

Ovipositing females of a short-lived gall midge take time to assess suboptimal grass seed heads

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Abstract. The short-lived adult wheat midge *Sitodiplosis mosellana* deposits eggs on the seed head of various grasses close to the developing seeds on which larvae feed. The time taken to make egg-laying decisions is investigated using three types of wheat *Triticum aestivum* seed heads. Young Roblin, Old Roblin and Young Key differ in their effects on ovipositing females (72%, 22% and 6% of eggs in choice tests, respectively) and effects on feeding larvae (75%, 25% and 5% larval survival, respectively). Within seconds of arriving, the female is able to distinguish Young Roblin from the two lower-ranked types. However, the lower-ranked types are not rejected at this time. Instead, all head types are examined before the female eventually flies away. On Young Roblin, probing with the ovipositor is the first behaviour that occurs. Thereafter probing and insertion of the ovipositor occupy most of the female's time and behavioural transitions tend to be 'progressive', signalling a shift from low to high intensity examining. Differences between females visiting Old Roblin and Young Key are significant but take longer to emerge. On both, sitting is the first behaviour but, over the next 5–10 min, the female on Young Key exhibits more sitting, walking and 'regressive' transitions than the female on Old Roblin. It is suggested that, when the ovipositing female is short-lived and incapable of controlled flight in all but essentially windless conditions, her behaviour is designed to thoroughly, rather than rapidly, examine a suboptimal host before abandoning it for the uncertain future of finding a better host.

Key words. Cecidomyiidae, behavioural mechanisms, focal animal sampling, host selection, Poaceae, time-limited, wheat midge.

Introduction

In phytophagous insects, the ovipositing female makes several important decisions when she visits a plant: a yes/no decision about whether eggs will be deposited and, if the answer is yes, a decision about how many eggs. Both decisions are assumed to show some degree of optimization through natural selection (Thompson, 1988). Thus, the female should make the 'yes' decision only on plants that provide some degree of offspring survival. Numbers of eggs then should be adjusted according to how the plant functions as a food resource relative to other hosts.

Mechanistic studies of behaviour reveal how the female makes these decisions. In such studies, the female is observed continuously during host finding and selection.

Behaviours then are related to the eggs that are eventually deposited. At this mechanistic level, the species that are best understood are those most amenable to being observed. For the most part, these species have adult females that are relatively large and easy to find in the field or rear in the laboratory (tephritid fruit flies: Prokopy *et al.*, 1982; Abrahamson & Weis, 1997; butterflies: Jones, 1977; Singer, 1986). Typically, the female has an adult lifespan of 3–6 weeks and has excellent flight skills. Several groups of eggs are matured.

Less understood is the behaviour of phytophagous species having a short-lived adult female. This female has much less time to find and examine potential hosts (sometimes only 1–2 days) and, typically being small, are slow flyers. Relative to a butterfly (Jones, 1977), only a small area can be searched for hosts. Bad weather also has a greater impact on small insects and can further restrict time for host finding. The only advantage of the short-lived female is that adult feeding is rare. This eliminates the need to interrupt host-finding

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with the search for food, a common constraint for butterflies (Janz *et al.*, 2005). Because of these time limitations, researchers have predicted that the small short-lived female is less 'fussy' about host selection (Abrahamson & Weis, 1997). Thus, the female has enough time to sense the major differences between hosts and nonhosts (e.g. two plants providing 80% versus 0% larval survival) but not enough time to sense the more subtle differences between two plants that serve as hosts to varying degrees (e.g. two plants providing 50% versus 20% larval survival).

