



Influence of oviposition strategy of *Nemorilla pyste* and *Nilea erecta* (Diptera: Tachinidae) on parasitoid fertility and host mortality

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HIGHLIGHTS

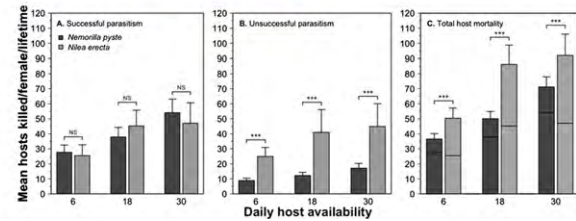
- ▶ *Nemorilla pyste* is a tachinid parasitoid that oviposits onto hosts.
- ▶ *Nilea erecta* is a tachinid parasitoid that ovularviposits onto substrate around hosts.
- ▶ The influence of host density on parasitoid fertility and host mortality was examined.
- ▶ Species had similar fertilities, but *N. erecta* caused higher host mortality.
- ▶ Increased host mortality from *N. erecta* was attributed to superparasitism.

GRAPHICAL ABSTRACT

Nemorilla pyste oviposits directly onto the host and develops gregariously.



Nilea erecta ovularviposits onto foliage and develops solitarily. This strategy resulted in higher rates of unsuccessful parasitism and higher host mortality.



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ABSTRACT

This study examined fertility of the tachinid (Diptera) parasitoids *Nemorilla pyste* (Walker) and *Nilea erecta* (Coquillett), and mortality of the host, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae). These are common parasitoids of mature *C. rosaceana* larvae in apple and sweet cherry orchards in central Washington where *C. rosaceana* is an important pest. The parasitoids have similar phenology and use the same stages of the host, but their modes of attack and development in the host differ. *N. pyste* oviposits on the host and develops gregariously, while *N. erecta* ovularviposits on foliage near the host and develops solitarily. Life tables were used to compare adult longevity and fertility of these flies at three different host densities in laboratory cage experiments. Results suggested that although population growth rates of these species were similar under the experimental conditions, *N. erecta* had a greater effect on mortality of the host population. This additional mortality was attributed to superparasitism because it occurred in host larvae at a higher rate than expected from natural causes, and it occurred more frequently when fewer hosts were available to *N. erecta* females. A supplemental experiment demonstrated that host larvae that were manually infected with *N. erecta* maggots to simulate superparasitism expressed high rates of larval mortality. Thus, even though *N. erecta* had a greater effect on the host population, reproductive opportunity was lost to superparasitism. These findings may demonstrate certain situational disadvantages of ovularviposition as a reproductive strategy, and illustrate how parasitism effects may be underestimated in field sampling.

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1. Introduction

Host attack strategies of parasitoids in the family Tachinidae (Diptera) vary widely and play an important role in the population dynamics of species (O'Hara, 2008), making these flies interesting models for demographic study and comparison. Although tachinids

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lack the piercing ovipositor and venom-producing accessory glands that are thought to be key to the success of hymenopteran parasitoids, several adaptations allow them to parasitize hosts from more orders and phyla than any other group of parasitoids (Eggleton and Belshaw, 1993; Feener and Brown, 1997; Sivinski and Aluja, 2001). These include full embryonation of eggs within an uterus/ovisac, structures for stabbing the host to allow partial insertion of eggs, production of stationary or free-living planidial larvae, and the production of microtype eggs, which must be consumed by the host for infection to occur (Clausen, 1940; Wood, 1987).

Demographic data for tachinids is scarce, and understanding of how fecundity determined by dissections of gravid females translates into fertility is limited. The different oviposition strategies likely have important effects on fertility and population dynamics. For example, egg mortality thought to be highest in species that produce microtype eggs that are scattered on host food plants, because the eggs are minute and are produced in great numbers (Price, 1975). Other species produce fewer eggs, but females invest more in them. For example, females of some species produce larger maggots that can wander freely in search of hosts.

Because most species of Tachinidae are beneficial, demographic data are invaluable for improving biological control in ecosystems where tachinids play an important role. This study examined how different oviposition strategies in the tachinid parasitoids *Nemorilla pyste* (Walker) and *Nilea erecta* (Coquillett) affect population dynamics on the host *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae). These tachinids are the most abundant parasitoids of late instar *C. rosaceana* and *Pandemis pyrusana* Kearfott (Tortricidae) larvae in apple and sweet cherry orchards in central Washington where the leafrollers are important pests. *N. pyste* and *N. erecta* accounted for 33% and 23% of all parasitism (56% total) recorded on *C. rosaceana* over three years in central Washington in unsprayed apples (VPJ, unpublished data).

