Assessing a method for rearing North American yellowjackets

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ABSTRACT

Studying yellowjackets is challenging due to their cryptic nesting behaviour, short field season, and extreme variation in population density. Developing or perfecting techniques for rearing yellowjackets would greatly increase the opportunity of studying the communication ecology of yellowjackets and the evolution of eusociality in the Hymenoptera. Our objective was to assess a method for rearing the five *Vespula* congeners *V. acadica* (Sladen), *V. alascensis* (Packard), *V. atropilosa* (Sladen), *V. germanica* (F.), and *V. pensylvanica* (Saussure). In early spring 2014, we collected queens of each of the five species from the field and placed them singly in a plywood nest box connected to a mesh cylinder that served as a foraging arena and provided constant access to water and food (honey, live flies, and live caterpillars). For each queen, we recorded nest initiation, the attachment site of the nest pedicel, and the stage of nest development at the end of the experiment, nine weeks after the last collection date of queens. Queens of *V. germanica* (n=18), *V. alascensis* (n=11), *V. acadica* (n=4), *V. pensylanica* (n=23), and *V. atropilosa* (n=11) had nest-inititation rates of 61%, 50%, 25%, 17%, and 0%, respectively. The mean number of nest cells built by queens of *V. germanica*, *V. alascensis*, *V. acadica*, *V. pensylvanica*, and *V. atroplisoa* were 21.6 ± 4.6, 17.8 ± 6.3, 8.0, 26.5 ± 8.3, and 0, respectively. Two *V. germanica* queens and one *V. pensylvanica* queens established nests that produced a few worker wasps. Although our rearing method compares favorably to, and in some aspects improves, previous rearing methods, further refinements are needed to generate the large numbers of wasp workers that are essential for experimental testing of hypotheses pertinent to life-history traits of yellowjackets.

Key Words: Yellowjacket, *Vespula*, rearing, nesting

INTRODUCTION

Yellowjackets and hornets are intensely studied because they can be (*i*) invasive and pestiferous species in many ecosystems (Landolt 1998; D'Adamo *et al.* 2001; Day and Jeanne 2001; Landolt *et al.* 2005; Brown *et al.* 2014), (*ii*) potential biological control agents (Hoffmann *et al.* 2000), (*iii*) vectors of microorganisms (Davis *et al.* 2012; Stefanini *et al.* 2012), and (*iv*) threats to citizens with venom (hyper)sensitivity (Nakajima 1986; Schmidt 1986; Ono *et al.* 2003). Furthermore, wasps are model organisms for studying the evolution of eusociality (Landolt *et al.* 1998) and chemotaxonomy (Bruschini *et al.* 2007). However, studies of wasps are challenging due to a short field season, extreme variation in wasp-population densities, and the often cryptic nesting behaviour of wasps (Edwards 1980).

There are seven accounts of establishing vespine nests in the laboratory (Table 1). Ishay *et al.* (1967) reared *Vespa orientalis* L. with field-collected nests and overwintered gynes in their "Vespiaries", but did not comment on the success rate of either method. Ross *et al.* (1981) attempted to rear nests of five *Vespula* species under laboratory conditions, and recorded the percent of nest initiation for each of these species. None of the nests developed beyond the emergence of the first workers. Following up on the work by Ross *et al.* (1981), Matthews *et al.* (1982) reared 14 nests of *V. maculifrons* (Buysson), five nests of *V. germanica* (F.), one nest of *V. vulgaris* (L.) [=*V. alascensis* (Packard)], and one nest of *V. vidua* (Saussure) under environmentally controlled conditions. All of these nests progressed to producing at least two queen larvae. Using the method by Ross *et al.* (1981), Ross (1983) reared and studied queen foraging behaviour of *V. germanica, V. vulgaris* (=*V. alascensis*), and *V. maculifrons,* using 3–5 nests of each species. Vetter and Visscher (1995) successfully reared nests of *V. pensylvanica* (Saussure) from field-collected gynes, reporting the first and only account of laboratory-reared

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Table 1

Description of methodologies and success rates for rearing yellowjackets and hornets in the genera *Vespa* and *Vespula* (V.)