Gall midges (Diptera: Cecidomyiidae) have a short-lived adult female and are an important component of the insect communities that attack plants (Gagné, 1989). Finding hosts and distinguishing locations within hosts are critical for the survival and growth of larval offspring (Harris *et al.*, 2001, 2003). The newly-eclosed larva has limited mobility and must be placed near the feeding site. Here, the larva remains in a small area (e.g. < 1 cm²), using its small mandibles to pierce the outer cell wall of epidermal cells (Rohfritsch, 1992). On a suitable host, the result of this attack is the creation of a gall. In its simplest form, the gall is the nutritive tissue that the larva induces and then feeds upon (Bronner, 1992). In more complex galls, the nutritive tissue is surrounded, partially or completely, by a macroscopic gall structure (Stone & Schönrogge, 2003). Each gall-maker species can only induce galling on a narrow range of plant species (Gagné, 1989; Abrahamson & Weis, 1997; Stone & Schönrogge, 2003) and, on the host, has additional requirements for a specific developmental stage of a particular structure (e.g. young rapidly expanding leaves; Gagné, 1989). Although some gall midge species attack a perennial host and do not have to travel far to find oviposition sites (Larsson & Eckbom, 1995), many others attack annual hosts (Gagné, 1989) and presumably have to fly considerable distances not only from the eclosion site to oviposition sites (Sylvén, 1970), but also from one oviposition site to the next (Withers & Harris, 1996; Withers *et al.*, 1997a). At the time of eclosion, the female can carry several hundred mature eggs but commonly deposits only one to two eggs at each larval attack site (Harris & Rose, 1989; Smith & Lamb, 2001). This makes sense because competition between larvae reduces survival and growth (Withers *et al.*, 1997b).

For the small number of gall midge species that have been investigated, adult females appear to have the behaviour and physiology necessary for effective host selection. Females

fly upwind to volatiles produced by the correct developmental stage of the requisite plant structure (Galanihe & Harris, 1997; Birkett *et al.*, 2004). After landing on a plant, the female walks on plant surfaces, bringing the antennae and the tip of the abdomen in contact with chemical and physical features of the plant surface (Åhman, 1985; Waquil *et al.*, 1986; DeClerk & Steeves, 1988; Harris & Rose, 1989; Kanno & Harris, 2000). How long the gall midge female takes to examine a plant before making an oviposition decision has not been studied.

In the present study, host selection behaviour of the wheat midge *Sitodiplosis mosellana* (Géhin) is investigated. Throughout its distribution in Europe, Asia and North America (Harris *et al.*, 2003), the wheat midge larva is found feeding on developing seeds of grasses (family Poaceae), including seventeen wild and domesticated species in the genus *Triticum* (Wise *et al.*, 2001). Wheat midge eggs are deposited near larval attack sites (Smith & Lamb, 2001), typically in groups of one to two eggs placed on the proximal surfaces of the two modified leaves (i.e. the glume and lemma) that protect the developing seed. Immediately after hatching, the larva moves a short distance (< 5 mm) to locate the developing seed. Attack either kills the seed or greatly reduces germination and seedling growth (Lamb *et al.*, 2000). Egg counts have shown that females can distinguish between *Triticum aestivum* L. genotypes (Lamb *et al.*, 2003) and also between developmental stages of a single host genotype (Ding & Lamb, 1999; Lamb *et al.*, 2003).

The present study aims to determine how long it takes the wheat midge female to make oviposition decisions. The nature of these decisions and underlying behavioural mechanisms also are investigated. The three seed head types are pre-flowering and post-flowering heads of the *T. aestivum* cultivar Roblin (hereafter referred to as Young Roblin and Old Roblin, respectively) and pre-flowering heads of the *T. aestivum* cultivar Key (referred to as Young Key). Survival of wheat midge larvae is excellent on Young Roblin heads (70–90%; Ding & Lamb, 1999; Lamb *et al.*, 2003), poor on Old Roblin heads (25–30%; Ding & Lamb, 1999) and very poor on Young Key heads (5–10%; Lamb *et al.*, 2003). The six behaviours that are discussed (Table 1) originate from a previous study that developed methods for observing wheat midge females during host finding and examination (Ganehiarachchi & Harris, 2007).