Tortricid leafrollers are important pests in many regions of North America where pome and stone fruits are grown, and tachinid parasitoids are often associated with them. The Okanagan and Similkameen Valleys of British Columbia are adjacent to the current study region, and *N. pyste* and *N. erecta* occur there on *C. rosaceana* and *P. pyrusana* [as *P. limitada* (Robinson)] in apples, but are rare relative to hymenopteran species (Vakenti et al., 2001; Cossentine et al., 2004, 2007). In the two major fruit growing areas of Michigan, tachinids were the second most important family of parasitoids on *C. rosaceana* in apples, accounting for 36% of all parasitism, and *N. erecta* ranked among the most important tachinid species (Wilkinson et al., 2004). In New York, tachinid parasitism of leafrollers in apples was as high as 54% and was partially attributed to *N. erecta* (Westbrook, 2003). In a more recent study examining the potential for conservation biological control of parasitism of *C. rosaceana* in New York, tachinids were as important as hymenopteran parasitoids (Sarvary et al., 2007). In abandoned apple orchards in southern Ontario, *N. erecta* was an infrequent parasitoid of *C. rosaceana* (Hagley and Barber, 1991). In Poland, where a different leafroller pest complex affects apples, tachinids were rare on most leafroller species, but were the most important group parasitizing the European leafroller, *Archips rosanus* L. (Pluciennik and Olszak, 2010).

Host ranges of *N. pyste* and *N. erecta* are broad and overlap extensively, with most hosts found in Tortricidae and Pyralidae (Lepidoptera) (Arnaud, 1978; O'Hara, 2005). The flies are also similar in that they attack primarily late instar host larvae, and lifetime fecundities of both species are predicted to be ≈ 100 eggs per female (O'Hara, 2005). In both species, the maggots typically complete feeding in the pupal stage of the host, and emerge from dead host pupae to form their puparium nearby (Wiman, 2011). The flies also have similar appearances and body size.

However, despite many apparent similarities, these two flies have very different oviposition and host use strategies. *N. pyste* is oviparous and develops gregariously in the host (O'Hara, 2005; Wiman, 2011). The large, unembryonated plano-convex egg is glued directly to the host, and requires several days of incubation before the maggot emerges and penetrates. Eggs are allocated to hosts either individually, or in clutches, and gregarious broods constitute 2–3 parasitoids per host (Wiman, 2011). In contrast, *N. erecta* is ovolarviparous and solitary (O'Hara, 2005; Wiman, 2011). Ovolarviparity is a more evolutionarily advanced attack strategy where the eggs are held and fully embryonated in an ovisac, which is a heavily tracheated evagination of the common oviduct (Wood, 1987; Stireman, 2002; O'Hara, 2008; Tachi and Shima, 2009). In *N. erecta*, the eggs hatch immediately when deposited on foliage, and the fully developed 1st instar maggots seek out hosts, burrowing inside immediately on contact.

One goal of this research was to determine how the different attack and host use strategies of *N. pyste* and *N. erecta* affect population growth of the two parasitoid species. Population dynamics of the two flies were investigated with life tables for cohorts that were presented with different numbers of hosts. A secondary goal was to determine the effect of the parasitoids on host populations in terms of mortality. Previous studies have shown that immature survival of *N. pyste* is poor, but additional mortality is inflicted on hosts even when parasitoids fail to develop successfully (Wiman, 2011). The impact of flies on host populations was investigated by following mortality in hosts exposed to flies. This research was intended to contribute to a better understanding of the population dynamics of these parasitoids, which are largely unknown in a demographic context. These data may help quantify the value of these species as biological control agents of leafroller pests in deciduous tree fruits at a time when the tree fruit industry in Washington is becoming increasingly receptive to biological control (Jones et al., 2009). Moreover, these efforts may assist conservation biological control efforts in other pome and stone fruit growing regions, in other crop systems affected by tortricid leafrollers (e.g., cane fruits), and forest ecosystems where these Tachinidae occur on *Choristoneura fumiferana* and *C. occidentalis*, the eastern and western spruce budworm, respectively (see O'Hara, 2005).

2. Materials and methods

2.1. Source of parasitoids and hosts

Colonies of *N. pyste* and *N. erecta* were established from flies reared from *C. rosaceana* larvae collected in commercial apple and cherry orchards in central Washington between 2006 and 2010 (Wiman, 2011). Flies were reared in climate controlled growth rooms (16:8 h L:D at ~ 900 lux, 22 °C, $\sim 75\%$ RH). Host *C. rosaceana* larvae came from a laboratory colony maintained on artificial diet (Shorey and Hale, 1965) at the Washington State University Tree Fruit Research and Extension Center in Wenatchee, WA (WSU-TFREC).

2.2. Experimental protocols

On the day of eclosion, male and female flies were paired in cages (0.25 m³) that were provisioned with ≈ 25 ml of a 10% honey-water solution dispensed through a cotton wick (Patterson Dental Supply, Saint Paul, MN) in small cups (30 ml, P125 Solo Cup Company, Lake Forest, IL). Preliminary studies indicated that the flies do not reproduce sooner than 4 days after pairing. Thus, after allowing a mating and pre-oviposition period of 3 days, a bouquet of apple foliage infested with *C. rosaceana* larvae was introduced to