Species (# gynes)	Collection dates	Gynes nest-building	Nests producing Workers[3]	Gynes/males produced	Overwint. generation	Size (cm^3) of nest box	Diet	Rearing condition	Ref[4]
Vespa orientalis; numbers not specified	N/A[2]	Yes; numbers not specified	Yes; numbers not specified	N/A	Yes	55×55×10 55×55×5	Free forage; bee hive	Indoor shed; near-outdoor conditions	1
V. germanica (22 OW; 21 FC) *V. maculifrons* (54 FC) *V. alascensis* (11 FC) *V. flavopilosa* (14 FC) *V. vidua* (7 FC)	May-June 1980; Overwint. gynes from fall 1979	28% (12/43) 48% (26/54) 27% (3/11) 0% (0/14) 28% (2/7)	42% (5/12) 15% (4/26) 33% (1/3) (0/0) 50% (1/2)	No	No	1.1– 0.6-L paper cylinder lined with soil & root surrogate	Water; honey; live flies; frozen crickets; cat food; frozen fish; hamburgers	Photoperiod: L: 15h, 20min D: 8h, 40min Temp: L: 24±2°C D: 18±2°C RH: 65-90%	2
V. germanica (N/A[2]) *V. maculifrons* (N/A[2]) *V. alascensis* (N/A[2]) *V. vidua* (N/A[2])	May-June 1980	5 14 1 1	Yes; numbers unspecified	Yes	No	As in Ref 2	As in Ref 2	As in Ref 2	3
V. alascensis (N/A[2]) *V. maculifrons* (N/A[2]) *V. germanica* (N/A[2])	April- May 1983	3 5 5	N/A[2]	No	No	As in Ref 2	As in Ref 2	As in Ref 2	4
V. pensylvanica (24)	March-June 1994	46% (11/24)	45% (5/11)	2/4 produced gynes; 2/4 produced males	No	0.4-L cylinder lined with paper; root surrogate on lid	Honey/water; cat food; chicken; bee larvae/pupae; salmon; crickets; cabbage looper larvae	Temp.: 25°C Photoperiod: as outdoors	5
V. vulgaris (11) *V. germanica* (5 OW; 33 FC)	Springs of 1994-95	45% (5/11) 29% (11/38)	40% (2/5) 9% (1/11)	No	No	15×10×10; soil-lined	Honey/water; live blowflies	Indoors with near-outdoor conditions	6
Vespa crabro (5)	October 1998	20% (1/5)	1/1 nest produced workers	12 males; 3 gynes	Yes	30×20×40	Bee drones & pupae	Temp: 20-30°C; RH:70%	7
V. alascensis (10) *V. acadica* (4) *V. germanica* (18) *V. atropilosa* (11) *V. pensylvanica* (23)	March-May 2014	50% (5/10) 25% (1/4) 61% (11/18) 0% (0/11) 17% (4/23)	20% (1/5) 25% (1/4)	No	No	15×15×30	Honey/ syrup; house flies; cabbage looper larvae	Natural temp., RH & light	8

1OW = Overwintered; FC = Field collected; 2N/A = No information provided; 3Subset of those queens that initiated nests; 4(1) Ishay et al. 1967; (2) Ross et al. 1981; (3) Matthews *et al.* 1982; (4) Ross 1983; (5) Vetter and Visscher 1995; (6) Leathwick 1997; (7) Hoffmann et al. 2000; (8) this study

Vespula nests from spring-captured queens through to males and gynes. In New Zealand, Leathwick (1997) reared one *V. germanica* nest and two *V. vulgaris* nests, which produced workers. Finally, Hoffmann *et al.* (2000) mated gynes and males from two feral *Vespa crabro* L. nests and over-wintered five mated gynes, of which one established a nest that produced next-generation gynes.

Both transplanting feral nests into research areas and in situ observations and experimentation are means of studying eusocial wasps (Spradbery 1973; Edwards 1980; Akre *et al.* 1980; Akre 1982) and advancing our understanding of their ecology. However, the cryptic nesting behaviour of vespine wasps and extreme variation in population density make it difficult to locate sufficient numbers of nests for rigorous and replicated observations and experiments (Edwards 1980; Aldiss 1983). Furthermore, the process of excavating subterranean nests and transplanting them to new locations may damage the brood comb and nest envelope, thereby possibly affecting the behaviour of nest mates or leading to the loss of queens (Vetter and Visscher 1995).