Table 1. Behaviours scored for individual wheat midge females interacting with wheat seed heads.

| Behaviour | Terminal abdominal segments | Ovipositor relative to plant surface | Body position and behaviours |
|-----------|-----------------------------|--|---|
| Arrival | Extended | Not touching | Wings moving |
| Probe | Extended | In direct contact and moving over surfaces | Antennation and walking up and down seed head |
| Insert | Fully extended | In direct contact and inserted between modified leaves | Oviposition |
| Walk | Telescoped | Not touching | Moving up and down seed head |
| Sit | Telescoped | Not touching | Stationary |
| Depart | Telescoped | Not touching | Wings moving |

Materials and methods

Insects

The adult females used in behavioural studies developed from larvae that fed on hard red spring wheat. Mature larvae were collected either directly from the field (Mohall, North Dakota, 48°76' N, 101°51' W) or from the greenhouse-reared progeny of field-collected larvae (Wildrose, North Dakota, 48°59' N, 103°21' W). Greenhouse rearing has been described previously (Ganehiarachchi & Harris, 2007). Wheat heads were air-dried for 2–3 weeks and then broken apart to extract quiescent third-instar larvae. Larvae were collected with a camel's hair brush and placed in a plastic cup on the surface of a layer of moist silica sand (depth 1 cm), with this layer of sand sitting on top of a 4-cm deep layer of potting soil. During the next 2–3 weeks at $20 \pm 3^\circ\text{C}$, larvae became active and burrowed through the sand into the soil. The cup was transferred to a cold room ($2.5 \pm 2.0^\circ\text{C}$) and remained there for a minimum of 4 months.

When adults were needed, cups containing larvae were transferred to a cage held in an environmental chamber ($20\text{--}24^\circ\text{C}$, 60–80% RH) having lighting conditions similar to those of mid-summer in North Dakota (LD 16.8 h; lights off 23.00 h). The cage consisted of a clear plastic box ($35 \times 23 \times 27$ cm) with a ceiling of fine mesh and a floor of moist soil. Adults emerged 3–7 weeks later. Males and females eclosed in the mid-afternoon and late afternoon, respectively, and were held in the same cage to ensure mating (Pivnick & Labbé, 1992). Flight and visits to seed heads start at dusk and continue through the night (Pivnick & Labbé, 1993). The wheat midge female carries 60–80 mature eggs and begins oviposition on the second night after adult eclosion. The majority of eggs are oviposited on the third night, the night when behaviour was observed. Each female was observed only once and had not been exposed to plant material before being observed.

Plants

Two hard red spring wheat (*T. aestivum*) genotypes were used, cultivar Roblin and cultivar Key 97-10. Roblin heads were of two developmental stages, either Young Roblin at the pre-flowering stage (the head had emerged from the flag leaf but the anthers had not yet appeared, Zadoks' stage 58–59; Zadoks *et al.*, 1974) or Old Roblin at a post-flowering stage (i.e. approximately 5–6 days after anthers emerge from central florets, the latter being Zadoks' stage 60). Key heads were at the pre-flowering stage (i.e. Young Key).

Wheat was grown under insect-free controlled conditions (20°C , $590\text{--}710 \mu\text{M m}^{-2} \text{s}^{-1}$; LD 16 : 8 h) in an environmental chamber (Model E-8, Conviron, Canada). A single seed was planted in soil (Sunshine SP 100, Sun Gro Horticulture Inc., Washington, District of Columbia) supplemented with a slow-release fertilizer (Scotts, 15 : 9 : 12 N : P : K, Scott's Sierra Horticultural Product Company, Maysville, Ohio) held in a 6.4×25 cm Cone-tainer (Stuewe & Sons Inc., Corvallis, Oregon). Plants were watered daily. Excised grass heads

were used in all tests (Lamb *et al.*, 2003). Thus, 2 h prior to the start of behavioural observations, an intact wheat stem was submerged in water and cut 20 cm below the distal end of the seed head. When still under water, the stem was moved to a water-filled tube (length 7 cm length; Floral Pik, Dakota Plastics, Watertown, South Dakota).