the cage. Each bouquet consisted of four Red Delicious apple stems (≈ 25 – 35 cm long) collected from unsprayed trees at WSU-TFREC. The leaves on the bottom 10 cm of each stem were removed and the stems inserted into 250 ml floral tubes filled with water (Dakota Plastics Co., Watertown, SD). Bouquets were infested with 6, 18, or 30, fifth instar *C. rosaceana* larvae. These densities were chosen to represent scarce to excess host numbers based on preliminary oviposition observations on *N. pyste*. Fifth instars were selected because this was the most frequently attacked stage in the field (Wiman, 2011). Sex ratios of host larvae were balanced at 1:1 to account for potential body size bias of the flies because female *C. rosaceana* are larger than males. When removed from their diet cups and placed on foliage bouquets, colony *C. rosaceana* larvae disperse over the bouquets and establish protected feeding sites by rolling leaves around their bodies using silk. Fifth instar larvae were initially identified by measuring larval head capsule widths using an ocular micrometer ($\bar{x} = 1.11$ mm, range = 0.09–1.40 mm; Jones et al., 2005), but subsequent host selections were made by eye after sufficient experience with larval sizes. Sex of *C. rosaceana* larvae was determined by identification of the male gonads that are visible through the abdominal cuticle.

2.3. Determination of sterility

To evaluate fertility of species, cage data were excluded when females failed to produce offspring, or the reproductive period was interrupted. Thus, life tables represented only those fertile females that were successfully followed through the entirety of adult life. Failure to produce offspring was considered to indicate a problem with mating or fertilization. Considering the complexity of tachinid mating systems, results are focused on fertile females only. Interruption of the reproductive period was caused by premature death of the female by natural causes, accidental death by researcher error, or the escape of the fly from the cage. Escape of flies or accidental death tended to occur when foliage bouquets were exchanged.

2.4. Determination of fertility

For each day of the adult female parasitoids' life, a new bouquet with the same number of larvae was introduced, while the larvae from the previous day were collected. For *N. pyste*, only the host larvae that were parasitized, evident from the external eggs, were collected for rearing. These were placed in individual 30 ml cups (P125 Solo Cup Co.) with ≈ 10 ml of diet and monitored daily for host death, moth eclosion, or parasitoid emergence. Thus, for *N. pyste*, fecundity and fertility were determined. For *N. erecta*, a reliable method for assessing which of the host larvae that were exposed to gravid female flies were parasitized could not be established. Although a droplet of hemolymph often appears on the host following maggot penetration, this is ephemeral. The penetration site on the host tends to melanize, but the scars resemble symptoms of viral infection, physical injuries, or even cannibalism by *C. rosaceana* larvae. Thus, fecundity and survival of maggots of *N. erecta* could not be determined, so data are limited to estimates of fertility only for this species. As a necessity for determining fertility, all host larvae that were exposed to gravid *N. erecta* females were reared individually in the small diet cups (30 ml). Preliminary observations indicated that *N. erecta* would only oviposit during the day in response to intense light, and the maggots desiccated rapidly once they were out of the female and emerged from the chorions (within an hour in a petri dish). Thus, we expected that exchanging the bouquets in the morning of each day did not limit parasitism by preventing maggots from the previous day from finding hosts. All 30 ml cups with host larvae parasitized by *N. pyste*, or potentially parasitized by *N. erecta*, were checked daily for mortality or changes

in host or parasitoid life stage. Because of the laboriousness of rearing and monitoring each host that was exposed to *N. erecta* (thousands of host larvae), this approach was maintained for the first two years of the experiment only (2008–2009). To save time and labor, in the final year of the experiment (2010), hosts exposed to *N. erecta* were cupped together in groups of five in larger diet cups (96 ml, P325 Solo Cup Co.) and were monitored for parasitoid emergence only (host survival was not followed).

2.5. Demographic analyses

Fertility data for flies from each of the three host availability treatments were combined according to female age in cohort life tables, so that there were three life tables for each species. From these life tables, age-specific survival (L_x) and fertility ($L_x h_x M_x$) were calculated, where h_x is the hatch rate, and M_x is the maternity rate (Carey, 1993). Because exact hatch rate and maternity rate could not be determined for *N. erecta* because fecundity could not be determined (see Section 2.3), h_x and M_x are estimates that account for survival of immature endoparasitic stages as these parameters are based on the number of parasitoids that emerged from hosts. Log-rank analysis was used to test for differences in the survival trajectories for each parasitoid species for the host availability treatments (Harrington and Fleming, 1982; Therneau and Lumley, 2009). A life table entropy value for survival was calculated for each species (Goldman and Lord, 1986). All analyses and plots were executed in the open-source statistical environment R (R development core team, 2009).

