant at the $P <$ 0.05 level (Table 3). Variation in foliar water content is often an important factor in insect growth and development (Scriber, 1984) but was not significant

and did not provide an explanation for the results in our study (Table 1). Because nitrogen is often a limiting factor for insects (Mattson, 1980), the lack of improvement in weight gain by *T. ni* larvae was surprising, given the elevated percent total nitrogen in plants exposed to 2.0 pH fogs and the increases in soluble proteins and specific free amino acids in plants subjected to 2.5 pH fogs (Tables 1 and 2). However, because nitrogen was added to the system in the form of fertilizer, the nitrogen availability may not have been as limiting in these plants as compared to other systems. Insects feeding on foliage from plants in either acidic fog treatment, therefore, would not have been limited by percent total nitrogen, but the ratios of amino acids conceivably could affect the acceptance or osmotic balance of treated foliage (House and Barlow, 1964; Broadbeck and Strong, 1987).

Several alternative explanations exist for the lack of increased weight gain of *T. ni* larvae on plants with higher percent total nitrogen, soluble protein, or specific free amino acids. One explanation amenable to further testing is that the nitrate form of nitrogen is unsuitable for *T. ni*. A second explanation is based on the observation that test plants from the pH 2.0 and 2.5 fogs had (by subjective evaluation) more necrotic areas, which reasonably could be expected to interfere with palatability or phagostimulation. Large necrotic regions on

TABLE 3. IMPACT OF ACIDIC FOGS ON LARVAL FEEDING, HOST PREFERENCE, LARVAL WEIGHT GAIN, AND PERCENT SURVIVAL OF *T. ni* EGGS

	pH of test fog					
Source	2.0		2.5		3.0	
Eaten on intact plants (cm^2)[a]		*df*, *t* value		*df*, *t* value		*df*, *t* value
Control plants	8.81	52, −1.043	4.07	52, −1.702[b]	5.28	55, −2.363*
Test plants	10.16		5.62		8.61	
Eaten in 24-hr leaf choice test (cm^2)[c]						
Control plants	0.66	18, −0.404			1.09	18, −0.388
Test plants	0.73				1.25	
Larval weight gain[a]						
Control plants	0.030	49, 1.503	0.030	42, 0.403	0.019	25, 4.323**
Test plants	0.037		0.028		0.093	
Survival of eggs (%)[a]						
Control plants	83.33	10, 1.209			85.33	10, 1.785
Test plants	75.83				75.83	

[a] Analyzed with an unpaired *t* test; *significance at the $P < 0.05$ level; **$P < 0.01$.
[b] Significant at $P < 0.1$ level.
[c] Analyzed with a paired *t* test.

Phaseolus species caused by acidic fogs typically have no intact cells and the cellular material coagulates into an undifferentiated mass less than one third the volume of undamaged tissue (Evans et al., 1977; Trumble and Walker, unpublished). Thus, larvae on these plants would spend more time in transit between acceptable or palatable regions and less time feeding than larvae on foliage with minimal damage. A third explanation is that the changes in nitrogen content and quality were at least partially offset by increases in other compounds (phenolics, phytoalexins, etc.) produced in response to damage that deterred feeding and/or assimilation by the larvae. Finally, because *T. ni* is a generalist feeder, the variation in nitrogen form and content between stressed and unstressed plants within the species observed in this study may be small relative to variation among species within the insect's broad host range.

Long-term effects of acidic fogs on the larval population dynamics of insects would be dependent, among other things, on the time following acidic fog exposure, the duration and acidity of the fog, and the rate at which nitrate nitrogen was converted to insect usable or acceptable forms. In our study, no significant ($P < 0.1$) effects of fogs were seen on the survivorship of the egg stage (Table 3) or on suvivorship of larvae in any treatment–control comparison from the larval weight gain study or the leaf area consumption study (t test, P was always greater than 0.2). However, the survivorship of larvae was not followed through to pupation, nor were any direct effects of acidic fogs on adult oviposition or larval behavior monitored. Thus, additional research must be conducted before the impact of acidic fogs on the life history or population dynamics of *T. ni* can be estimated with confidence.

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