Behavioural observations

During two summers (2005 and 2006), observations began in late June and finished in early August. This is the time period when wheat midge adults are present in North Dakota fields. Observations were conducted in a greenhouse but under natural rather than artificial light conditions (sunset in the range 21.20–20.50 h). Observations began at 20.00 h and continued until approximately 20 min after sunset, when it became impossible to see the fine details of female behaviour. If, during observation of a single female, light conditions deteriorated before the female flew away from the seed head, the observation was abandoned.

To compare Arrival frequency, one of each of the three head types was placed at a uniform height in a cage (diameter 15 cm, height 17 cm; glass walls and a mesh ceiling). Observations began immediately after 15–20 females had been aspirated into the cage (20.00 h). For each head type, Arrival was scored each time a female landed on the head. Heads were removed immediately after the observation ended. Eggs were counted using a microscope. To do this, each of the modified leaves (i.e. the glume, lemma and palea) comprising each spikelet of the seed head were removed one at a time and examined for eggs. Subsequently, the rachis was examined. The location of the egg within the seed head was not recorded. This test was conducted 11 times, with different heads and different groups of females used for each test.

Behavioural observation methods of Martin & Bateson (1993), including focal animal sampling and continuous recording, were used to quantify female behaviour during a visit to a seed head. Each evening that observations were made, three cages were set up, each containing a single head type. The aim was to complete three to five observations each evening (i.e. one female visiting Young Roblin and one to two females visiting Old Roblin and Young Key). However, because visits to Young Roblin could be quite lengthy, this experimental design was not always achieved. The cage consisted of a plastic cup (diameter 9 cm, height 12 cm) filled with moist sand and covered with an inverted plastic cup of the same dimensions but having a mesh insert in the ceiling. The seed head was positioned in the middle of the cage, its distal end 4 cm below the cage ceiling. Because a wheat midge female is much more likely to land on a seed head when other females are present (Ganehiarachchi & Harris, 2007), five to ten females were introduced into the cage. However, when the first of these females landed on the seed head, this cage along with its 'extra' females was removed and replaced with an empty cage. This procedure did not appear to disturb the female examining the seed head and prevented other females from interrupting the observation.

A tape recorder was used to score six behaviours (Table 1). Arrival (or Time Zero) was the moment that the female landed on the seed head and folded her wings. Depart was scored when the female flew away from the head. The head was immediately removed and eggs were counted. The taped record of the female's visit was transcribed, noting to the nearest second when the female switched to a different behaviour. The number of females visiting Young Roblin, Old Roblin and Young Key were 21, 26 and 37, respectively.

Statistical analysis

Behavioural transitions were calculated in one of two ways. For transitions that occurred only once during the visit (i.e. Arrival to the next behaviour or the behaviour preceding Depart), the transition percentage represents the proportion of all the females visiting that head type that exhibited the transition. Here, the means and errors were estimated by scoring zero or one for the probability of each individual female exhibiting the transition. For behavioural transitions that were exhibited several times during the female's visit, the transition frequency was calculated by dividing the number of times the female exhibited the transition (e.g. Walk to Probe) by the total number of transitions that the female exhibited from the first behaviour (e.g. Walk to all behaviours, including Probe). Latencies were calculated using Arrival as time zero. Percentage of time was calculated by adding the durations of all occurrences of the behaviour, dividing this by the length of the visit, and multiplying by 100.