A consistent supply of wasp nests would greatly benefit the study of vespine ecology, particularly the biology and ecology of the nest as a super-organism (Wilson 1971; Moritz and Bürgin 1987). This is most obvious in studies of alarm-pheromone systems among social wasps, where the presence of the nest is essential to observe nest-defense behavior. Of the nine species of yellowjackets and hornets that reportedly use alarm pheromones, pheromone components have been identified for only four species (Maschwitz 1964a,b; Saslavasky *et al.* 1973; Veith *et al.* 1984; Maschwitz 1984; Maschwitz and Hanel 1988; Heath and Landolt 1988; Landolt *et al.* 1995; Landolt *et al.* 1999; Ono *et al.* 2003), and the pheromone effect has often been tested with only a single nest.

Our objective was to assess a method for rearing Vespula congeners targeting for diversity *V. acadica* (Sladen), *V. alascensis*, *V. atropilosa* (Sladen), *V. germanica*, and *V. pensylvanica*.

MATERIALS AND METHODS

Collection of queens

We sweep-netted queens of unknown mating status in the Greater Vancouver area and Lillooet, both British Columbia (B.C.), during sunny clear days between 10:00 and 16:00 hours, capturing most queens while they were prey-hunting or collecting nectar from English hedge laurel, *Prunus lauresianus*. Between 20 March and 15 May 2014, we collected a total of 66 yellowjacket queens [*V. acadica* (4), *V. alascensis* (10), *V. atropilosa* (11), *V. germanica* (18), and *V. pensylvanica* (23; Table 2)], 55 of which in Vancouver, and 11 of which during a 2-day trip (13–15 May) to Lillooet. We immediately placed captured queens singly into glass jars (0.3–0.5 L) containing foliage of *P. lauresianus* or Western red-cedar, *Thuja plicata*, on which they commonly rest. Whenever possible, we kept jars in a cool and dark area for <2 h before placing them in rearing units (see below) that we kept inside a fenced area of Simon Fraser University's (SFU's) insectary annex. This approach minimized the queens' stress of confinement.

Rearing units

Nest-rearing units resembled those described by Ross *et al.* (1981), but had several modifications (Figure 1). Each unit consisted of a plywood box nesting cavity (15 cm high × 15 cm wide × 30 cm long) with one side panel hinged for periodic observations. A 2.5-cm hole in the top (dorsal) panel of the nest box provided entry into a mesh screen cylinder (15 cm diam × 20 cm tall), the top and bottom of which was hot-glued to a Petri dish (15 cm diam) for rigidity and stability. A hole (5 cm diam) in the bottom Petri dish of the cylinder corresponded with the dorsal hole of the nest box, allowing the foundress and potential workers to exit the box and to enter the mesh cylinder for foraging. The top Petri dish of the cylinder had one hole (3 cm diam) to accommodate an inverted 50-mL falcon tube with a cotton-filled pipette tip containing the water supply, and a second hole (2 cm diam) that was plugged with a cork or rubber stopper and allowed intermittent insertion of live flies and cabbage looper larvae (see below) as food sources. The top of the falcon tube was cut off to replenish water as it was consumed or evaporated (Figure 1).

Table 2

Numbers of overwintered *Vespula* (V) queens field-collected between 20 March and 15 May 2014, the proportion of queens that initiated nests and attached the nest pedicel to a twig in the nest box (Figure 1b) or the nest box roof, and the mean number of cells built by queens that initiated nests.

