

Predictability of Stored-Wheat Insect Population Trends from Life History Traits

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ABSTRACT The extent to which the relationship between environmental factors (temperature and grain moisture content) and life history traits can predict insect population trends in stored wheat was investigated by comparing nine measured population trends with predictions from population dynamics simulation models. Regression of measured against predicted insect population densities indicated that the predictions of the simulation model explained 87, 93, and 96% of the changes in the mean measured densities of *Cryptolestes ferrugineus* (Stephens), *Rhyzopertha dominica* F., and *Tribolium castaneum* (Herbst), respectively. Thus, for the population trends of the three species presented here, only 13, 7, and 4% of the changes in mean measured population densities remains to be explained by other factors.

KEY WORDS Insecta, stored wheat insects, life histories, simulation model

THE RELATIONSHIPS between environmental factors and life history traits are important to population ecologists because environmental factors influence population trends through their effects upon developmental time and egg production. Temperature and grain moisture content are major influences on the developmental time and egg production of stored-grain insects. Hagstrum & Milliken (1988) fitted equations to data from 45 published studies on the effects of these environmental factors on the developmental time of stored-grain insects. Data also are available on the effects of these factors on egg production by *Cryptolestes ferrugineus* (Stephens) (Smith 1965), *Rhyzopertha dominica* F. (Birch 1953), and *Tribolium castaneum* (Herbst) (Howe 1962). Earlier studies by Longstaff & Cuff (1984) used similar data to simulate the population trends of another stored-product insect pest, *Sitophilus oryzae* (L.). Population dynamics simulation models were used in this study to determine the extent to which the relationships between environmental factors (temperature and grain moisture content) and life history traits can predict population trends of three of the most important insect pests in stored wheat.

Materials and Methods

Population trends of single-species populations of *T. castaneum* and *R. dominica* were observed in the laboratory, and population trends of multiple-species populations of *C. ferrugineus*, *T. castaneum*, and *R. dominica* were observed in two cylindrical metal bins on a farm near Enterprise, Kans. Population trends from these two sets of data

and a third set of data reported by Hagstrum (1987) were compared with population trends predicted by the population dynamics simulation models described in this paper.

Population Estimates. The study of insect populations in the laboratory was conducted in 19-liter (5 gal) metal cans (38 cm high, 30 cm diameter) containing 19 kg of hard red winter wheat. Three cans of wheat were infested with five pairs of adult *R. dominica* and two cans were infested with five pairs of adult *T. castaneum*. The study was conducted in a room maintained at 27°C and 70% RH (14% wheat moisture content). Nine samples of 0.12 kg of wheat were taken from each of the *T. castaneum* populations after 30, 50, and 70 d and from each of the *R. dominica* populations after 42, 56, and 70 d using a grain trier. The grain trier is a double-walled tube (2.5 cm outside diameter, 50 cm long) with seven oblong holes (1.5 by 5.4 cm). The inner tube is divided into seven compartments that collect grain samples when the inner tube is turned so that the holes in the two tubes match. In taking a sample, the holes are closed as the trier is pushed into the grain, they are opened to allow grain to enter the seven compartments, and then they are closed again before withdrawing the trier. One sample was taken in the center, four samples were taken near the edge of the container in four compass directions, and the other four samples were taken halfway between the center and edge sample locations. Adult insects were separated from the wheat with an oblong-hole grain sieve (0.18 mm by 1.27 cm) (Seedburo, Chicago) and counted.

On the farm, two cylindrical metal bins (6.4 m diameter), each with 82 t of hard red winter wheat 3 m deep, were sampled 3, 7, and 14 d after newly harvested wheat was placed in storage in late June and then at 2-wk intervals until the end of Sep-

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tember 1986 when the grain was fumigated. On each occasion, 24 2.9-kg samples of wheat were taken with a commercial pneumatic grain sampler (Cargill Probe-A-Vac, Minneapolis) from three locations near the center and three locations two-thirds of the distance between the center and outer wall in each of four vertical 0.75-m layers of grain. Adult insects were separated from the wheat with an oblong-hole grain sieve (Seedburo, 0.18 mm by 1.27 cm), identified to species, and counted. The average initial moisture levels of wheat samples was 10.4% in both bins, and moisture decreased 0.1% per week in the top and bottom layers but not in the middle layers. The average initial temperatures measured at sampling locations with thermocouple junctions spaced on cables at depth intervals of 0.9 m were 33.0 and 36.3°C, and temperatures decreased 0.7 and 0.8°C, respectively, per week.

Simulation Model. The population dynamics computer simulation model used in this study consisted of four major parts: equations describing the relationship between temperature and insect development, a delay process for moving the immature insects through the stages and simulating variation in developmental rate, a 70-element array for keeping track of adult age, and equations describing relationship between temperature and insect egg production. Insect survivorship was not explicitly included in the model. However, for *R. dominica* egg production was calculated in such a way as to include survivorship.

Temperature–developmental time equations used to calculate the time that insects spent in each stage were from Hagstrum and Milliken (1988). For *T. castaneum* and *C. ferrugineus*, significantly different temperature–developmental time equations had been fitted to data collected at high (14%) and low (10%) grain moisture contents. For these two species, the effects of intermediate moistures on developmental time were simulated by linear interpolation between the two calculated developmental times.

