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EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON *Nephapsis bicolor*, A PREDATOR OF *Aleurodicus* spp.

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Abstract: The spiralling whitefly *Aleurodicus dispersus* Russell was introduced to Africa and Asia during the 1990s. Among its potential biological control agents is the predatory coccinellid, *Nephaspis bicolor* Gordon. Since the coccinellid may be required to control *A. dispersus* under variable climatic conditions, four temperature (20-34°C) and two RH (78% and 90%) (55% RH at 26°C only) regimes were evaluated under laboratory conditions in Trinidad and Tobago. Temperature (but not RH) had significant effects on the development rate of all stages of *N. bicolor* and on the pupal and adult sizes. Interactions between temperature and RH were significant only for egg incubation and total duration and for size of the pupa. Mortality during development was highest at 30°C/90% RH and lowest at 23°C/78% RH. Both temperature and RH significantly affected the preoviposition period, while temperature alone affected longevity and lifetime fecundity. Adult survival at 26°C and 55% RH as well as 90% RH was significantly higher compared to all the other treatments. Based on the life table statistics, the best performance of female *N. bicolor* was at 26°C and 55% RH. Although feeding, survival, development and reproduction occurred under all the temperature and RH regimes, constant low and high temperatures were not conducive to the coccinellid since the survival of immature stages and adults was greatly reduced. Thus, the introduction of *N. bicolor* into such environments may not result in long-term establishment. It may be necessary to 'thermally adapt' the beetles prior to release and/or to time the field releases to coincide with favourable environmental conditions. Another solution may lie in maintaining laboratory cultures of *N. bicolor* and making periodic (inoculative/augmentative) releases.

Key words: *Aleurodicus cocois,* Coccinellidae, Coleoptera, Hemiptera, *Nephaspis bicolor*, relative humidity, Sternorrhyncha, temperature

INTRODUCTION

During the last decade of the twentieth century, the spiralling whitefly *Aleurodicus dispersus* Russell (Aleyrodidae: Sternorrhyncha) was introduced to several countries of Africa and Asia (Alam *et al.,*1997; Anon., 1993; Gungah *et al.*, 2005; Kajita *et al.,* 1991; Kiyindou, 1993; Mani and Krishnamoorthy, 1996; Wen *et al.*, 1994). The whitefly had been introduced to Hawaii and Pacific islands of the Pacific in the 1980s, where it was successfully controlled by exotic natural enemies: a parasitic wasp *Encarsia* sp. nr. *haitiensis* Dozier (Aphelinidae) and two predatory beetles, *Nephaspis bicolor* Gordon and *N. indus* Gordon (as *N. amnicola* Wingo = *N. oculata* Blatchley) (Coccinellidae) from Trinidad (Greathead and Greathead, 1992; Kumashiro *et al.*, 1983; Suta and Esguerra, 1993; Tauili'-ili and Vargo, 1993). Field studies established that *A. dispersus* did not occur in in Trinidad and Tobago and that *N. bicolor* was the predominant

species responsible for the effective control of these whiteflies in Trinidad and Tobago (Lopez and Kairo, 2003). Furthermore, *N. bicolor* was the only species encountered during field collections in Hawaii in 1996, more than ten years after the initial release of both *N. bicolor* and *N. oculata.* Therefore, the coccinellid *N. bicolor* was considered for introduction to countries invaded by *A. dispersus* and other *Aleurodicus* spp. and was evaluated in Trinidad and Tobago (Lopez, 2003; Lopez *et al*., 1997; Lopez and Kairo, 2014).

Knowledge of climatic suitability and adaptability of candidate biocontrol agents is useful, as it allows climatic matching and provides much-needed information on rearing. Many countries have a range of environments, depending on geography/topography and the pest species may be present in several environments. Even in seemingly favourable environments, one or more of the climatic factors may deter the establishment of a particular species. In Hawaii, high *A. dispersus* population levels were present in two areas with distinctly different climatic patterns, which strongly influenced the effectiveness of *N. indus* in controlling *A. dispersus* in the two ecological zones (Kumashiro *et al.* 1983). When introduced, *N. bicolor* will be required to control *A. dispersus* and/or other species under a range of environmental conditions. Since temperature and RH are among the major environmental factors that can affect the survival, and thus establishment, of an introduced species, the study reported here was undertaken to determine temperature and humidity effects on the development, reproduction and survival of *N. bicolor*. Four temperature ranges (20-34°C) used in the study represent the tropical / subtropical conditions under which *N. bicolor* may be introduced and expected to be effective.

MATERIALS AND METHODS

Experimental conditions and prey species

Air-tight glass chambers (Patil *et al.,* 1994) were designed with dimensions of 30 cm x 23 cm x 22 cm which provided an internal volume of 15,180 cu. cm. Four glass tubes, 10 cm long each, were glued to the bottom of the chamber to form a square of side 12 cm. A plastic tray, 28 cm x 16 cm, placed on the tubes provided the platform on which petri dishes were placed inside the chamber. The cover of the chamber was made of glass and was 0.5 cm longer and wider than the chamber. A one-cm wide glass strip was glued on the inside of the cover, at a distance of 0.75 cm from the edge on all four sides, which prevented the cover from sliding off. The chamber was made airtight by applying a layer of Vaseline® along the perimeter of the cover (Figure 1).