Species	# Queens collected	# Queens initiating nests*	Attachment site of pedicel in nest box[a]		Cells built[a] Mean (SE)
			Twig	Roof	
V. acadica	4	1/4 (25%)	1/1 (100%)	0/1 (0%)	8
V. alascensis	10	5/10 (50%)	3/5 (60%)	2/5 (40%)	17.8 (6.3)
V. atropilosa	11	0/11 (0%) **	-	-	-
V. germanica	18	11/18 (61%) **	5/11 (45%)	6/11 (55%)	21.6 (4.6)
V. pensylvanica	23	4/23 (17%)	3/4 (75%)	1/4 (25%)	26.5 (8.3)

[a]Subset of those queens that initiated nests

* Significant difference between nest inititation rates (p=0.0013, FET)

** Significant difference in nest initiation rates between queens of *V. germanica* (61%) and *V. atropilosa* (0%; p=0.012, FET)

In previous studies for rearing yellowjackets, root surrogates as potential attachment sites for the nest pedicel were affixed to the top of nesting boxes (Ross *et al.* 1981; Matthews *et al.* 1982; Ross 1983, Vetter and Visscher 1995, Leathwick 1997). Accordingly, we hot-glued a few twigs as surrogate roots to the roof of nesting boxes, and tracked whether queens attached the nest pedicel to a twig or the roof of the box.

Some of the most vigorous nests of *V. alascensis* and *V. germanica* we observed in the field were built in straw composts, as previously reported (Spradbery 1971). Therefore, we lightly packed the 6.5-L nest box with (untreated) organic animal bedding straw for insulation. We supplied the mesh cylinder with decaying wood and filter paper to encourage pulp gathering for nesting material.

To prevent predation by ants, we placed each rearing unit on a brick in a water-filled tray on a table about 1 m above ground in a south-facing, rain-sheltered area.

Food provisioning of queens

Starting on the day of capture, we fed each queen daily with (i) honey and/or corn syrup (Akre *et al.* 1976; Ross *et al.* 1981) that we smeared on the mesh cylinder of the rearing unit, (ii) 3–5 common house flies, *Musca domestica* L., or the bottle flies *Lucilia sericata* (Meigen) or *Phormia regina* (Meigen) (Akre *et al.* 1976), and (iii) 5–10 2nd or 3rd instar larvae of the cabbage looper, *Trichoplusia ni* (Hübner), the latter also used as prey for yellowjackets by Vetter and Visscher (1995), although these were rarely consumed in our study. Although we often observed queens hunting flies, consuming honey and water, and entering or exiting the nesting box, we never observed violent flights against the mesh screen cylinder in attempts to escape the confinement. To reduce disturbance of nesting behaviour, we checked for nest initiation only once per week, always when the queen was foraging in the mesh cylinder.

Statistical analyses of data

We analysed all data with the statistical software R (version 3.1.3). We used a Pearson's X^2 test, binomial exact test, or Fisher's exact test (FET), depending on the data constraints and the specific hypothesis, to test for a difference in (*i*) nest-initiation rate between species (FET), followed by pairwise comparisons of proportions using the Bonferroni P-value adjustment method using the fmsb R package (Nakazawa 2014), (*ii*) site of pedicel attachment between species

(FET), and (*iii*) for a deviation from a 50/50 chance of pedicel attachment to the twig or nest box roof for each species (X^2 goodness of fit test or binomial exact test), addressing the question whether queens have an innate preference for root-like substrates to attach the nest pedicel. To test for differences in the mean number of cells built by queens of the five species we studied, we performed an ANOVA.

RESULTS

Percent nest initiation

Queens of all five species, except *V. atropilosa*, initiated a nest. Queens of *V. germanica*, *V. alascensis, V. acadica,* and *V. pensylvanica* had nest-inititation rates of 61%, 50%, 25%, and 17%, respectively. The significant difference between these five nest-inititation rates (p=0.0013, FET; Table 1) can be attributed to significantly different nest initiation rates between queens of *V. germanica* (61%) and *V. atropilosa* (0%) (p=0.012, FET).