Variation in development rate was simulated with a time-varying distributed delay (Manetsch 1976). The basis for this method is that there is a mean (DEL) and variance (s^2) associated with the time required to complete development of a stage at a given temperature. The shape of the curve describing the number of individuals leaving a stage over time can be approximated by a member of the Erlang family of density functions. The specific function is designated in the model by the parameter K , which is calculated as DEL^2/s^2 . There were few measures of variation in rate of development reported in the literature, so a variance was estimated for each mean duration of development using the equation of Shaffer (1983), where

$$s = 0.209x^{0.73}$$

A K for each stage was determined by first calculating a K for each temperature for which mean

developmental times were available, then taking average of K 's at the different temperatures. However, K for each stage was not allowed to exceed $DEL \cdot 5$, otherwise the delay would be unstable (i.e., would give erroneous results).

Eclosing adults, upon leaving the pupal stage, entered a 70-element adult array at a 1:1 sex ratio. Adults were moved each day to the next element of the array and were no longer considered in calculating egg production after 70 d.

Daily egg production rate was calculated using the equations in Table 1 based upon data for *C. ferrugineus*, *R. dominica*, and *T. castaneum* published by Smith (1965), Birch (1953), and Howe (1962), respectively. Daily egg production equalled the sum of the number of females multiplied by their daily lifetime average or age-specific egg production rate. For *R. dominica*, the equations described the effects of only grain temperature and moisture content on the daily average lifetime egg production rate, because insufficient data were available on age-specific changes in egg production. Daily egg production of *R. dominica* was calculated by dividing the rate of multiplication per generation (R_0) by the generation time (T) in days. For *T. castaneum*, egg production at 10% moisture was 60% of egg production at 14% moisture (Howe 1962) and for *C. ferrugineus* egg production at 10% moisture was 40% of egg production at 14% moisture (Smith 1965). Egg production rates at intermediate moistures were obtained by linear interpolation. Because White (1987) reported that daily egg production of *T. castaneum* on whole-kernel wheat was only half of that on the wheat flour and yeast diet used by Howe (1962), the egg production calculated using fitted equations was divided by two in the model.

Model inputs were the initial number of adult insects and the temperature and grain moisture conditions for each day of the simulation. Because estimates of initial population densities were quite variable for farm bins, all simulations were begun with five pairs of adults. The predicted numbers for each sampling date were then adjusted, so that for each of the populations the sums of predicted equalled the sums of mean measured population densities. This procedure used all of the measured mean densities for each population to establish what the initial population density must have been.

We used programs from the SAS Institute (1982) to fit regression equations for egg production and to calculate means and standard errors for each of nine insect populations on each sampling date. Bonferroni t tests (Milliken & Johnson 1984) were performed to compare measured with predicted mean population densities. An SAS regression program also was used to compare measured and predicted mean population densities.

Results and Discussion

The measured population trends of three species of adult stored-grain insects were quite similar in

Table 1. Equations describing egg production (Y) in relation to age of adults (A), and temperature in °C (T) for two species of stored-product insects and temperature and percentage of grain moisture (M) for *R. dominica*

Species	df	Equation ^a	r ²
<i>C. ferrugineus</i> ^b	72	$Y = e^{0.76T - 0.026T^2 - 0.00062T^2A + 0.38A - 0.034A^2 - 4.38}$	0.8729
<i>R. dominica</i>	9	$Y = 1.45T - 0.024T^2 + 0.37M - 24.64$	0.8086
<i>T. castaneum</i> ^b	40	$Y = e^{-23.96 \text{ LOG } T + 8.70T - 0.43T^2 - 0.00022T^2A + 0.0085T^3 + 5.41}$	0.9359

^a Equations for *C. ferrugineus*, *R. dominica*, and *T. castaneum* are based upon data published by Smith (1965), Birch (1953), and Howe (1962), respectively.

^b T = T - 19°C.