Saturated salt solutions were used to obtain different RHs (Winston and Bates, 1960). Based on the size of the chambers, the volume of the solutions required was calculated to be 425 ml per chamber. Four chambers were set up with saturated solutions of sodium chloride (NaCl) for 75-80% RH and four with potassium chloride (KCl) for 85-90% RH. Four controlled-temperature (CT) Rooms were maintained at temperatures ranging between 20-24°C, 24-28°C, 29-33°C and ambient, respectively. Two humidity chambers (one each with KCl and NaCl) were placed in each laboratory. A layer of salt crystals was maintained at the bottom of the chambers at all times. It was not possible to set up the treatment of 55-60% humidity despite several trials. Therefore, data collected on the biology of *N. bicolor* at 26°C and 55% RH were used to compare the effects of humidity at this temperature.

A maximum-minimum thermometer was placed in each laboratory and temperatures were recorded daily. A calibrated Fisher Hair Hygrometer was used to measure the humity inside the chamber. Observations on the duration of development were recorded daily. Throughout the experiment, *Aleurodicus cocois* (Curtis) reared on coconut plants (Lopez *et al.,* 2005) was used as the prey for feeding *N. bicolor*. The cultures were maintained outdoors under sleeve cages. Plants harbouring the required whitefly stages were brought into the CT Room at least 48 h prior to being used in order to acclimatize them.

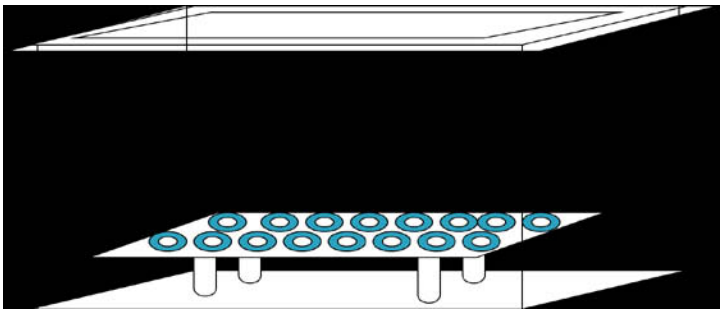

Figure 1. Line diagram of a humidity chamber (not drawn to scale).

Developmental biology

Oviposition was obtained on coconut leaves harbouring larvae/pupae of *A. cocois* by releasing large number of mated *N. bicolor* females overnight to obtain uniform-age eggs. The leaves were observed under a microscope and the flocculent material carefully removed to locate the eggs, which were gently detached with a fine paintbrush. The eggs were placed individually on leaf discs with mixed whitefly stages and moved to a 4-cm diameter petri dish. The petri dishes were arranged on the 28 x 16 cm plastic tray, which was then placed on the tubes inside the humidity chamber. Under each of the nine experimental conditions, 57-78 eggs was set up and observations were recorded daily. When the eggs hatched, the newly emerged larvae were gently transferred to fresh leaf discs with a fine brush and the duration of development for each of the four stages recorded. Leaf discs were changed as required ensuring that there was always an excess of food available for the developing larvae. Measurements were made on three stages in order to assess the effect of experimental conditions on insect growth. These were 4^{th} instar larva (width of head capsule at the widest point), pupa (width at the widest point) and newly emerged adult (length and width at the longest and widest point, respectively).

Reproductive biology

Newly emerged adults were paired and released for oviposition on leaf discs harbouring mainly $3^{rd}/4^{th}$ instars and pupae. The leaf discs were changed every three days. The number of eggs laid during this interval was recorded. The insects were observed daily for mortality. If males died during the course of the experiment, they were replaced with mature males from general cultures. The experiment was terminated when all the females died.

Data analysis

The data recorded on various developmental and reproductive biology parameters were subjected to a General Linear Model (GLM) analysis using SPSS® (Norusis, 1993). The Analyses of Variance (ANOVAs) from the GLM output were used to determine the effect of the various temperature and RH regimes as well as interactions between temperature and RH on the various parameters. Data from the third RH treatment (55%), which was available for 26°C only, was subjected to a separate ANOVA using EXCEL® to determine differences among the three RH treatments (90%, 78% and 55%) for all parameters. Since there were unequal replications, paired t-tests were used for comparing means and evaluating levels of significance for those treatments where significant F values were obtained.

Log linear models were fitted to obtain approximate X^2 (chi-squared) values in order to evaluate the effects of, and interactions between, the temperature and RH treatments on the survival of the immature stages of *N. bicolor.* Data on the survival of adult female *N. bicolor* were subjected to the Kaplan Meir analysis using SPSS® (Lopez and Kairo, 2003). The correlation between longevity and fecundity was determined using SPSS®. The data on survival and egg production of females was used to compute life table parameters, according to Birch (1948): net reproductive rate $R_0 = \sum_0^\infty l_x m_x$; mean generation time GT = $ln\ R_0/r_m$; intrinsic rate of increase r_m = $\sum e^{-r_m x} l_x m_x = 1$; finite rate of increase $\lambda = e^{r_m}$ and doubling time DT = $ln\ 2/r_m$.

RESULTS

The average maximum and minimum temperature, overall mean temperature and RH regimes during the course of the experiment were as follows:

Temperature regimes: minimum - maximum (average)	Humidity regimes:
1. 20 - 26° C (23.3°C) 2. 21 - 31° C (25.9) 3. Ambient 23.3 - 31.7 (27.7) 4. 27 - 34°C (30.4)	1. NaCl: 75-81% (all temperatures) 2. KCl: 88.5-92% (all temperatures) 3. CT Room 50-60% (only at 25.9° C)

For convenience of presentation, the treatments are rounded off to the nearest whole number, e.g. temperatures are referred to as 23°C, 26°C, 30°C and ambient, and RH as 90%, 78% and 55%.