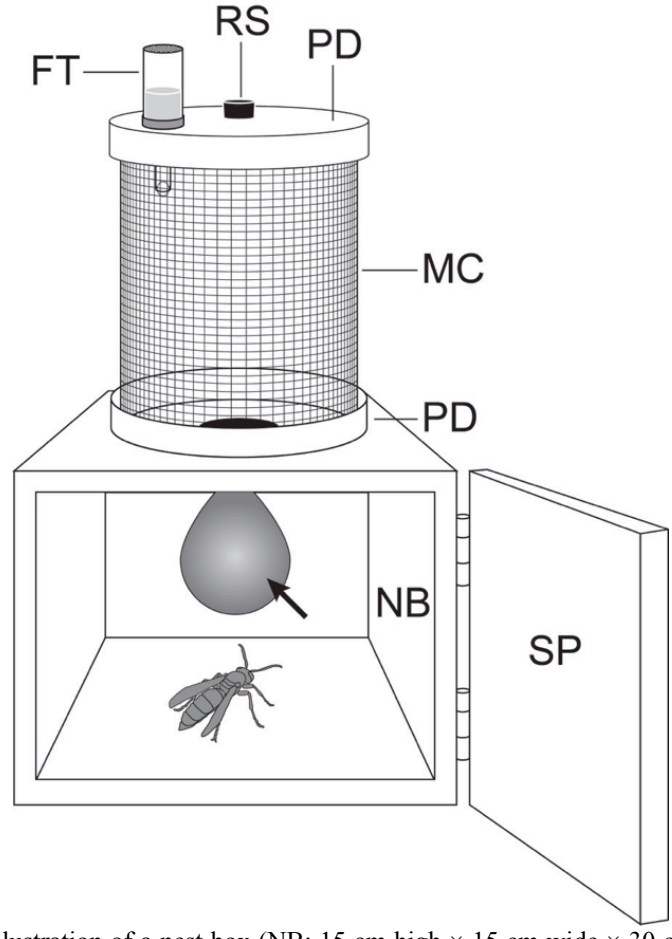

Figure 1. Graphical illustration of a nest box (NB; 15 cm high × 15 cm wide × 30 cm long) with hinged side panel (SP) connected to a mesh-cylinder (MC) foraging arena (15 cm in diam × 20 cm tall), the top and bottom of which reinforced by Petri dishes (PD) for stability and to accommodate a 50-mL falcon tube (FT) with a cotton-filled pipet tip as a water reservoir. The "feeding hole" in the top Petri dish was closed with a rubber stopper (RS) and allowed intermittent insertion of live blow fly and caterpillar prey. The arrow depicts an embryo nest started by the queen.

The effect of collection date on nest initiation could not be tested statistically, because dates could not be assigned to those wasps that failed to initiate a nest. Of the queens we had captured on 15, 17, and 29 April 2014 (57% of the total), six, three, and three, respectively, initiated a nest.

Attachment site of nest pedicel

There was no significant difference in the proportion of queens that attached the nest pedicel to a twig or the roof of the nest box (Figure 2a,b,c; p=0.77, FET), between the four species that initiated a nest (Table 2). Of the nest-initiating queens, three of four *V. pensylvanica* queens, three of five *V. alascencis* queens, and five of 11 *V. germanica* queens attached the nest pedicel to a twig. The single nest-initiating *V. acadica* queen did the same.

Within each of the three species (*V. alascensis, V. germanica, V. pensylvanica*) in which more than one queen initated a nest, there was no significant deviation from a 50/50 chance in the proportion of queens that attached the nest pedicel to a twig or the roof of the nest box [*V. alscensis*: binomial exact test, p = 1.0; *V. germanica*: X^2 goodness-of-fit (1, N = 11) = 0.09, p = 0.76; *V. pensylvanica*: binomial exact test, p = 0.63].

Cells built by queens

Of the five species we studied, queens of four species intitated nests. Of nest-building queens, the mean number of cells they had built (*V. acadica*: 8.0, N=1; *V. alascensis*: 17.8 ± 6.3, N=5; *V. germanica*: 21.6 ± 4.6, N=11; *V. pensylvanica*: 26.5 ± 8.3, N=4; Table 1) did not differ at the time we terminated the study [$F_{(3,17)}$ = 0.502, p= 0.686]. One queen each of *V. alscensis* and *V. germanica* constructed only the nest pedicel and quickly abandoned further attempts of nest building. When we terminated the study (July 20), all larvae and workers had died, and queens had suspended any further nest-building attempts. All queens were dead by mid-July or early August.

Workers produced

Of the 66 queens in our study, each of two *V. germanica* queens maintained a nest that produced worker wasps (two worker wasps from one nest, and six from the other), and one *V. pensylvanica* queen produced a nest from which one worker emerged. All workers appeared at times when the first feral workers appeared in the field, around the first week of June.