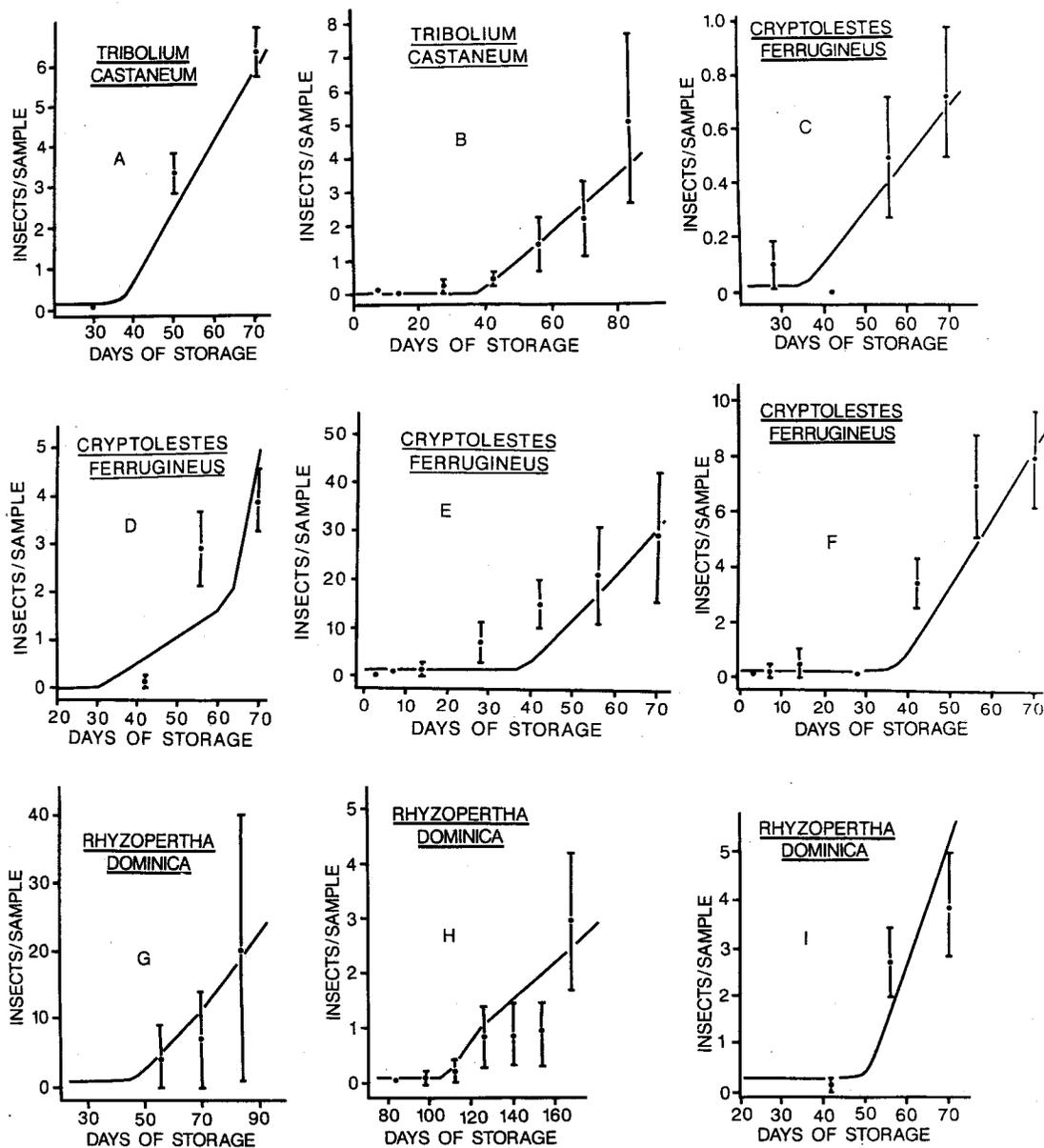


Fig. 1. Comparison of measured seasonal changes in the mean number of adult insects per sample (·) ± SE (vertical bar) with the numbers of insects predicted by the simulation model (—). The population trends include new laboratory data (A and I), new on-farm data (B, E-G), and on-farm data (C, D, and H) collected by Hagstrum (1987).

Table 2. Parameters for regression of measured (y) against predicted (x) population densities for three insect species

Parameter	Species		
	<i>C. ferrugineus</i>	<i>R. dominica</i>	<i>T. castaneum</i>
df	20	12	9
Slope \pm SE	1.01 \pm 0.088	0.95 \pm 0.078	1.12 \pm 0.081
t for H_0 : slope = 1	0.1474	-0.576	1.502
Probability > t	0.8843	0.5753	0.1673
Intercept \pm SE	0.95 \pm 0.72	-0.30 \pm 0.51	0.052 \pm 0.20
t for H_0 : intercept = 0	1.320	-0.579	0.254
Probability > t	0.2025	0.5743	0.8056
R^2	0.8750	0.9309	0.9603

form (Fig. 1). Few insects were found in samples during the first 30 to 33 d for *C. ferrugineus*; 35 and 38 d for *T. castaneum*; and 42, 48, and 102 d for *R. dominica*. The only adults present during this initial period were those that were introduced during initial infestation in laboratory studies (Fig. 1A and I) or those that immigrated into newly harvested wheat stored on the farm (Fig. 1B-H). The duration of this period of low initial density was generally determined by the developmental times for a species. However, a much longer 102-d period of low adult numbers for *R. dominica* (Fig. 1H) was probably the result of either delayed immigration of this species into the bin or the first-generation adults being too scarce to be found in samples taken. After a period of low initial adult insect densities, the adult population then increased rapidly as the offspring of these initial adults reached the adult stage.

The measured mean insect population densities increased as the predicted population densities increased (Fig. 1). When the Bonferroni t test was used to compare measured mean population densities to predicted, the differences were not significant at the 1% level for any of the nine populations. However, in some cases, the large standard errors of measured mean densities may have been responsible for the lack of significant differences. Another approach to comparing means, regression of measured (y) against predicted (x) insect population densities, indicated that the simulation model explained 87, 93, and 96% of the changes in mean measured densities of *C. ferrugineus*, *R. dominica*, and *T. castaneum*, respectively (Table 2). Thus, for population trends of the three species presented here, only 13, 7, and 14% of the differences between predicted and measured mean population densities remains to be explained by other factors. A slope of <1 indicated that the predicted densities tended to exceed the measured densities of *R. dominica*, whereas a slope of >1 indicated that the measured densities of *C. ferrugineus* and *T. castaneum* tended to exceed predicted densities. However, none of the slopes were significantly different from 1. Also, the intercepts of regression equations were not significantly different from zero for any of the three species, indicating that as densities approached zero, neither measured nor pre-

dicted densities were consistently higher or lower than one another. Consistently high survivorship is not too surprising under the favorable conditions encountered in this study. The similarity between measured and predicted numbers of insects per sample is particularly encouraging in that these comparisons include data from four different bins of farm-stored wheat, from two different crop years, and from single- and multiple-species populations.

This capability to predict insect infestation levels from the initial adult density and the expected grain temperature and moisture conditions can be valuable in evaluating the relative effectiveness of various pest management programs. Thorpe et al. (1982) used a simulation model to study the effects of aeration on *S. oryzae*. However, with stored grain, pest management decisions often must be made for several insect species simultaneously. The next step is to validate the model over a wider range of environmental conditions and to add various management practices such as parasites, aeration, fumigation, and insecticide applications. With such further development, the models described here should be useful in evaluating the relative effectiveness of a pest management program on different pest species.