2.6. Evaluation of superparasitism in *N. erecta*

Because parasitism rates could not be directly determined for *N. erecta* as discussed above, a separate experiment was conducted to determine potential effects of superparasitism on host mortality and parasitoid emergence. Superparasitism describes the circumstance when a parasitoid attacks a host that has previously been attacked by the same species. In this study, females were confined to forage alone in individual cages, and thus superparasitism is more aptly defined as self-superparasitism. Although superparasitism can be adaptive, it is typically associated with costs for the developing parasitoids. In extreme examples, parasitoids produce many more eggs than can successfully develop from a single host (see Godfray, 1994). To examine effects of self-superparasitism for *N. erecta*, females were allowed to mate in laboratory cages and were held for 8–10 days without opportunity to oviposit on hosts (honey-water was provided). At the end of this period, females were anesthetized by placement in a refrigerator for 5 min. Females were then pinned through the sternum onto a dissection plate. A pin-vise (BioQuip Products, Rancho Dominguez, CA) with an insect pin formed into a hook was then used to pull on the ovipositor until the ovisac was everted under a dissecting microscope. The ovisac was covered with a few drops of deionized water to delay desiccation, and mature larvae (indicated by the fully developed cephalopharangeal skeleton) were teased from the ovisac. These maggots were stimulated with insect pins until they emerged from the chorions. At this point the maggots were transferred via insect pins to 5th instar *C. rosaceana* hosts held individually in 30 ml plastic cups (P125 Solo Cup Company, Lake Forest, IL) provided with approximately 10 ml of pinto-bean diet medium. Similar in vitro methods of maggot transfer have been used to control parasitism by other ovularviparous species of Tachinidae (e.g., Rodriguez-del-Bosque and Smith, 1996; Lauziere et al., 2001). For this experiment 1, 2, or 3 or more (5 maximum) maggots were placed on the dorsal abdomen of the host. Subsequent survival of the host, and emergence of the parasitoid were then observed.

2.7. Calculation of host mortality

Mortality of host *C. rosaceana* larvae was determined experimentally for cases of successful parasitism and for cases where hosts died but no parasitoids were produced. This latter form of mortality was termed “incidental mortality”, where incidental mortality = natural mortality + unsuccessful parasitism. Because the cause of death could not be determined from observation of hosts, natural mortality was estimated. In previous studies, natural mortality of immature *C. rosaceana* larvae was determined to be approximately 30% for the period from egg hatch to the adult (Jones and Wiman, 2008, MD Doerr and JF Brunner, unpublished data). If this mortality were evenly distributed between six larval instars and pupae, approximately 4% mortality would be expected at any single immature stage. The 5th instar host larvae used in this study either pupated directly or they went through the 6th instar before pupating (the 6th instar is not obligatory for *C. rosaceana* larvae, and is more common in female larvae [Jones et al., 2005]). Thus, there were three immature lifestages with opportunity for natural mortality. For larvae proceeding from the 5th instar to pupae, the expectation was for approximately 8% natural mortality. For larvae molting from the 5th instar to the 6th instar before pupating, the expectation is for 12% mortality. Thus, the average natural mortality rate for the *C. rosaceana* larvae used in these experiments was expected to be about 10%.

To tease out the different sources of host mortality for *N. pyste*, 10% of the total number of parasitized larvae that were reared was subtracted from the number of hosts that were parasitized but died prematurely. This provided an estimate of the number of hosts that died from unsuccessful parasitism and were unlikely to have died had they not been parasitized. Natural mortality for all hosts that were offered to *N. pyste* was then estimated by taking 10% of the total number of hosts that were provided to the flies over their lifetime.

There were two host mortality data sets for *N. erecta* (see Section 2.3); one set representing mortality from successful parasitism and incidental mortality of all hosts (2008–2009), and one representing successful parasitism only (2010). Thus, natural mortality was estimated by taking 10% (expected natural mortality) of the hosts that were reared from the former data set, and subtracting this number from the number of hosts that died prematurely. The result gave an estimate of host mortality from unsuccessful parasitism. Addition of means and pooled variance and was used to illustrate total mean mortality from the different sources (successful parasitism, unsuccessful parasitism, and natural causes) because of the two data sets. Proportional differences in mortality were tested using the *G*-test, also known as the log-likelihood ratio test, where values of *G* are approximately χ^2 distributed (Sokal and Rohlf, 1995).

3. Results

3.1. Sterility rates

For *N. pyste*, fertility data were successfully collected from 45 of 103 pairs (15 viable females for each host availability treatment). There were 47 females that were excluded for failure to produce offspring, so that 51% of pairings where females did not die or escape either failed to mate successfully, or had other reproductive problems. Of 108 pairs of *N. erecta*, fertility data were successfully collected for 46 females (15–16 per host availability treatment), and 33 females failed to produce offspring (42% unsuccessful pairs).

3.2. Adult survival

Log-rank analysis determined that intraspecific survival (L_x) trajectories of adult female *N. pyste* and *N. erecta* were statistically

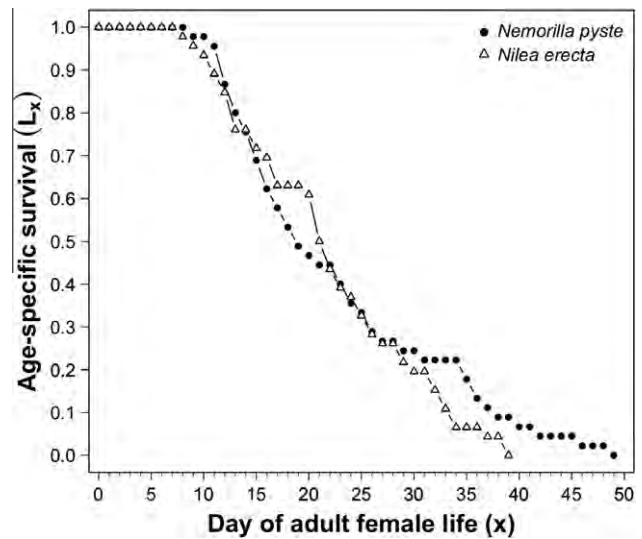


Fig. 1. Age-specific survival (L_x) of 45 *Nemorilla pyste* and 46 *Nilea erecta* females pooled from each host availability treatment.