Developmental biology

The means (±SE) for the duration of development and size of *N. bicolor* (Table 1) are based on the output from the General Linear Model (GLM). Under both RHs, the rate of development of *N. bicolor* generally increased as the temperature increased from 23°C to 30°C.

Table 1. Duration of development (days±SE) and size (mm±SE) of *N. bicolor* under four temperature and two humidity regimes

	23°C		26°C		Ambient		30°C	
	90%	**78%**	**90%**	**78%**	**90%**	**78%**	**90%**	**78%**
Duration of development:								
Egg	6.11 ± 0.061 n = 66	6.27 ± 0.062 n = 64	5.34 ± 0.061 n = 67	5.05 ± 0.064 n = 60	5.20 ± 0.058 n = 74	5.03 ± 0.064 n = 61	4.5 ± 0.061 n = 66	4.57 ± 0.057 n = 75
1st instar	3.07 ± 0.122 n = 54	2.95 ± 0.115 n = 61	1.84 ± 0.113 n = 63	2.06 ± 0.131 n = 47	1.78 ± 0.018 n = 69	2.02 ± 0.126 n = 51	2.13 ± 0.121 n = 55	1.96 ± 0.121 n = 55
2nd instar	2.94 ± 0.140 n = 48	3.07 ± 0.126 n = 59	1.93 ± 0.126 n = 59	1.93 ± 0. 149 n = 42	2.25 ± 0.118 n = 67	2.00 ± 0.143 n = 46	2.02 ± 0.140 n = 48	1.96 ± 0.132 n = 54
3rd instar	3.77 ± 0.208 n = 44	3.44 ± 0.183 n = 57	2.54 ± 0.183 n = 57	2.62 ± 0.221 n = 39	2.60 ± 0.142 n = 65	2.65 ± 0.210 n = 43	2.67 ± 0.213 n = 42	2.65 ± 0.191 n = 52
4th instar	4.83 ± 0.233 n = 41	5.13 ± 0.203 n = 54	3.49 ± 1.36 n = 51	3.32 ± 0.242 n = 38	3.17 ± 0.203 n = 54	3.60 ± 0.230 n = 42	3.42 ± 0.249 n = 36	3.47 ± 0.223 n = 45
Total larval	20.7 ± 0.38 n = 41	20.9 ± 0.33 n = 54	15.4 ± 0.34 n = 51	15.1 ± 0.39 n = 38	14.9 ± 0.33 n = 54	15.4 ± 0.37 n = 42	14.5 ± 0.41 n = 36	14.7 ± 0.36 n = 45
Pre-pupa	1.90 ± 0.132 n = 40	2.06 ± 0.121 n = 48	1.91 ± 0.13 n = 43	1.43 ± 0.14 n = 35	1.32 ± 0.143 n = 34	1.39 ± 0.135 n = 38	1.24 ± 0.167 n = 25	1.26 ± 0.135 n = 38
Pupa	6.15 ± 0.138 n = 33	6.24 ± 0.123 n = 41	4.94 ± 0.134 n = 35	4.45 ± 0.138 n = 33	4.26 ± 0.152 n = 27	4.38 ± 0.136 n = 34	3.74 ± 0.182 n = 19	3.93 ± 0.144 n = 30
Total overall	28.3 ± 0.48 n = 33	28.7 ± 0.43 n = 41	22.4 ± 0.46 n = 35	20.9 ± 0.048 n = 33	20.6 ± 0.53 n = 27	21.1 ± 0.47 n = 34	18.7 ± 0.63 n = 19	20.0 ± 0.50 n = 30
Size								
4th instar[a]	0.421 ± .002 n = 39	0.425 ± .002 n = 51	0.42 ± .002 n = 49	0.424 ± .002 n = 36	0.422 ± .002 n = 64	0.424 ± .002 n = 40	0.420 ± .002 n = 40	0.419 ± .002 n = 49
Pupa[b] (width)	1.14 ± 0.013 n = 36	1.10 ± 0.011 n = 47	1.10 ± 0.012 n = 40	1.12 ± 0.013 n = 35	1.12 ± 0.013 n = 33	1.10 ± 0.012 n = 38	1.07 ± 0.015 n = 24	1.09 ± 0.012 n = 38
Adult[c] (length)	1.48 ± 0.018 n = 27	1.49 ± 0.015 n = 41	1.44 ± 0.017 n = 30	1.47 ± 0.017 n = 32	1.46 ± 0.020 n = 23	1.46 ± 0.017 n = 33	1.41 ± 0.025 n = 15	1.46 ± 0.018 n = 27
Adult[b] (width)	1.06 ± 0.013 n = 27	1.04 ± 0.010 n = 41	1.02 ± 0.012 n = 30	1.04 ± 0.012 n = 32	1.04 ± 0.014 n = 23	1.02 ± 0.012 n = 33	1.00 ± 0.017 n = 15	1.01 ± 0.013 n = 27

n = as in Table 1.; a = width of the head capsule of 4th instar larva (at the widest point); b = width of pupa / adult (at the widest point); c = length of adult (at the longest point).