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Larval Trails of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) as Kairomonal Host-Finding Cues for the Parasitoid *Cephalonomia waterstoni* (Hymenoptera: Bethyidae)

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ABSTRACT Wandering late fourth instars of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), leave a substrate-borne chemical trail that serves as a kairomone for their host-specific bethylid parasitoid *Cephalonomia waterstoni* Gahan. Male *C. waterstoni* show no trail-following behavior to this kairomone. Experienced *C. waterstoni* females follow trails of ≥ 0.0001 larval equivalents per centimeter (LE/cm), whereas naive females show no trail following responses to dosages of < 0.1 LE/cm. A dose-dependent response for time spent on the trail and length of trail followed was shown by the parasitoid to natural trails. The time spent by the parasitoid on artificial trails produced by using beetle extracts also showed a dose dependent response. The parasitoid shows weak or nonexistent trail-following responses to hexane extracts of larvae of the flat grain beetle *Cryptolestes pusillus* (Schonherr) or the red flour beetle *Tribolium castaneum* (Herbst). The trails of *C. ferrugineus* larvae are only moderately persistent. Natural trails aged on filter paper elicit trail-following responses by the parasitoid for less than one week. Artificial beetle extract trails on ground glass plates elicited trail following by the parasitoid for almost two weeks. The disappearance of both natural and solvent extract kairomonal trails follows an exponential decay model.

KEY WORDS Insecta, biological control, behavior, trail-following

THE RUSTY GRAIN BEETLE, *Cryptolestes ferrugineus* (Stephens), is a widespread and important pest of stored cereal grains and is often one of the most abundant pests of on-farm storage facilities in the American midwest (Hagstrum 1987). The general biology of *C. ferrugineus* has been thoroughly studied by Rilett (1949b), Smith (1965), and Hagstrum (1987, 1989). Although the adult beetles feed primarily on the exposed wheat germ, the larvae are internal seed feeders, and pupae are enclosed in silk cocoons. As such, the species is considered to be relatively free from predators or parasites. However, as noted by Smith (1972), both the first and late fourth (final) instars wander substantial distances, and thus are susceptible to attack. The ectoparasitic bethylid wasp *Cephalonomia waterstoni* Gahan paralyzes and oviposits upon late fourth-instar rusty grain beetle larvae and readily feeds upon, but does not oviposit upon, earlier instars. The general bionomics of *C. waterstoni* are well described by Rilett (1949a) and Finlayson (1950a).

Although *C. waterstoni* will attack and oviposit on a few other species of *Cryptolestes* under a no-choice laboratory situation, it is normally host-specific (Finlayson 1950a,b). The mechanisms by which this host-specificity is achieved, and the evolutionary pathways leading to it, are of interest. Finlayson (1950b, 1952) conducted an extensive series of

experiments on host preference and host location, and suggested that a chemical cue derived from the cuticle of the larval *C. ferrugineus* was possibly being used by the parasitoid. He was unable, however, to identify the chemical(s) or to clarify further other long- or short-range proximal cues that would account for the host-finding, host-recognition, and host-acceptance behavior of the parasitoid.

As part of a larger program aimed at elucidating the chemical and physiological ecology of stored product insects and their parasitoids and predators, we have observed that wandering fourth instars of *C. ferrugineus* appear to mark their path by frequent "dragging" of their abdomen as they move among the wheat kernels. Such marking behavior suggested to us that the beetles might be leaving a kairomonal cue that could be used by *C. waterstoni* for host location. We now report that *C. ferrugineus* larvae do indeed leave a chemical trail as they seek their pupation site and that this trail is a potent species-specific kairomonal cue for *C. waterstoni* females.

Materials and Methods

Insects. All colonies of *C. ferrugineus*, *C. waterstoni*, *Cryptolestes pusillus* Schonherr, and *Tribolium castaneum* (Herbst) were collected from

farm-stored wheat in Kansas, and have been in culture in our laboratory for at least 1 yr. Insects are maintained on whole wheat flour with 5% brewer's yeast, and are held in incubators maintained at 30°C, about 70% RH, and constant darkness.

C. ferrugineus Trail Deposition and Bioassay.

One, four, or eight fourth instars were placed in an arena consisting of a glass ring (4.6 cm i.d. by 2.5 cm high) with a solid acrylic cylinder (4.2-cm diameter by 3.5 cm high) centered within the glass ring, both sitting on Whatman No. 41 filter paper (9-cm diameter). The larvae wandered freely in the channel between the glass ring and the acrylic cylinder for 30 min, and then were removed. The location of the outer ring was marked on the paper with a light pencil streak, and the glass and acrylic cylinders then were removed. One experienced female parasitoid (see below) was placed in the center of the test arena, and the filter paper was covered with a petri dish lid (7-cm diameter) to exclude air currents. Afterward, the behavior of the parasitoid upon contacting the presumptive beetle larvae trail was noted. Behavior scored included the amount of time out of 2 min spent on trail, distance traversed on the trail during the 2-min, and trail-following behavior (antennal movements and kinetic movements). Trail deposition and subsequent trail-following bioassays were conducted in a cabinet illuminated with an incandescent red light bulb (40 W) located approximately 0.75 m from the assay arena. The cabinet was held at a temperature of 29 ± 1°C. In this and all subsequent experiments each female was used only once and then was returned to stock cultures. A fresh trail was always provided for each female.

Kairomone Extraction. *C. ferrugineus* cultures of the proper age to contain predominately fourth instars were sieved through a 35-mesh sieve to remove medium. The larvae were weighed, and three subsamples were counted and weighed to estimate total number of larvae. Larvae then were killed by freezing at -25°C, warmed to room temperature, and extracted three times for 1 min each with 5-ml portions of hexane. The combined hexane extracts were pooled and concentrated with a stream of nitrogen to give a final volume of one larval equivalent (LE) per microliter of solution. Appropriate serial dilutions of this stock solution were then made and used in bioassays.