equivalent for the three host densities (*N. pyste*: $\chi^2 = 0.5$, d.f. = 2, $P = 0.782$; *N. erecta*: $\chi^2 = 2.5$, d.f. = 2, $P = 0.281$). Thus, survival data from each life table were pooled for each respective species in Fig. 1. Both species had long-lived females; maximum survival was 49 days for *N. pyste* and 39 days for *N. erecta* (Fig. 1). The average expectation of life for newly eclosed females was similar for both species (*N. pyste*: $E_0 = 22.9$ d; *N. erecta*: $E_0 = 22.0$ d) and median survival occurred between days 19 and 20 for *N. pyste*, and between days 18 and 19 for *N. erecta*. The entropy value for survival of adult *N. pyste* females ($H = 0.45$) indicates that survival was approximately linear ($H = 0.50$; Goldman and Lord, 1986). Thus, deaths of *N. pyste* were roughly evenly distributed over time. For *N. erecta*, entropy for survival of females ($H = 0.37$) was slightly closer to zero, indicating that deaths were slightly more clumped over time, i.e., more females died closer to the same age relative to *N. pyste*.

3.3. Fertility

In both species, net fertility ($L_x h_x M_x$) increased over the first few days of the reproductive period before peaking between days 8–10, and declined thereafter (Fig. 2). This general pattern held for each species regardless of host availability. Even though the number of hosts available in the intermediate (18 hosts) and high (30 hosts) host density treatments differed from the low (6 hosts) host density treatment by a factor of 3 and 5, respectively, net fertility trajectories for these treatments differed primarily in the magnitude of the peak at the beginning of the reproductive period. Thus, oviposition strategy had little bearing on the time and rate of parasitization. For *N. erecta* females provided with 30 hosts per day, there was a steeper decline in fertility from the peak than females with 6 or 18 hosts. Gross and net fertility of *N. erecta* and *N. pyste* were similar for all host availability treatments examined (Table 1).

3.4. Evaluation of superparasitism in *N. erecta*

For the solitary species, *N. erecta*, the transfer of maggots dissected from females to hosts indicated that the proportion of hosts dying prior to pupation was nearly 4 times higher in hosts that received 2 maggots ($n = 89$) relative to mortality of hosts infected with 1 maggot ($n = 130$) ($G = 10.25$, d.f. = 1, $P = 0.001$; Fig. 3). No further increase in host mortality resulted from transfer of more than 2 maggots ($n = 94$) to a host ($G = 0.09$, d.f. = 1, $P = 0.767$).

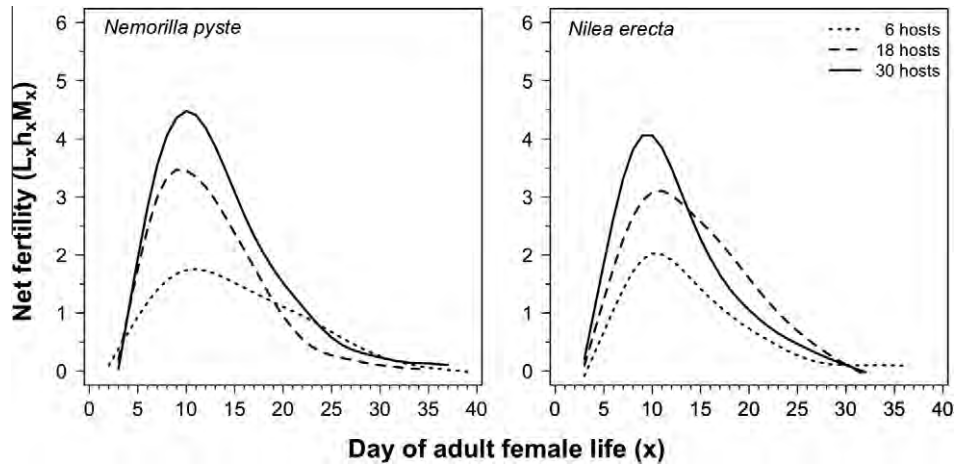


Fig. 2. Superimposed spline interpolations for net fertility of *Nemorilla pyste* and *Nilea erecta* females from the three host availability treatments.

Table 1
Fertility life table summary statistics for *Nilea erecta* and *Nemorilla pyste* from each of the three host availability treatments (6, 18, or 30 hosts per day).