Some behavioural measures had a single value for each female: length of visit, eggs per visit, latency to first exhibiting a behaviour, behavioural transitions (excluding transitions from Arrival to the next behaviour and from all behaviours to Depart), percentage of total time and total number of occurrences. For these measures, the homogeneity of variances across females visiting the three head types was tested using O'Brien's test at $P < 0.05$ (JMP IN, Version 4.04; SAS Institute, 2002). When variances were homogeneous, one-way analysis of variance (ANOVA) then tested for effects of head type (JMP IN, Version 4.04). When variances were heteroscedastic, data were transformed (log or arcsin) and again tested for homogeneity of variances. If the problem persisted, a Welch ANOVA was used, a test for equality of means that allows for heterogeneous variances (JMP IN, Version 4.04). When the ANOVA showed that seed head treatment had significant effects, the Tukey–Kramer honestly significant difference test ($P < 0.05$) was used to compare female responses on the three head types (JMP IN, Version 4.04).

For the percentage of females that exhibited the behaviour and for the behavioural transitions that were exhibited only once per visit (i.e. the transition from Arrival to the next behaviour and the transition preceding Departure), females visiting different head types were compared by logistic regression (SAS, Version 9.1; SAS Institute, 2004). However, because the maximum likelihood assumptions were violated in a number of cases, an exact conditional analysis was used throughout (Agresti, 1990). When this test found significant

differences, behaviours on the three head types were compared in a pairwise manner using exact odds ratios.

Results

Visits to heads during a choice test

When groups of females were given a choice between the three head types, numbers of females visiting the head were not influenced by head type ($F_{2,30} = 0.19$, $P = 0.82$). By contrast, numbers of eggs per head were influenced by head type ($F_{2,30} = 11.13$, $P < 0.001$). For Young Roblin, Old Roblin and Young Key, numbers of visits per head (mean \pm SE) were 16.18 ± 3.12 , 14.45 ± 2.95 and 16.18 ± 2.98 , respectively, whereas numbers of eggs per head were 63.63 ± 14.96 , 18.90 ± 5.12 and 4.54 ± 2.42 , respectively.

Behaviour during visits to seed heads

Seed head type had significant effects on the length of the female's visit, as well as eggs per visit (Fig. 1). For length of visit (Fig. 1a), head types were ranked Young Roblin $>$ Old

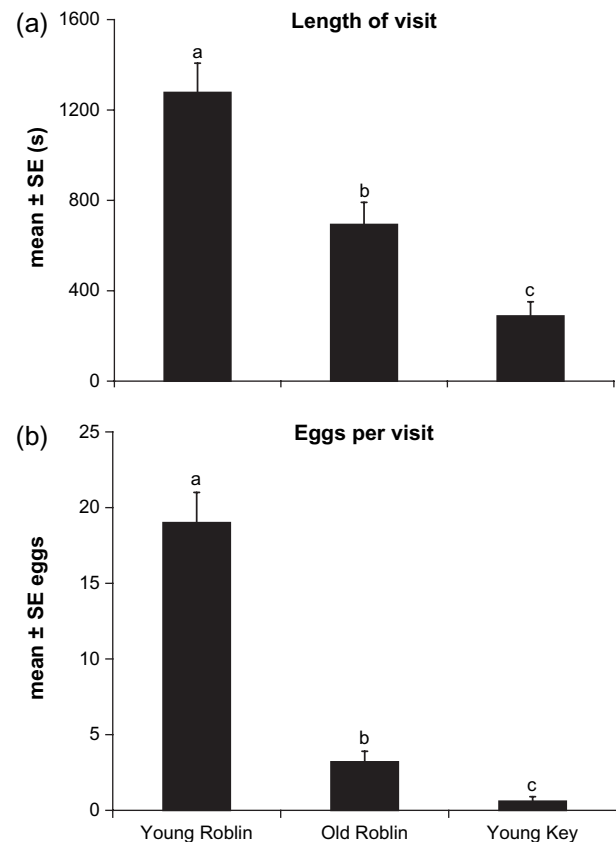


Fig. 1. For wheat midge visits to three seed head types, the length of the visit in seconds (a) and eggs oviposited per visit (b). Means are significantly different if they do not share the same letter (Tukey–Kramer honestly significant difference test, $P < 0.05$).