Trail Bioassay. A trail 10 cm in circumference was penciled onto a piece of paper, and a ground glass plate (12 cm by 12 cm) was placed on top of the paper. Using a 1 µl disposable pipet a trail was streaked on the ground glass plate using the pencil trail on the paper as a guide. Resulting trails (expressed as LE/cm) were one-tenth the nominal concentration of the test solution. Control trails of hexane only were assayed also. Solvent was allowed to evaporate for 1 min, and a single female *C. waterstoni* was gently released in the center of the ground glass plate, and a petri dish lid (7-cm di-

ameter) was placed over the plate. Upon first contacting the test trail a stopwatch was started and the total time spent by the parasitoid on the trail in the next 2 min was recorded. In some cases (see below) the bioassay was recorded on videotape for subsequent analysis of distance traveled on the test trail by the parasitoid. Preliminary trials with male *C. waterstoni* elicited no trail following behavior under any circumstances.

Videotaping of Trail Bioassay. Some bioassays were videotaped. Data were stored on ¼-in videocassette tapes. All recordings were made in the real-time mode. Lighting was provided by an incandescent red light bulb (40 W) about 0.75 m from the assay arena and about 2 m from the video camera.

Persistence of Larval *C. ferrugineus* Trails.

Natural trails deposited by 1, 4, or 8 beetle larvae on filter paper during a 30-min time period were held in darkness at 29 ± 1°C for 1 wk. The trail-following responses of experienced *C. waterstoni* females to these natural trails after 0, 3, and 6 d during the 1-wk period were determined. In addition, beetle extracts of 0.01 LE/cm were streaked on ground glass plates, held in darkness at 29 ± 1°C for 2 wk, and bioassayed using experienced *C. waterstoni* females at various times during the 2 wk. At least five females were tested at each time for both natural and extract trails.

Effect of Experience on Parasitoid Trail Following. Newly emerged to 1-wk old female wasps were allowed to mate and then were divided into two groups. One group was provided only with honey streaks and was held 24 h before being used in the trail bioassay. These animals are called *naive*. The second group was provided fourth instar *C. ferrugineus* larvae to sting and oviposit upon before being held for 24 h with a honey streak and then used in the trail bioassay. These animals are called *experienced*. Wasps from each group were individually tested for trail following using serially diluted beetle extracts. Trails between 1.0 LE/cm and 0.0001 LE/cm were tested for eliciting trail following behavior. At least five females were tested at each concentration for both groups. Total time out of 2 min spent on the trail and trail following behavior was recorded.

Species Specificity of Kairomone Extracts. Larvae of *C. pusillus* and *T. castaneum* were removed from their culture media in the same manner as described above for *C. ferrugineus*. Hexane extracts were prepared as described above. The trail-following responses of experienced *C. waterstoni* females to 0.01 to 4 LE/cm trails of each of the above extracts were recorded. Wasps were also tested with 0.01 LE/cm trails of larval *C. ferrugineus* as a positive control.

Scanning Electron Microscopy (SEM). Fourth instars of *C. ferrugineus* were prepared for SEM using the methods of Grodowitz et al. (1982). After critical point drying all specimens were sputter-coated with gold-palladium and viewed with an

Table 1. Trail-following responses of *C. waterstoni* to natural kairomonal trails deposited by varying numbers of wandering *C. ferrugineus* fourth instars

No. <i>C. ferrugineus</i> larvae	\bar{x} response (± 1 SE) ^a	
	Time, s	Distance, cm
0	2.50 (0.47)	1.30 (0.40)
1	23.80 (6.06)*	6.00 (1.10)*
4	45.80 (8.25)*	10.30 (2.48)*
8	53.20 (8.33)*	12.45 (2.03)*

^a Means in the same column followed by an * are significantly different from the control trails (0 larvae) at $\alpha = 0.05$.

ETEC Auto Scan U-1 Electron Microscope (ETEC Corporation, Hayward, Calif.).¹

Statistical Methods and Voucher Specimens. All bioassays were replicated at least five times unless otherwise indicated. Data were analyzed by one-way analysis of variance or by regression analysis using the Statgraphics Statistical Package for personal computers (Statistical Graphics Corporation, Rockville, Md.). Voucher specimens of all taxa are deposited in the research collection of the Department of Entomology, Kansas State University, Manhattan, and in the research collection of the Department of Entomology, University of Wisconsin, Madison.

Results

Larvae of *C. ferrugineus*, when placed in the trail-laying arena and allowed to wander for 30 min, deposited a chemical trail on the filter paper, as shown by the strong dosage-dependent, trail-following response of the parasitoids (Table 1). Close observation of the beetle larvae during this wandering period indicated that every few seconds while walking and while resting they lowered their abdomen very close to the substrate and touched the substrate with what appeared to be long setae. Similar intermittent touching of substrate was evident when *C. ferrugineus* larvae were observed crawling among wheat kernels. No body parts except the tarsi were observed to touch the substrate during movement or resting periods. Examination of the ventral abdominal surface of these larvae by SEM revealed on each abdominal segment a symmetrically placed pair of long and stout socketed setae corresponding in location to the setae observed being dragged by the beetle larvae while they walked (Fig. 1).