The duration of development of all stages was significantly ($p<0.01$) affected by temperature but not by RH (Table 2) and was longer for all stages at 23°C. The interactions between temperature and RH were significant only for the egg stage and total development. Significant temperature effects were recorded for pupal and adult width, while RH had no effect on the size of the insect. The interaction between temperature and RH was significant only for the width of the pupa.

Table 2. ANOVAs from General Linear Model (GLM) to investigate the effects of temperature and relative humidity (RH) on the duration of development and size of *Nephaspis bicolor*

	Temperature	**RH**	**Temperature * RH**
Duration of development:			
Egg	187 (254***) [1]	0.44 (1.78)	4.3 (5.8***)
1st instar	95 (39***)	0.21 (0.26)	3.9 (1.6)
2nd instar	78 (28***)	0.22 (0.24)	2.1 (0.7)
3rd instar	71 (13***)	0.31 (0.16)	2.6 (0.5)
4th instar	170 (24***)	2.02 (0.91)	4.8 (0.7)
Total larval development	2254 (129***)	1.44 (0.25)	6.6 (0.4)
Prepupa	24 (12**)	0.22 (0.32)	4.9 (2.3)
Pupa	199 (106***)	0.02 (0.04)	4.8 (2.6)
Total development	3223 (143***)	1.88 (0.25)	59.4 (2.6*)
Size			
4th instar[a]	0.0007 (1.05)	0.0004 (1.78)	0.0003 (0.48)
Pupa[b]	0.065 (3.90**)	0.002 (0.32)	0.049 (2.93*)
Adult length[c]	0.065 (2.40)	0.031 (3.64)	0.017 (0.63)
Adult width[c]	0.039 (2.10*)	0.0003 (0.07)	0.017 (1.28)

1 = Sum of squares (F values); *, **, *** = significant at $p<0.05$, $p<0.01$ and $p<0.001$, respectively Degrees of freedom (df) were as follows: Temperature = 3; Humidity = 1; T x H = 3; a, b and c = as in Table 1.

At 26°C, no significant differences were found in the rate of development of the 4th instar and prepupal stages under the three humidity regimes (Table 3), while paired t-tests revealed significant differences between the other stages (Table 4). Significant differences in the size of 4th instars ($p<0.001$), pupal width ($p<0.001$) and adult width ($p<0.05$) (Table 5) were attributable mainly to the differences between the sizes at 55% and the higher RH (Table 6).

Table 3. ANOVA for the duration of development of *N. bicolor* under three RH regimes at 26°C

Stage	**Duration of development (days±SE)**			**F (df)**
	90% RH	**78% RH**	**55% RH**	
Egg	5.3 ± 0.06 (n=67)	5.05 ± 0.05 (n=60)	5.4 ± 0.09 (n=55)	8.82*** (179)
1st instar	1.8 ± 0.07 (n=63)	2.1 ± 0.14 (n=47)	2.2 ± 0.07 (n=54)	3.13* (161)
2nd instar	1.9 ± 0.12 (n=59)	1.9 ± 0.12 (n=47)	2.4 ± 0.18 (n=54)	3.44* (152)
3rd instar	2.5 ± 0.13 (n=57)	2.6 ± 0.16 (n=39)	3.1 ± 0.19 (n=51)	3.10* (145)
4th instar	3.5 ± 0.19 (n=51)	3.3 ± 0.22 (n=38)	3.8 ± 0.21 (n=51)	1.52 (137)
Total (larval)	10 ± 0.24 (n=51)	10 ± 0.32 (n=38)	11.4 ± 0.32 (n=51)	8.03*** (137)
Prepupa	1.9 ± 0.18 (n=43)	1.4 ± 0.11 (n=35)	1.6 ± 0.11 (n=48)	2.83 (123)
Pupa	4.9 ± 0.17 (n=35)	4.5 ± 0.12 (n=33)	4.2 ± 0.08 (n=47)	10.35*** (112)
Total (overall)	22.4 ± 0.39 (n=35)	20.9 ± 0.42 (n=33)	22.4 ± 0.39 (n=47)	4.07* (112)

*, *** = as in Table 2. n = as in Table 1; df = degrees of freedom

Table 4. Results from t-tests for the duration of development (in days) of *N. bicolor* under three RH regimes at 26°C

RH 1	RH2	Stages significantly different between RH1 & RH2
90%	78%	Egg**, 1st instar*, Pupa**, Overall total*
	55%	1st instar*, 2nd instar*, 3rd instar*, Total larval duration**, Pupa**
78%	55%	Egg**, 2nd instar*, 3rd instar*, Total larval**, Overall total duration**

*, ** = as in Table 2.

Table 5. ANOVA for the size of 4th instar larva, pupa and adult length & width (all in mm ± SE) of *Nephaspis bicolor* under three RH regimes at 26°C

Stage	Size (in mm ± SE) under			F value (df)
	90% RH	78% RH	55% RH	
4th instar[a]	0.42 ± 0.001 (n=49)	0.42 ± 0.002 (n=36)	0.40 ± 0.003 (n=52)	39.51*** (134)
Pupa[b]	1.10 ± 0.009 (n=40)	1.12 ± 0.006 (n=35)	1.04 ± 0.007 (n=47)	17.68*** (119)
Adult length[c]	1.44 ± 0.019 (n=30)	1.46 ± 0.021 (n=33)	1.48 ± 0.013 (n=47)	1.24 (107)
Adult width[c]	1.02 ± 0.011 (n=30)	1.04 ± 0.013 (n=32)	0.99 ± 0.010 (n=47)	4.33* (106)

*, *** = as in Table 1; a, b, c = as in Table 1; df = as in Table 3.