Roblin > Young Key ($F_{2,80} = 29.16$, $P < 0.001$), with each step of the ranking representing a two-fold change. For eggs per visit (Fig. 1b), the ranking of head types was similar (log transformed data, $F_{2,80} = 62.32$, $P < 0.001$) but there was greater fold change (i.e. a six-fold change between Young and Old Roblin and a five-fold change between Old Roblin and Young Key). Length of visit, seed head type and the interaction between visit length and head type all had significant effects on eggs (effect of visit length: $F_{1,77} = 70.12$, $P < 0.001$; head effect: $F_{2,77} = 29.72$, $P < 0.001$; visit length \times head effect: $F_{2,77} = 13.4$, $P < 0.001$).

Differences in behaviour were observed immediately after the female arrived on the seed head (Fig. 2 and Table 2). Thus, on Young Roblin, the transition that was exhibited most commonly was Arrival to Probe, whereas, on the two low-ranked heads, Old Roblin and Young Key, the most common transition was Arrival to Sit.

For some but not all behaviours, head type had significant effects on the proportions of females exhibiting the behaviour (Fig. 3 and Table 3). Thus, head type had significant effects on proportions of females exhibiting Probe, Insert and Oviposit but not on females exhibiting Sit or Walk. All females oviposited on high-ranked Young Roblin. On the two low-ranked heads, Young Key and Old Roblin, only 13% and 60% of females oviposited, respectively. However, when oviposition did occur on Young Key and Old Roblin, numbers of eggs were similar (mean \pm SE: 4.60 ± 0.93 and 5.20 ± 0.98 eggs per visit, respectively; $F_{1,19} = 0.11$, $P = 0.74$).

Seed head type also influenced when various behaviours were first exhibited by the female. Thus, latency to Sit (Fig. 4a and Table 3) was very short for the low-ranked types, Old Roblin and Young Key, but greater than 300 s for females visiting Young Roblin. A similar contrast was observed for Walk (Fig. 4b). Latency to Probe (Fig. 4c) was less than 50 s for all three head types and latency to Insert (Fig. 4d) was longer on Young Key than on Young or Old Roblin.

Behavioural transitions were also influenced by head type (Fig. 5 and Table 2). After exhibiting Sit (Fig. 5a) or Walk

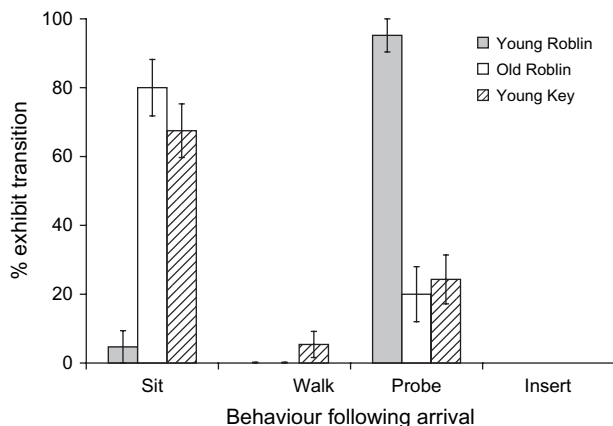


Fig. 2. Behaviour exhibited by wheat midge females immediately after arriving on the seed head. The effect of head type is shown in Table 2.

Table 2. Effects of seed head on behavioural transitions.