	<i>Nemorilla pyste</i>			<i>Nilea erecta</i>		
	6	18	30	6	18	30
Gross fertility ^a	46.29	67.31	92.97	44.36	55.19	80.57
Net fertility ^b	28.00	47.58	61.06	29.02	39.20	53.83
Mean age gross fertility ^c	18.11	15.20	16.75	17.02	15.36	16.55
Mean age net fertility ^d	14.38	12.65	13.58	13.84	13.30	13.33

a $\sum_{x=z}^{\beta} h_x M_x$
 b $\sum_{x=z}^{\beta} L_x h_x M_x$
 c $\sum_{x=z}^{\beta} x h_x M_x / \sum_{x=z}^{\beta} h_x M_x$
 d $\sum_{x=z}^{\beta} x L_x h_x M_x / \sum_{x=z}^{\beta} L_x h_x M_x$

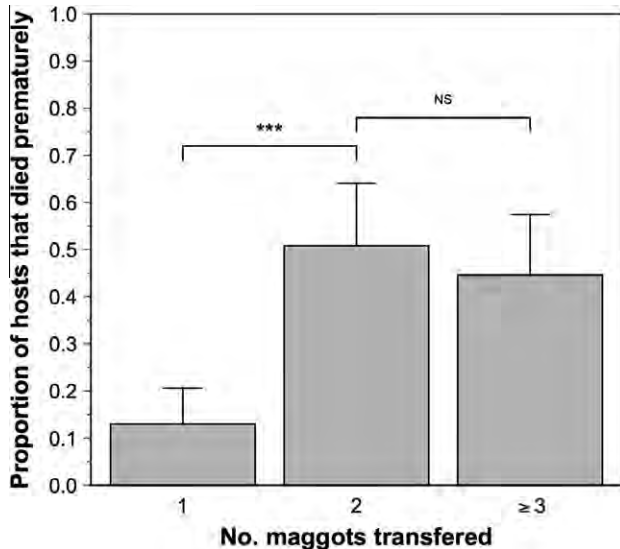


Fig. 3. Mortality of *Choristoneura rosaceana* larvae that were manually infected with different numbers of *N. erecta* maggots. Asterisks indicate significant differences in host mortality ($P < 0.017$; post-hoc *G* test with Bonferroni correction for $\alpha = 0.05$).

3.5. Host mortality rates

Incidental mortality (natural mortality + unsuccessful parasitism) of host larvae that were used in these experiments depended on the daily host density for each parasitoid species (Table 2). After making the adjustment for the expected level of natural mortality

for each treatment (see Section 2.6), unsuccessful parasitism was determined to be an important mortality factor for hosts exposed to *N. erecta*, and to a lesser extent for hosts parasitized by *N. pyste*. Within species, the proportion of *C. rosaceana* hosts dying before pupation from unsuccessful parasitism depended on daily host availability (*N. erecta*: $G = 92.02$, d.f. = 2, $P < 0.001$; *N. pyste*: $G = 27.21$, d.f. = 2, $P < 0.01$). For *N. erecta*, the rate of premature host death was highest among hosts presented to flies in groups of six larvae (20.4%), and mortality decreased significantly with each increase in host availability (to 9.4% and 6.4%, respectively; Fig. 4). In hosts parasitized by *N. pyste*, substantial mortality from unsuccessful parasitism only occurred when flies were limited to six hosts daily (6.9%); hosts that were presented in groups of 18 or 30 had low mortality from unsuccessful parasitism (2.9% and 2.2%, respectively; Fig. 4).

There were no significant differences in the mean number of hosts killed by *N. pyste* and *N. erecta* per female fly over adult life from successful parasitism within each host availability treatment (6 hosts: $F = 1.58$, d.f. = 1, 22, $P = 0.22$; 18 hosts: $F = 0.35$, d.f. = 1, 23, $P = 0.58$; 30 hosts: $F = 3.49$, d.f. = 1, 21, $P = 0.08$; Fig. 5A). Successful parasitism indicates that the host was killed by the parasitoid that emerged from its body. However, incidental mortality from unsuccessful parasitism was significantly higher for *N. erecta* relative to *N. pyste* (6 hosts: $F = 8.82$, d.f. = 1, 22, $P = 0.007$; 18 hosts: $F = 5.31$, d.f. = 1, 23, $P = 0.03$; 30 hosts: $F = 5.61$, d.f. = 1, 21, $P = 0.02$; Fig. 5B). Thus, the total mean host mortality (successful parasitism + unsuccessful parasitism) caused by *N. erecta* was higher than the mortality caused by *N. pyste* at each respective host availability treatment despite the fact that *N. pyste* lived longer and had more opportunity to encounter hosts (Fig. 1). For *N. erecta*, roughly half of the total mortality of hosts due to the flies was derived from unsuccessful rather than from successful parasitism (Fig. 5C).

4. Discussion

Fertilities of *N. pyste* and *N. erecta* were remarkably similar given the divergent oviposition strategies of the two species. A separate analysis of clutch size in *N. pyste* indicates that the number of eggs produced per day was dependent on host availability (Wiman, 2011). Female *N. pyste* withheld eggs rather than increasing clutch sizes when hosts were fewer, suggesting that *N. pyste* is capable of regulating oviposition according to host availability, or is able to avoid superparasitism (Wiman, 2011). These abilities are complimentary with the attack strategy of *N. pyste*, which entails direct

Table 2Premature mortality (death prior to pupation) in *Choristoneura rosaceana* larvae exposed to *Nilea erecta* (parasitism status unknown), or parasitized by *Nemorilla pyste*.