Table 6. Results from t-tests for size (mm) of *N. bicolor* reared under three RH regimes at 26°C

RH 1	RH2	Sizes significantly different between RH1 & RH2
90%	78%	None
	55%	4th instar**, pupal width**, adult width*
78%	55%	4th instar**, pupal width**, adult width**

*, ** = as in Table 2.

Overall cumulative mortality (%) was the highest at 90% RH, with 71.2% and 63.7% deaths occurring at 30°C and ambient, respectively (Table 7) and lowest mortality (40.6%) at 23°C and 78% RH. The approximate-χ^2 values for the survival of immature stages revealed Temperature (T) x Survival (S) and Humidity (H) x S interactions to be significant for 1st instar, prepupa and pupa. In addition, T x S interactions were significant for the 4th instar. High variations in some

Table 7. Cumulative mortality (%) and approximate χ^2 values for the survival of immature stages *Nephaspis bicolor* under two humidity and four temperature regimes.

	23°C		26°C		Ambient		30°C		χ^2	χ^2	χ^2
Stage	**90%**	**78%**	**90%**	**78%**	**90%**	**78%**	**90%**	**78%**	**$T^1 \times S^2$**	**$H^3 \times S$**	**$H \times T$**
1st instar	18.2	4.7	6.0	31.7	6.8	16.4	16.7	26.7	13.93**	7.67**	6.14*
2nd instar	27.3	7.8	11.9	40.0	9.5	24.6	27.3	28.0	6.30	1.31	19.96**
3rd instar	33.3	10.9	14.9	45.0	12.2	29.5	36.4	30.7	7.32	0.52	23.29***
4th instar	37.9	15.6	23.9	46.7	27.0	31.2	45.5	40.0	9.32*	0.29	11.08*
Prepupa	39.4	31.3	35.8	51.7	54.1	37.7	62.1	49.3	17.29***	4.21*	4.16
Pupa	50.0	40.6	47.8	55.0	63.7	44.3	71.2	60.0	16.14**	6.86**	1.89
Rank (mortality)	5	8	6	4	2	7	1	3	-	-	-

T = Temperature; 2. S = Survival; 3. H = Humidity; Degrees of freedom (df) for both T × S and H × T = 3; df for H × S = 1; *, **, *** = approximate χ^2 values significant at p<0.05, 0.01 and 0.001, respectively

of the treatments may have resulted in significant, unexpected approximate-χ^2 values for T x H interactions for instars 1-4. Under the three RH regimes at 26°C, approximate-χ^2 values for survival were highly significant for all stages (Table 8).

Table 8. Cumulative mortality and approximate χ^2 values for the survival of immature stages of Nephaspis bicolor under three humidity regimes at 26°C

Stage	90%	78%	55%	χ^2
1st instar	6.0	31.7	1.81	14.336***
2nd instar	11.9	40.0	3.64	18.485***
3rd instar	14.9	45.0	7.27	17.474***
4th instar	23.9	46.7	9.09	14.005***
Prepupa	35.8	51.7	14.55	14.029***
Pupa	47.8	55.0	16.37	18.451***

*** = approximate χ^2 values significant at $p<0.001$; All approximate χ^2 had 2 degrees of freedom.

Reproductive biology

Means (±SE) for pre-oviposition period, longevity and lifetime fecundity are presented in Table 9. The number of females that died without ovipositing ranged from 0 (78% and 90% RH at 26°C and 78% RH at 30°C) to 7 (78% RH at ambient). Based on the ANOVAs, both temperature and RH had significant effects on the pre-oviposition period, while temperature alone affected longevity and lifetime fecundity (Table 10). Temperature x RH interactions were significant only for the pre-oviposition period.

The ANOVA for the three reproductive parameters at 26°C revealed that both longevity and lifetime fecundity were affected by RH (Table 11). Female *N. bicolor* maintained at 55% RH lived significantly longer than those maintained at 78% RH. They also oviposited more eggs than those at the higher RH treatments did (Table 12).

Results from the Kaplan Meier analysis for the survival (days ± SE) of female *N. bicolor* under the nine treatment regimes are presented in Figure 2. Breslow statistics revealed that survival at 26°C and 55% RH was significantly higher compared to all the other treatments except 26°C and 90% RH (Table 13). Survival rate under the latter treatment was, in turn, significantly higher than that under the higher temperature regimes (30°C and ambient) at both RHs. Under other regimes, survival was variable and did not appear to follow any particular pattern.

Life-table statistics for each parameter were ranked in descending order for purposes of evaluation (Table 14). The best performance of *N. bicolor* was at 26°C and 55% RH, which recorded the highest values for net reproductive rate (R_O), intrinsic rate of increase (r_m) and innate capacity for increase (λ), and the lowest for doubling time (DT). The reverse was true for the combination of 30°C and 90% RH. Reduction in the humidity to 78% at 30°C resulted in improved values for most parameters. The combinations 26°C at 78% and at 90% RH were superior to all the remaining treatments, ranking 2 or 3 for most parameters (Table 14).