DISCUSSION

The differences in nest-initiation rates that we observed between queens of *V. atropilosa* and *V. germanica* (Table 2) reflect the ecological diversity of the genus *Vespula* (Akre *et al*. 1980; MacDonald *et al*. 1980; Akre 1982; Macdonald and Matthews 1984; Landolt *et al*. 1998).

In Pullman (Washington, USA), queens of *V. atropilosa* begin nesting on average 10 days earlier than queens of *V. pensylvanica* (Akre *et al*. 1976). Nests of *V. atropilosa* also decline one month earlier than the nests of most, if not all, members of the *Vespula vulgaris* group (Akre *et al*. 1976). We captured all queens of *V. atropilosa*, which invariably failed to establish nests (Table 2), in late spring (May 15), possibly at a time when these queens could have had established a nest already or could have been tending a nest at an embryo stage, thereby resulting in no (repeated) nest-initiation attempts in our study. However, rearing of *V. atropilosa* nests from over-wintered field-collected queens has never been attempted before, and we may have simply failed to provide one or more essential requisites for successful nesting. Therefore, it remains inconclusive whether *V. atropilosa* queens cannot be reared using the method described here or whether we simply captured *V. atropilosa* queens too late in the season.

We report the first account of nest initiation in a nest box for *V. acadica* and the second account of nest initiation for a member of the *V. rufa* group, the first account being *V. vidua* (Ross *et al*. 1981; Matthews *et al*. 1982; Ross 1983). In our study, the nest initiated by one of four *V. acadica* queens stood out from all other *Vespula* nests in that the queen incorporated prey body

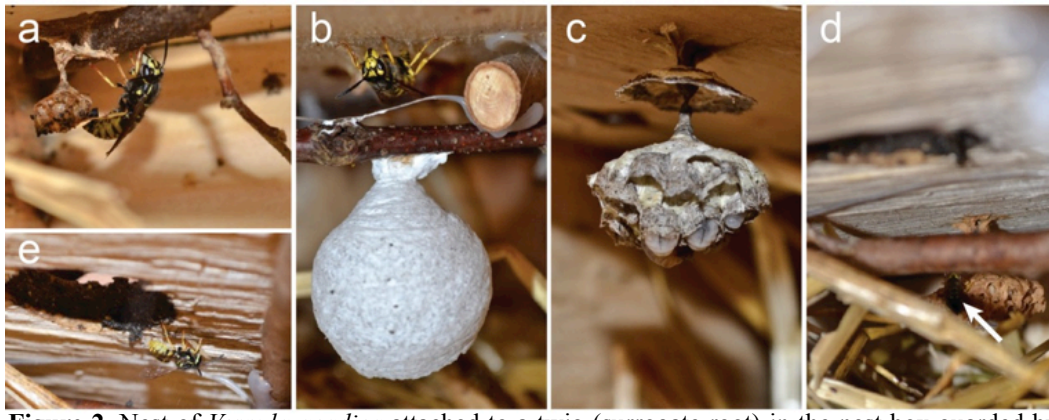

Figure 2. Nest of *Vespula acadica* attached to a twig (surrogate root) in the nest box guarded by the queen (note black pieces of prey incorporated into the nest); (b, c) Embryo nests of *V. germanica* with envelope (b) and of *V. pensylvanica* without envelope (c); (d, e) nest of *V. germanica* with one worker wasp (arrow) tending eggs and larvae (d) and one worker wasp wing fanning at the entrance of the nest box (e).

parts in the nest (Figure 2a). Whether this is typical for *V. acadica* queens will become apparent in further rearing studies or careful inspections of feral nests.