Species	Daily host density	No. dead host larvae	No. hosts pupated	Total hosts (<i>n</i>)	Prop. dead host larvae ^a	95% CI
<i>N. erecta</i>	6	249	570	819	0.304a	0.273, 0.337
	18	491	2038	2529	0.194b	0.179, 0.210
	30	359	1846	2205	0.163c	0.148, 0.179
<i>N. pyste</i>	6	124	607	731	0.170a	0.143, 0.199
	18	176	1180	1356	0.130b	0.112, 0.149
	30	185	1326	1511	0.124b	0.106, 0.140

^a Different letters indicate significant intraspecific differences in the proportion of larvae dying prematurely (post-hoc *G* test, with Bonferroni correction for $\alpha = 0.05$).

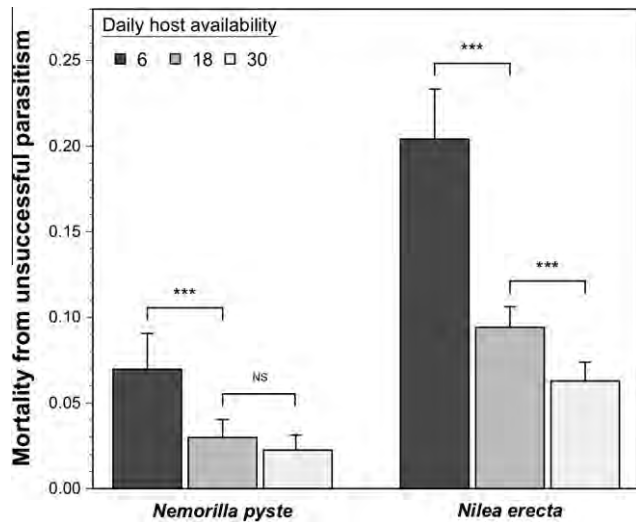


Fig. 4. Proportion of *C. rosaceana* larvae that were killed by unsuccessful parasitism by *Nemorilla pyste* and *Nilea erecta*. Error bars represent exact binomial 95% confidence intervals. Asterisks indicate significant differences within species ($P < 0.017$; post-hoc *G* test with Bonferroni correction for $\alpha = 0.05$).

physical interaction with hosts in order to place the egg. In contrast, in *N. erecta*, ovularviposition does not require physical interaction between adult parasitoids and hosts, and a reduced ability to adjust oviposition to host availability was expected for this species. It is unlikely that an indirect oviposition strategy could evolve in *N. erecta* if females oviposited completely randomly (Godfray, 1994). Observation of oviposition in *N. erecta* indicates that females tend to place their eggs near host feeding sites, and it is likely that females make some use of host cues. However, the high rate of

unsuccessful parasitism in the current experiments suggests that the ability of females to oviposit according to host density is either very basic, or was biased by experimental conditions. Our results suggest that high rates of superparasitism occurred when host availability was limited, and this was manifested as elevated rates of mortality of host larvae and limited fertility. It is possible that confinement in cages may have interfered with the ability of *N. erecta* females to distinguish host cues that normally allow females to estimate density of hosts and ovularviposit accordingly.

Previous studies have demonstrated elevated host mortality with increasing rates of superparasitism by solitary Tachinidae such as *N. erecta* (King et al., 1976; Gross and Young, 1984). Similarly, in gregarious oviparous species such as *N. pyste*, clutch sizes can exceed the number of maggots that are able to develop successfully. In *N. erecta*, premature host death from superparasitism appears to be caused by active fighting of the solitary endoparasitoids, which possibly disrupts critical functions in the host larva (Wiman, 2011). For the gregarious species *N. pyste*, we observed a similar, but less intense, effect of superparasitism on host mortality. With *N. pyste*, hosts are attacked directly and the number of eggs per host was determined in these experiments. Net clutch size ($\sum L_x M_x / L_x C_x$) for hosts attacked by *N. pyste* was 2.34, 1.79, and 1.14 for host availabilities of 6, 18, or 30 larvae per day, respectively (Wiman, 2011). In the current study, the highest host mortality from unsuccessful parasitism occurred in the lowest host density treatment for *N. pyste* (6 hosts). Thus, higher host mortality from unsuccessful parasitism was positively associated with higher clutch size. Because development is gregarious in *N. pyste*, host mortality from unsuccessful parasitism is not the result of physical contests between endoparasitoids, but may instead reflect other stresses placed on a host supporting too many maggots.

Price (1975) ranked tachinid parasitoids in order of likely increasing juvenile mortality to demonstrate that species with