| First behaviour | Second behaviour | Seed head effect | | |
|-----------------|------------------|------------------|----------------|----------|
| | | d.f. | Test statistic | <i>P</i> |
| Arrive | Sit | – | 29.77 | < 0.001 |
| Arrive | Walk | – | 3.82 | 0.080 |
| Arrive | Probe | – | 33.94 | < 0.001 |
| Arrive | Insert | – | – | – |
| Sit | Walk | 2,72 | 14.12 | < 0.001 |
| Sit | Probe | 2,72 | 13.14 | < 0.001 |
| Sit | Insert | 2,72 | 1.54 | 0.210 |
| Walk | Sit | 2,68 | 6.57 | 0.003 |
| Walk | Probe | 2,68 | 5.58 | 0.006 |
| Walk | Insert | 2,68 | 1.75 | 0.180 |
| Probe | Sit | 2,65 | 9.23 | < 0.001 |
| Probe | Walk | 2,65 | 3.27 | 0.044 |
| Probe | Insert | 2,65 | 23.87 | < 0.001 |
| Insert | Sit | 2,49 | 1.89 | 0.162 |
| Insert | Walk | 2,49 | 1.26 | 0.293 |
| Insert | Probe | 2,49 | 2.70 | 0.077 |
| Sit | Depart | – | 0.48 | 0.832 |
| Walk | Depart | – | 1.53 | 0.463 |
| Probe | Depart | – | 1.42 | 0.570 |
| Insert | Depart | – | – | – |

The test statistic is the *F*-value from the analysis of variance, except for transitions after Arrival and preceding Depart, which were analysed by logistic regression. If there is no test statistic, the transition did not occur. d.f., degrees of freedom.

(Fig. 5b), females visiting Young Roblin were more likely to transition to Probe, whereas females visiting the two low-ranked head types were more likely to transition to Walk or Sit. Moreover, after exhibiting Probe (Fig. 5c), the most common behaviour for females visiting Young Roblin was Insert, whereas, for females visiting the two low-ranked head types, it was either Sit or Walk. Some transitions were not influenced by head type (e.g. for females on all three head types, the most common transition after Insert was Probe) (Fig. 5d and Table 2).

Females visiting the two low-ranked head types also showed some differences in behavioural transitions. Relative to females

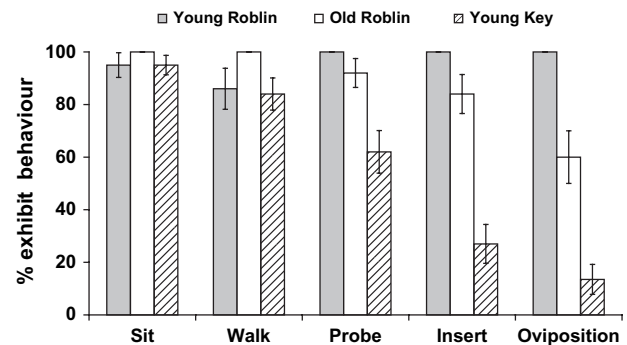


Fig. 3. Percentage of wheat midge females that exhibited each behaviour during visits to seed heads. The effect of head type is shown in Table 3.

Table 3. Effects of seed head on various behavioural measures.

| Measure | Behaviour | Seed head effect | | |
|------------------------------------|-------------|------------------|----------------|----------|
| | | d.f. | Test statistic | <i>P</i> |
| % Females exhibiting the behaviour | Sit | – | 1.34 | 0.607 |
| | Walk | – | 4.35 | 0.144 |
| | Probe | – | 15.06 | < 0.001 |
| | Insert | – | 37.00 | < 0.001 |
| | Oviposition | – | 40.51 | < 0.001 |
| Latency (s) | Sit | 2,80 | 34.70 | < 0.001 |
| | Walk | 2,79 | 10.04 | 0.001 |
| | Probe | 2,64 | 29.40 | < 0.001 |
| | Insert | 2,49 | 5.84 | 0.005 |
| % Total time | Sit | 2,80 | 42.68 | < 0.001 |
| | Walk | 2,80 | 13.05 | < 0.001 |
| | Probe | 2,80 | 21.34 | < 0.001 |
| | Insert | 2,80 | 57.52 | < 0.001 |
| Number of occurrences | Sit | 2,80 | 4.36 | 0.015 |
| | Walk | 2,80 | 6.90 | 0.002 |
| | Probe | 2,80 | 31.01 | < 0.001 |
| | Insert | 2,80 | 48.86 | < 0.001 |
| Duration (s) | Sit | 2,360 | 7.25 | 0.001 |
| | Walk | 2,388 | 1.29 | 0.277 |
| | Probe | 2,908 | 1.44 | 0.235 |
| | Insert | 2,424 | 4.07 | 0.017 |