Nest-inititation rates of 61% and 50%, respectively, by queens of *V. germanica* and *V. alascensis* (formerly *V. vulgaris*) in our study (Table 2) were twice as high as those previously reported for these two species (Ross *et al*. 1981) or for *V. germanica* (Leathwick 1997). Conversely, relatively fewer queens of *V. pensylvanica* initiated nests in our study (Table 2) compared to a previous study (Vetter and Visscher 1995). The underlying mechanisms contributing to this differential rearing success are difficult to determine. Unlike previous studies where rearing units resided indoors with small temperature oscillations and a constant photoperiod (Ross *et al*. 1981; Vetter and Visscher 1995; Leathwick 1997), we kept our nest boxes outdoors and thus exposed them to seasonal changes in photoperiod and to significant diel and seasonal temperature fluctuations. However, the straw inside the next boxes that we provided as insulation material may have been insufficient to keep *V. pensylvanica* queens warm and to induce more consistent nest building. The relatively high propensity of *V. germanica* queens to initiate nests irrespective of rearing conditions might be an intrinsic characteristic of *V. germanica* that may help explain why this wasp is so widely distributed and invasive in North and South America as well as in New Zealand (MacDonald *et al*. 1980; D'Adamo *et al*. 2001; Brown *et al*. 2014). Both feral and laboratory-reared nests of *V. germanica* have the fastest nest-development rates of all species studied in North America (MacDonald *et al*. 1980; Matthews *et al*. 1982)

The type of potential attachment sites for nest pedicels does not seem to matter critically, because the same number of queens attached the nest pedicel to the roof of the nest box or to a twig serving as surrogate root in a quasi-subterranean nest cavity. Considering, however, that the surface of roots was much smaller than the surface of nest box roofs, queens may indeed have preferred roots as potential attachment site for nest pedicels. Alternatively, the preference for pedicel attachment sites may vary between queens. If so, providing diverse and multiple sites for pedicel attachment could help increase rates of nest initiation.

The rate of cell building reflects queen quality and varies with species (Archer 2009). In our study, we could not consistently track nest development such as cells built per day, eggs laid, and number of cells with larvae or pupae, because 35% of the queens that initiated a nest built an envelope surrounding the cells (Figure 2c). Vetter and Visscher (1995) faced the same challenge with one of the four *V. pensylvanica* nests they reared. At the end of our study, however, we did record the number of cells per nest and did not find a significant difference in the mean number of cells built between species (Table 2). Apparently, all but three nesting activities (see below) were discontinued at the same point of brood development, just before the emergence of the first

worker wasps that would have continued all tasks except egg laying (Gambino and Loope 1992). Eggs and larvae died from unknown causes. How and why two *V. germanica* and one *V. pensylvanica* queens progressed to producing a few worker wasps (Figures 2d,e) remains unknown. We envision that the well-being of larvae could have been compromised by a fungal pathogen, although fungal growth was not apparent on food remains such as legs and wings of fed-on house flies that we left in the feeding cage. Alternatively, larvae may have suffered from a lack of nutritional diversity or key nutrients. Conceivably, free foraging eusocial wasps self-medicate in that they adjust their diet, or that of their offspring, in response to pathogens, as do caterpillars of *T. ni* and *Grammia incurrupta* (Edwards) (Singer *et al*. 2009; Shikano and Cory 2014).

CONCLUSION

Queens of the five *Vespula* species that we attempted to rear in nest boxes differed in nest-initiation rates, with *V. germanica* having a greater success rate than *V. atropilosa*. Whether these differences are due to intrinsic characteristics of these species, external factors such as ambient temperature during rearing, or the quality of the queens we had collected in the spring cannot be ascertained. The high propensity of *V. germanica* queens to initiate nests may be a contributing factor to the success of *V. germanica* as one of most pestiferous and invasive wasp species worldwide.

Most nests in our study failed to produce worker wasps. We speculate that these nests succumbed to a pathogen rather than to faulty rearing methodology, because all larvae visible in those nests that ceased to develop started to die within days of each other and showed similar signs of a fungal infection. We recommend that, in future attempts to rear yellowjackets, queens are allowed to forage freely as soon as they have initiated nest building. This would enhance the nutritional diversity for larval offspring, provide the essential nutrients at particular times during nest development, and possibly help curtail the effect of pathogens in the food or nest. Regardless, perfecting techniques for rearing yellowjackets in further studies is well justified, because it will greatly increase the opportunity of investigating the role of these intriguing predatory insects in ecosystems and the evolution of eusociality in the Hymenoptera.

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