The response of the parasitoids to the kairomonal trail was strikingly like that displayed by ants and termites engaged in trail following. The wasps stopped immediately upon first contacting the trail, briskly drummed the filter paper, then turned onto the trail and proceeded to follow it in a zigzag manner, constantly drumming the paper with their

antennae. The wasps sometimes left the trail to explore the area nearby for a few seconds, then returned to the trail and continued trail following. Occasionally, the wasps reversed direction and followed the trail in the opposite direction. The intensity of trail following on these natural trails (measured as either total time on trail or distance traveled on the trail) was clearly dose dependent (Table 1). Because time on the trail was easier to measure than distance, this parameter was measured in all subsequent bioassays.

The kairomonal trails left by *C. ferrugineus* are only moderately persistent. The natural trails deposited on filter paper by one to eight beetle larvae still elicited positive trail following by the parasitoid 3 d after deposition, but elicited little trail-following response by the parasitoid at 6 d after deposition (Fig. 2). In contrast, positive trail-following response occurred for almost 2 wk when the test trails were solution extracts applied to ground glass plates (Fig. 3). In both cases, regression analysis of the rate of loss of kairomone (as measured by time spent trail following by the parasitoid) indicated that the data were best fit by using an exponential decay model. For the natural trails on filter paper, $Y = e^{(2.911 - 0.146x)}$, $r^2 = 0.651$; $P = 0.0003$ for a one-beetle trail; $Y = e^{(3.701 - 0.023x)}$, $r^2 = 0.669$; $P = 0.0002$ for a four-beetle trail; $Y = e^{(3.539 - 0.020x)}$, $r^2 = 0.4533$; $P = 0.006$ for an eight-beetle trail; and for the 0.01 LE/cm extract on the ground glass plate, $Y = e^{(3.496 - 0.010x)}$, $r^2 = 0.673$; $P < 0.0001$.

To investigate further the biological properties of the kairomone, hexane extracts of larval *C. ferrugineus* were prepared and used for subsequent experiments. To ascertain whether the trail-following response of *C. waterstoni* females are enhanced by previous experience, trail-following bioassays were conducted using naive and experienced females. Naive females showed positive trail-following responses at dosages no lower than 0.1 LE/cm, whereas females that had been allowed to sting *C. ferrugineus* larvae before being tested followed trails greater than two orders of magnitude less concentrated than this (Table 2).

Studies were also conducted to ascertain whether *C. waterstoni* would respond to hexane extracts of larvae of other species of stored-product beetles, including *C. pusillus*, which *C. waterstoni* will attack if placed in a no-choice situation for an extended period of time (Finlayson 1950b). *C. waterstoni* showed only very weak trail-following responses to larval extracts of *C. pusillus* and no trail following was elicited by extracts of *T. castaneum* (Table 3).

Discussion

The biology of only a few species of *Cephalonomia* is well known (Evans 1964). Members of this genus are parasitoids of the larvae or pupae of

¹ Mention of a company or trade name is for identification purposes only and does not constitute an endorsement or a recommendation for its use by USDA.

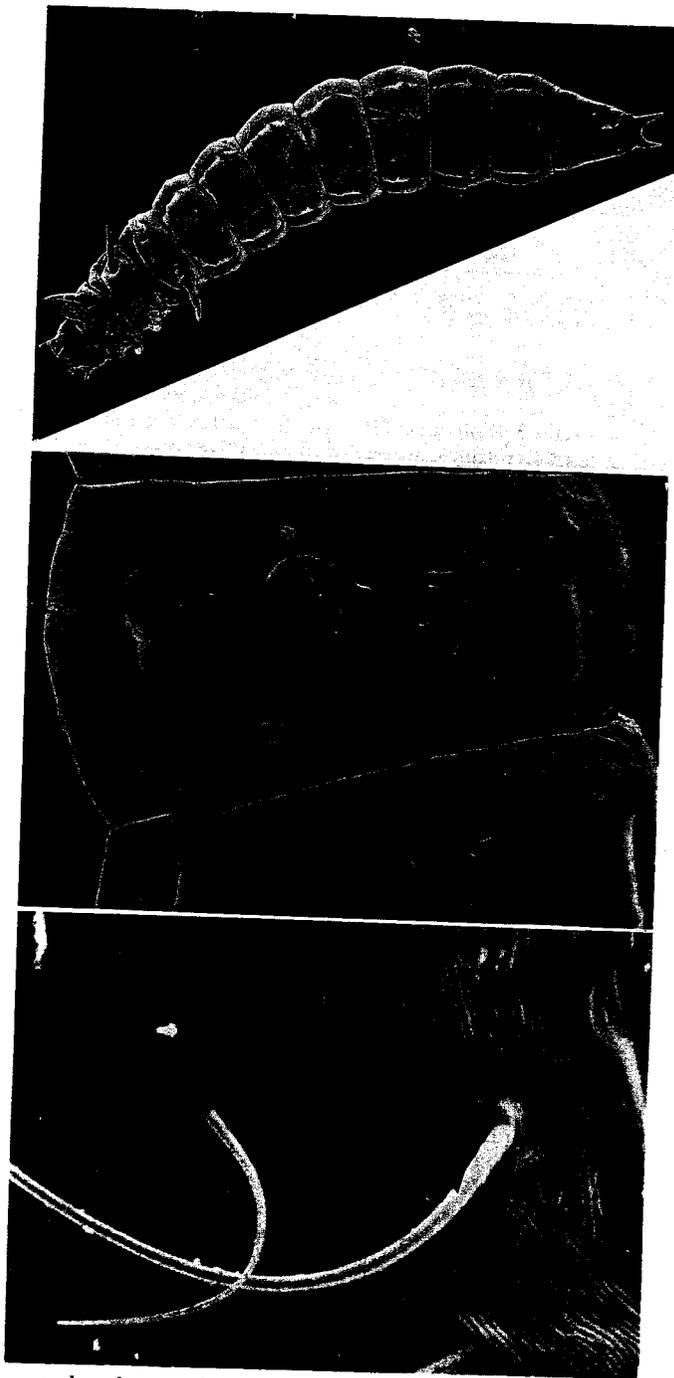


Fig. 1. SEM of the ventral surface of the abdomen of a *C. ferrugineus* fourth instar. Arrows point to the socketed setae occurring on each abdominal segment; these are thought to act as brushes transferring trail kairomone to substrate. (Top) Negative magnification, 13 \times . (Middle) Negative magnification 50 \times . (Bottom) Negative magnification 250 \times .