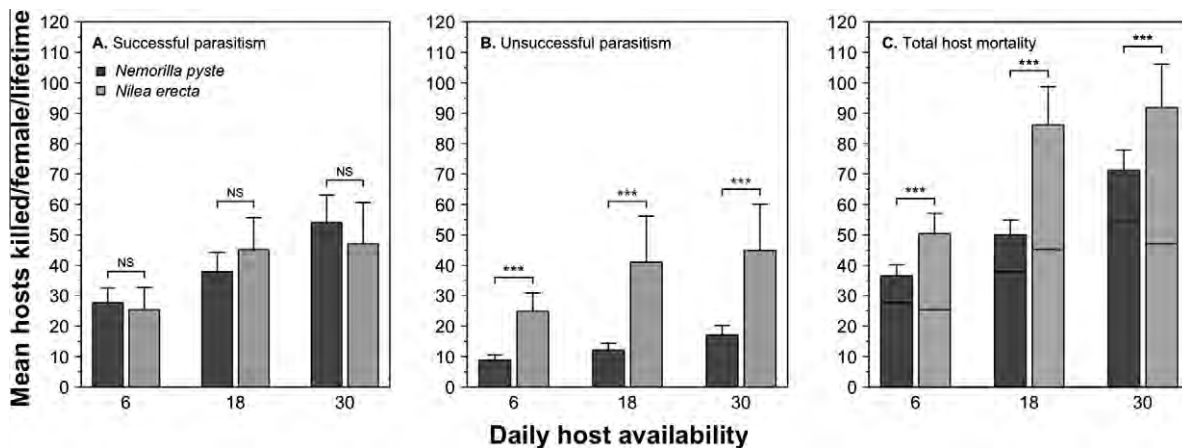


Fig. 5. The mean number of host larvae that died from successful parasitism was similar for *Nemorilla pyste* and *Nilea erecta* at each host density (A), while the number of hosts that were killed by unsuccessful parasitism was much higher for *N. erecta* (B), which led to a greater total mortality rate from parasitism among larvae exposed to *N. erecta* at each density (C; asterisks indicate $p < 0.05$; *F*-test).

the highest projected juvenile mortality had been selected to produce higher fecundities, determined by the number of ovarioles per ovary. According to this scheme, direct ovipositing species such as *N. pyste* are expected to display higher larval survival and lower fecundity than species with indirect modes of attack such as *N. erecta*. However, more recent studies have demonstrated that not only is larval mortality very high in tachinids with direct oviposition (Nakamura, 1995; Wiman, 2011), but a low number of ovarioles or capacity to store eggs does not limit fecundity for a long-lived synovigenic species such as *N. pyste*. *N. pyste* was found to produce up to 340 eggs in its lifetime, a comparable number to the maximum egg load of *N. erecta* determined by dissections (Wiman, 2011), but far more eggs than were previously thought to occur in *N. pyste* or in similar species (O'Hara, 2005). While *N. erecta* was found to have more eggs than *N. pyste* (Wiman, 2011), the current results demonstrate that this fecundity did not translate into higher realized fertility.

Although ovularviposition is a more evolutionarily advanced attack strategy than direct oviposition on hosts (Stireman, 2002; Tachi and Shima, 2009), embryonization of eggs in *N. erecta* did not have any particular advantages for the flies in this experiment, because reproductive rates (fertilities) in *N. pyste* were consistently higher. Selection is expected to favor embryonization of eggs when larval mortality is very high (Askew, 1971; Godfray, 1994). However, as discussed above, it appears that fertility of *N. erecta* suffered greatly because of superparasitism. These high levels of superparasitism were likely enhanced by the experimental methods (i.e., containment in cages limiting foraging opportunity). One important advantage of embryonization appears to be in competitive interactions, particularly in relation to species such as *N. pyste*, which have external embryonization of eggs. *N. erecta* enjoys a considerable temporal competitive advantage in competition for the host *C. rosaceana* because maggots are able to enter host before eggs of *N. pyste* have opportunity to hatch (Wiman, 2011).

The frequency of flies unable to produce offspring in these experiments suggests that optimal mating conditions were not achieved. In early attempts to induce mating of *N. pyste* and *N. erecta*, the most useful technique was to achieve sufficient lighting (~900 lux). Mating systems have been little studied in Tachinidae, but a consensus is that mating can take place far from where hosts are located on prominent landmarks such as hills and ridges (Wood, 1987; Alcock and Smith, 1995; Alcock and Kemp, 2006). Males assemble in these areas, defending distinct territories from other males while intercepting incoming females. Unfortunately, these conditions may not be easily replicated in the laboratory. Therefore, the “brute force” method of pairing the sexes in cages probably resulted in higher sterility than might occur in natural populations.

From the perspective of biological control, it is clear that while the reproductive rates of *N. pyste* and *N. erecta* were similar, more hosts were killed by *N. erecta* as a result of superparasitism and solitary development. Parasitism by *N. pyste* also resulted in higher levels of host mortality than would be predicted by parasitoid emergence alone. Although incidental host mortality does not benefit the flies, it is of interest for pest management. If these results were applied to field collections, estimates of parasitism determined by allowing flies to emerge from field-collected host larvae can be expected to yield erroneous accounts of the total effect of parasitism on host populations. Such an account of parasitism is predicted to be potentially conservative in the case of *N. pyste*, and highly conservative in the case of *N. erecta*. This could lead to underestimation of the benefits of biological control in tree fruit systems where these flies can potentially play an important role in protecting crops from damage by leafroller pests. Effects of parasitism on host populations can take many forms that must be considered when assessing the value of parasitoids to biological control

(Van Driesche, 1983). However, it is important to note that these results may not translate well to the field, where foraging conditions are quite different from laboratory cages. Further study would be necessary to investigate how mortality of field-collected *C. rosaceana* is affected by *N. pyste* and *N. erecta*. From the current results, it is reasonable to hypothesize that mortality from *N. erecta* would be much higher on a per-capita basis relative to *N. pyste*.

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