Although R_O was almost half at 90% RH under ambient (4.26) compared to 23°C (8.06), the two combinations were almost on par with regard to r_m, DT and λ. With few exceptions, the rankings for generation time (G) followed the same pattern as the other parameter.

Table 9. Reproductive biology parameters of female *N. bicolor* under four temperature and two humidity regimes (based on GLM)

	23°C		**26°C**		**Ambient**		**30°C**	
Parameter	**90%**	**78%**	**90%**	**78%**	**90%**	**78%**	**90%**	**78%**
Pre-oviposition period (days±SE)	13.3± 0.9 n=10[1]	18.0± 0.7 n=15	10.4±0.9 n=12	11.3±0.8 n=12	8.9± 1.0 n=8	9.8± 0.7 n=17	11.0±1.3 n=5	10.7 ±0.9 n=9
Longevity (days ± SE)	42.1± 6.5 n=12[2]	43.6± 4.9 n=21	59.1± 6.5 n=12	43.8± 6.5 n=12	25.3± 6.5 n=12	32.7±4.6 n=24	24.8±9.1 n=6	24.8± 6.5 n=9
Lifetime fecundity (no. of eggs / female ± SE)	34.8± 9.3 n=10[1]	28.0± 7.0 n=15	55.0±9.3 n=12	45.7± 9.3 n=12	23.0± 9.3 n=8	31.8±6.6 n=17	10.0±13.1 n=5	13.8± 9.3 n=9

1 = number of insects for which pre-oviposition period and lifetime fecundity were recorded; 2 = number of insects for which longevity was recorded

Table 10. ANOVA of General Linear Model (GLM) to investigate temperature and RH effects on reproductive biology in *N. bicolor*

Parameter	**Temperature**	**RH**	**Temperature * RH**	**Error (df)**
Preovipostion period	521*** (22.1)[1]	46.0* (5.8)	24.6 (3.1)*	622 (79)
Longevity	3459*** (6.9)	62.2 (0.1)	615 (1.2)	51557 (103)
Lifetime fecundity	5050** (4.883)	22.807 (0.022)	493.12 (0.477)	106525 (103)

1 and *, **, *** as in Table 2; df = as in Table 2.

Table 11. ANOVA for three reproductive biology parameters of female *N. bicolor* under three RH regimes at 26°C

Parameter	**90% RH**	**78% RH**	**55% RH**	**F value (df)**
Pre-oviposition period (days)	10.4±0.85 (n=12)	11.3±0.81 (n=12)	10.2±0.65 (n=10)	0.65 (31)
Longevity (days)	59.1± 6.46 (n=12)	43.8± 6.46 (n=12)	75.0±11.80 (n=10)	3.2* (31)
Fecundity (eggs/ female)	55.0±9.28 (n=12)	45.7± 9.28 (n=12)	93.0±12.60 (n=10)	4.51* (31)

* = significant at $p<0.05$; df = as in Table 3

Table 12. Results from paired t-tests for three reproductive biology parameters of *N. bicolor* females under three RH regimes at 26°C

RH 1	**RH2**	**Parameters with significant differences between RH1 & RH2**
90%	78%	None
	55%	Fecundity*
78%	55%	Longevity*, Fecundity*

* = significant at $p<0.05$.