small beetles that occur in cryptic environments. In general, these parasitoids are not highly host specific. For example, *C. gallicola* (Ashmead) readily parasitizes Anobidae and Ptinidae (Van Emden 1931, Kearns 1934), and *C. tarsalis* (Ash-

mead) parasitizes hosts from families as disparate as Cucujidae and Curculionidae (Powell 1938). The strong host specificity of *C. waterstoni* to larvae of *C. ferrugineus* (Finlayson 1950b) is thus noteworthy.

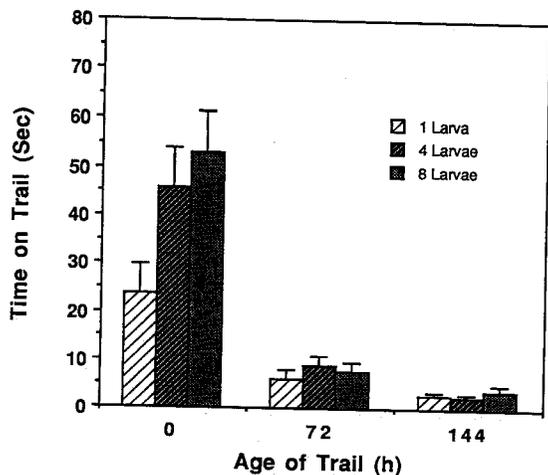


Fig. 2. Trail-following responses of *C. waterstoni* to aged natural trails of one, four, or eight *C. ferrugineus* larvae. Error bars are ± 1 SE.

As in many other bethylids, sib mating by *C. waterstoni* (Rilett 1949a, Griffiths & Godfray 1988) is common. Males usually emerge before females and often enter female cocoons to mate with the newly eclosed female. The subsequent reproductive success of this newly mated female is then totally dependent on her ability to locate *C. ferrugineus* fourth instars that have not yet spun their own cocoons. These larvae are rare in both space and time, occurring in patchily distributed sites within the mass of stored grain (Hagstrum 1987, 1989). Unless the beetle population has reached epidemic proportions, the *C. waterstoni* females face a formidable challenge in locating their hosts. Except for the top surface of the grain, the grain storage environment is totally dark, so the females are restricted to acoustical or chemical cues for locating the beetle larvae. Our observations of the marking behavior of wandering *C. ferrugineus*

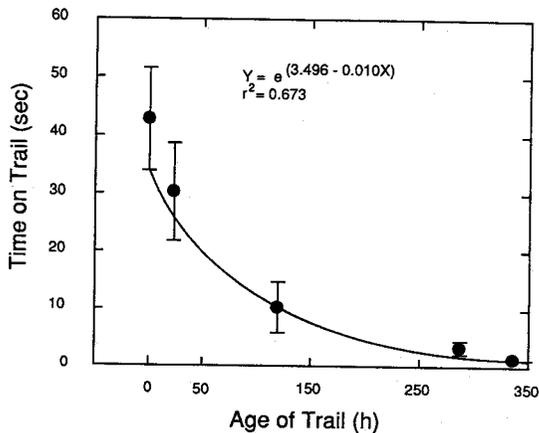


Fig. 3. Trail following responses of *C. waterstoni* to aged extract trails of *C. ferrugineus* larvae (0.01 LE/cm). Error bars are ± 1 SE.

Table 2. Duration of trail following by experienced and naive *C. waterstoni* to various levels of larval *C. ferrugineus* kairomone extracts

LE/cm	\bar{x} time, s (± 1 SE) ^a	
	Inexperienced (n = 15)	Experienced (n = 10)
0	0.63 (0.23)	2.65 (0.41)
0.0001	— ^b	11.40 (4.30)
0.0010	3.10 (1.32)	15.55 (3.70)*
0.0100	5.20 (1.07)	20.75 (4.67)*
0.1000	36.87 (7.83)*	35.80 (11.43)*
1.0000	38.87 (8.76)*	34.70 (7.00)*

^a Means in the same column followed by * are significantly different from the control trails at $\alpha = 0.05$.
^b Not tested.

larvae suggested that the chemical cue option was likely, and our results strongly support this hypothesis.

The kairomonal trail left by wandering *C. ferrugineus* larvae appears to be low in absolute abundance, noncontinuous, and ephemeral. As of yet, we have no evidence of its being used as a con-specific semiochemical. These abundance traits of the kairomone are advantageous for the beetle, but are disadvantageous for the parasitoid. To compensate, however, the sensitivity of the parasitoid to the kairomonal trail is high. Although accurate absolute abundance estimates of kairomone concentrations must await completion of our chemical identification studies, the present results using bioassays suggest that the parasitoid can detect and respond to *C. ferrugineus* kairomone at levels of a few nanograms or less. This estimate is based on a biomass of about 500 μ g for fourth instar *C. ferrugineus* and an assumption that the quantity of kairomone deposited by the larva is never >0.01% of its biomass at any given point on the trail (as would be the case if the socketed setae on each abdominal segment are responsible for physically depositing the kairomone onto the substrate). Our

Table 3. Duration of trail following by *C. waterstoni* to hexane extracts of larval *C. ferrugineus*, *C. pusillus*, and *Tribolium castaneum*

Test extract	\bar{x} duration of trail following (\pm SE) ^a	
	Concentration, LE/cm	s
Hexane	0	1.3 (0.5)a
<i>C. ferrugineus</i>	0.01	70.1 (10.5)b
<i>C. pusillus</i>	0.01	3.5 (0.5)a
<i>C. pusillus</i>	0.1	4.5 (1.2)a
<i>C. pusillus</i>	1.0	6.3 (2.4)a
<i>C. pusillus</i>	4.0	4.9 (1.2)a
<i>T. castaneum</i>	0.01	2.0 (0.4)a
<i>T. castaneum</i>	0.1	1.9 (0.2)a
<i>T. castaneum</i>	1.0	1.9 (1.2)a
<i>T. castaneum</i>	4.0	1.4 (0.7)a

^a Means followed by the same letter are not significantly different at $\alpha = 0.05$ (Fisher's LSD test) (Statgraphics 1986, p. 14-3). n = 5 for each mean.

observations have shown that if a *C. waterstoni* female wanders off the trail, she will search in the immediate environment until encountering it again. Thus, the discontinuous nature of the trail does not prevent its use as an effective orientation guide by the parasitoid. If trails become too discontinuous, however, the parasitoid acts as if the kairomone is simply an indication of a foraging patch (Van Alphen & Vet 1986) and proceeds to search the immediate environment actively in an apparently random fashion.

The intensity of trail following by the parasitoid clearly depends on the dosage of kairomone present and the prior experience of the parasitoid. The dosage is a reflection, in part, of the length of time that the trail has been on the substrate. Our studies using naturally deposited kairomone on filter paper indicate that kairomone either dissipates or degrades in <1 wk at 29°C. The apparent longer half-life of the kairomone when applied as a hexane extract to ground glass plates may be a result of the kairomone's being dissolved in extracted beetle lipids, which could retard evaporation. If the parasitoid can correlate dosage of kairomone on the substrate with probable length of time since the kairomone was deposited, then she has a mechanism for assessing whether it is "worthwhile" to continue following this trail. We have observed that experienced *C. waterstoni* actively search for the trail if they lose it when the trail is at a level of about 0.01 LE/cm. However, at trail levels of about 0.001 LE/cm or less, the parasitoid searches only briefly in the immediate vicinity for a lost trail and then wanders in a more random search pattern. This dosage-dependent searching behavior suggests that experienced parasitoids may indeed be able to assess the likelihood of finding a suitable host from the quantity of kairomone left on the grain. The ability of naive parasitoids to utilize this information efficiently is less clear. They do, however, respond to the kairomone without prior after-eclosion experience and hence are innately sensitive to the kairomone.

The host specificity of *C. waterstoni* could result from choices made at the hierarchical levels of host searching, host recognition, or host acceptance. The data from our studies comparing the trail following responses of the parasitoid to hexane extracts of larval forms of *C. pusillus*, *C. ferrugineus*, and *T. castaneum*, suggest that *C. waterstoni* minimizes its time searching for inappropriate hosts by responding only to the kairomonal trails of *C. ferrugineus* larvae. The slight amount of trail following elicited by the extracts of *C. pusillus* may be a result of low levels of *C. ferrugineus* kairomone also being present in *C. pusillus*, or it may reflect the presence of a related chemical in *C. pusillus*. In laboratory studies where the parasitoid was presented test larvae without any trails being present, the wasp was still able to discriminate between appropriate and inappropriate hosts at the behavioral level of either host recognition and/or host

acceptance (Finlayson 1950a, 1952). It is not apparent yet whether the kairomone used by *C. waterstoni* to track *C. ferrugineus* larvae is the same as the cue used by the wasp in assessing whether the beetle larva is a suitable host for her progeny.

To our knowledge, this is the first report of a parasitoid of stored product beetles using a kairomonal trail to locate their host. Indeed, we are aware of only two other examples of parasitoids utilizing naturally deposited kairomonal trails. In the first case, several species of stored product pyralid moth larvae have been reported to secrete 2-acyl-cyclohexane-1,3-diones from their mandibular glands while feeding and moving, and these ketones are potent semiochemicals which elicit both intraspecific dispersion and antennation and ovipositor probing by several parasitoids (Corbet 1971, Mudd & Corbet 1973, Mossadegh 1978, Mudd 1983, Mudd et al. 1984). Strand et al. (1989) have now shown that at least one parasitoid [*Bracon hebetor* (Say) (Hymenoptera: Braconidae)] of these pyralid larvae appears to locate its host, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), by following the trail of deposited mandibular gland secretion left by the moth's final instar as it wanders in search of a pupation site. In the second case, Klomp (1981) reported that the ichneumonid wasp *Poecilostictus cothurnatus* (Gravenhorst) locates its host, last instars of the pine looper, *Bupalus piniarius* L. (Lepidoptera: Geometridae), by following trails left on the pine needles by the wandering caterpillars.

A common feature of all three examples is that the host being sought by the parasitoid is a wandering-stage larva seeking a pupation site. Because such behavior is known from a multitude of larval forms in a diversity of taxa, we expect that future workers will uncover many additional examples of parasitoids locating their prey by tracking kairomonal trails left by their wandering hosts. Insufficient evidence now exists, however, to speculate on whether such kairomonal trails will prove to be used predominately by host-specific parasitoids such as *C. waterstoni*, or whether parasitoid generalists will also be found to commonly use this type of chemical cue.

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