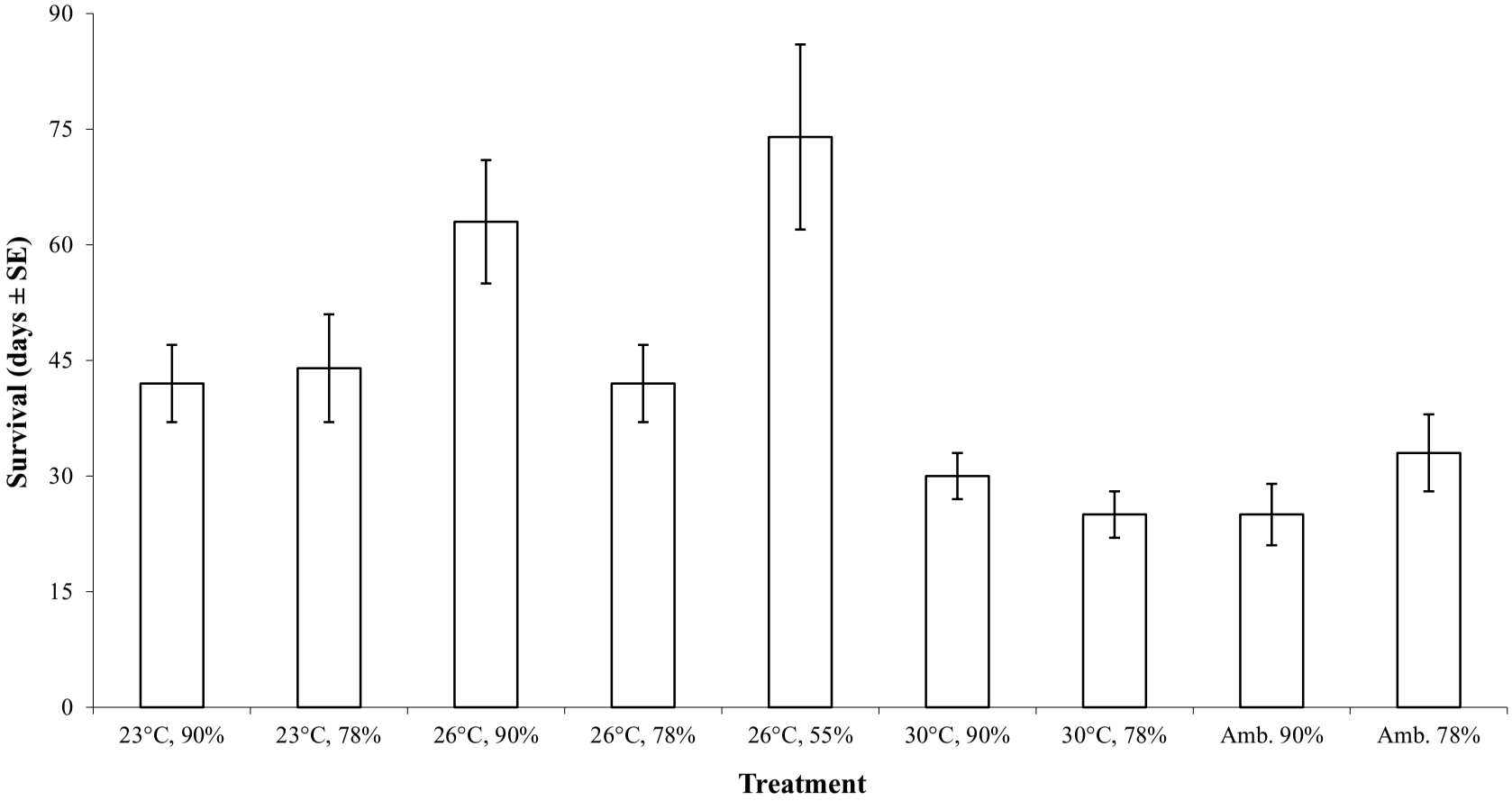

Figure 2. Survival (days ± SE) of *Nephaspis bicolor* females under nine temperature/RH regimes

Table 13. Breslow statistics for comparing the survival of female *Nephaspis bicolor* under nine treatment regimes

	23°C, 90%	**23°C, 78%**	**26°C, 90%**	**26°C, 78%**	**26°C, 55%**	**30°C, 90%**	**30°C, 78%**	**Amb., 90%**	**Amb., 78%**
23°C, 90%	-								
23°C, 78%	0.44								
26°C, 90%	4.80*	2.96							
26°C, 78%	0.00	0.66	2.80						
26°C, 55%	8.20**	4.33*	0.49	7.37**					
30°C, 90%	2.68	0.00	8.36**	1.49	16.74***				
30°C, 78%	6.35*	1.21	11.31***	4.75*	18.19***	0.68			
Amb., 90%	6.38*	1.00	11.89***	5.01*	17.20***	0.40	0.01		
Amb., 78%	2.11	0.91	7.28**	2.40	9.42**	0.03	0.46	0.43	-

*, ** and *** = differences between the treatments significant at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

Table 14. Life table statistics for *Nephaspis bicolor* under four temperature and three humidity regimes

	23°C		**26°C**			**Ambient**		**30°C**	
Parameter	**90%**	**78%**	**90%**	**78%**	**55%**	**90%**	**78%**	**90%**	**78%**
Net repro. Rate (R_O)	8.06	8.92	14.29	11.59	38.42	4.26	8.57	1.44	2.66
(Rank[1])	(6)	(4)	(2)	(3)	(1)	(7)	(5)	(9)	(8)
Intrinsic rate of increase (r_m)	0.0358	0.0314	0.0487	0.0493	0.0689	0.0353	0.0435	0.0084	0.023
(Rank)	(5)	(7)	(3)	(2)	(1)	(6)	(4)	(9)	(8)
Generation time (G)	61.61	75.92	64.85	54.26	67.82	42.04	54.43	43.40	42.54
(Rank)	(4)	(1)	(3)	(6)	(2)	(9)	(5)	(7)	(8)
Doubling time (DT)	19.36	22.08	14.23	14.06	10.06	19.64	15.93	81.87	29.88
(Rank)	(5)	(7)	(3)	(2)	(1)	(6)	(4)	(9)	(8)
Innate capacity for increase (λ)	1.036	1.032	1.050	1.051	1.071	1.036	1.045	1.009	1.024
(Rank)	(5)	(7)	(3)	(2)	(1)	(5)	(4)	(9)	(8)

1. Ranked in descending order based on performance for each parameter

DISCUSSION AND CONCLUSIONS

Under natural conditions in Trinidad, the average maximum and minimum temperature and RH range between 30.4-32.0°C and 20.3-22.4°C (mean 25.4-26.9°C), and 55-65% and 81-92% (mean 70-78%), respectively (based on meteorological data obtained from the University of the West Indies, St. Augustine, Trinidad). The presence of *N. bicolor* in the field throughout the year indicated that the coccinellid withstood existing temperature and RH ranges. Fluctuating field populations of *N. bicolor* appeared to be more a reflection of shifting prey populations than of any significant influence of climatic or weather factors (Lopez, 2003). Field studies in Hawaii revealed that the combination of low temperature and high RH were apparently not conducive to *N. indus*, even in areas with *A. dispersus* high populations (Kumashiro *et al*., 1983).

Results from the present study generally support these observations. Under both RH regimes, the duration of *N. bicolor* development was longest at 23°C and became shorter as the temperature increased to 30°C. Temperature also impacted on the size of the pupae and the adults, while temperature x RH interactions affected egg incubation, total duration of development and size of pupa. RH effects, on the other hand, were not significant both on duration of development and size of the adults. Both temperature and RH impacted survival in the 1st instar and the prepupal stages, while only the 4th instar was affected by temperature. At 26°C, all three RH treatments (90%, 78%, 55%) impacted development and reproduction, and had profound effect on the survival of all stages of *N. bicolor*. Overall, the regime 26°C and 55% RH best supported all stages of *N. bicolor*, indicating that these were optimum conditions for the survival of the coccinellid.

Somewhat similar effects have been reported on other tropical coccinellids. Temperatures of 25°C and 27±1°C, and RH of 75-85% and 65±5% were optimum for *Hyperaspis raynevali* Mulsant in Congo and *Chilocorus bijugus* Mulsant in India, respectively (Kiyindou *et al.,* 1987; Rawat *et al.*, 1992). *Scymnus frontalis* Fabricius developed in 80 days at 15°C and 17 days at 30°C, with higher survival at 19°C and 26°C (Naranjo *et al*., 1990). Temperatures of 25-30°C were optimum for the development of *Cryptolaemus montrouzieri* and there was a significant relationship ($R^2 = 0.82$) between temperature and duration of development (Jalali *et al*., 1999).

Temperature and RH effects on reproduction in *N. bicolor* were somewhat mixed and variable. Pre-oviposition period was the only parameter that was affected by temperature and RH as well as their interaction: it became shorter as temperatures increased from 23°C to Ambient (28°C), however, a further increase lengthened it significantly. Longevity and lifetime fecundity, on the other hand, increased as the temperature increased from 23°C to 26°C, but a further increase caused a decline in both parameters. Somewhat similar results have been reported for other coccinellids. Pre-oviposition period of *S. frontalis* was 20.5 and 7.7 days at 15°C and 30°C, respectively (Naranjo *et al*., 1990). The fecundity in *C. sexmaculata* increased from 348 eggs to 2611 eggs as temperatures rose from 26°C to 30°C, however, increase to 34°C resulted in a reduction to 356 eggs (Alikhan and Yousuf, 1986). The pre-oviposition period and the longevity of *Coccinella septumpunctata* L. decreased as temperatures increased from 20°C to 35°C (Xia *et al*., 1999). Lifetime fecundity, however, increased from 20°C to 25°C, at which it was the highest, and then declined rapidly as temperatures were increased to 30°C and 35°C.

In the present study, *N. bicolor* fed, survived, developed and reproduced at all the temperature and RH regimes tested. However, constant low or high temperatures were not conducive to the development and reproduction of the coccinellid since the survival of both immature stages and adults was greatly reduced. Further evidence to support this observation was obtained by reviewing the geographic and climatic conditions of the areas where *N. bicolor* has been successfully introduced. The majority of the introductions were made in the Caribbean and the Pacific, with warm, tropical climates (temperature ranges of 21-30°C and RH 50-90%, which are very similar to Trinidad). The related species, *N. indus* (as *N. amnicola*), did not establish in Bermuda and Fiji (Kamath, 1979). Thus, even within the genus, there appears to be some variability in the response to similar environments, since *N. bicolor* became established in Fiji and also reportedly displaced *N. indus* in Hawaii (Cock, 1985). Field-collected *Nephaspis* spp. from Hawaii in 1997 consisted entirely of *N. bicolor* (Lopez, 2003). In Trinidad, *N. bicolor* often comprised 90-100% of *Nephaspis* spp. collected in the field (Lopez and Kairo, 2003). It may thus be inferred that *N. bicolor* is more competitive and adaptable than *N. indus*.

RECOMMENDATIONS

Based on previous history of successful introduction as well as results from field studies, it is concluded that *N. bicolor* is a tropical species that can, upon introduction, become easily established in areas with similar environmental conditions as Trinidad.

Results from the present experiments also suggest that survival and long-term establishment may not occur in areas with temperature and RH ranges that are significantly different. It should be noted, however, that in many countries, there are climatic differences with seasons and altitude. It is crucial therefore that the time (season) and location of releases should coincide with the conditions that are optimum for *N. bicolor* survival in order to achieve establishment in the new environment.

Releasing climatically-matched species or biotypes of predatory coccinellids, including those adapted to local temperature conditions, is considered critical in biological control (Obrycki and Kring, 1998). Thus, if the introduction of *N. bicolor* becomes necessary in an area with seasonal lower temperatures for example, then it is suggested that further testing should be carried out simulating the conditions under which the introduction is proposed. It may also be possible to 'thermally adapt' *N. bicolor* by rearing through several generations at the required temperature prior to field release.

The example of *Cryptolaemus montrouzieri* Mulsant should also be borne in mind. It is well known that this coccinellid (like *N. bicolor*) is adapted to tropical temperatures (Peterkin *et al*., 1998), is unable to complete development at 10-17°C and requires at least 21°C to feed and oviposit (Babu and Azam, 1987; Coddling, 1977). Thus, although *C. montrouzieri* has been successfully introduced in temperate countries for the control of mealy bugs, predator populations usually die out during winter. Commercial insectaries in these countries maintain and produce the coccinellid in the laboratory for the control of various pest species under glass house conditions, and also to initiate or augment field populations. A similar solution may be applied in the case of *N. bicolor*, i.e. maintaining laboratory cultures of *N. bicolor* and making periodic (inoculative/augmentative) releases as necessary.

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