The test statistic is the *F*-value from analysis of variance, except for percentages of females exhibiting the behaviour, which were analysed by logistic regression exact tests. d.f., degrees of freedom.

visiting Young Key, females visiting Old Roblin were more likely to exhibit Sit to Probe (Fig. 5a), Walk to Probe (Fig. 5b) and Probe to Insert (Fig. 5c). By contrast, females visiting Young Key were more likely to exhibit Sit to Walk (Fig. 5a), Walk to Sit (Fig. 5b) and Probe to Sit (Fig. 5c).

Head type influenced percentages of time devoted to the behaviour, as well as numbers of occurrences and durations (Fig. 6 and Table 3). Females visiting the two low-ranked heads devoted more time to Sit, whereas females visiting Young Roblin devoted more time to Probe and Insert (Fig. 6a). For total occurrences, a similar pattern was observed (Fig. 6b). The duration of Insert was influenced by head type (Table 3), with Insert durations on Young Key being half as long as those observed on Roblin, regardless of whether Roblin was young or old (Fig. 6c). Durations of some behaviours were not influenced by head type (e.g. the duration of each occurrence of Probe did not differ) (Fig. 6c).

Regardless of head type, the female's departure by flight was almost always preceded by the behaviour Sit (Fig. 7 and Table 2).

Discussion

The three seed head types, Young Roblin, Old Roblin and Young Key, are equally likely to be visited but, after the female lands on the seed head, elicit different behaviours. This can be seen in two simple measures of what the female does on

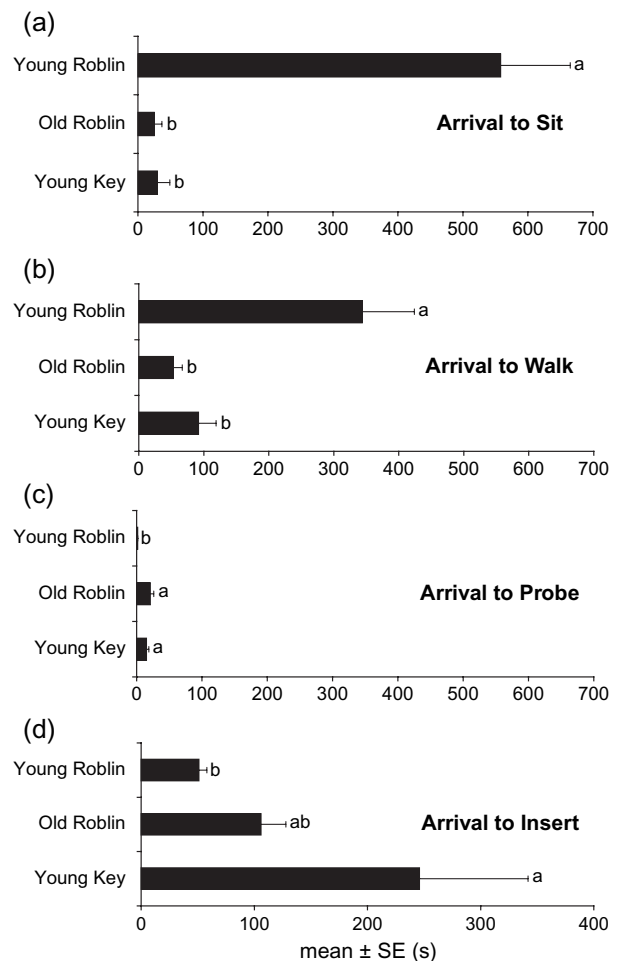


Fig. 4. Latency to four behaviours exhibited by wheat midge females on seed heads. The effect of head type is shown in Table 3. Means are significantly different if they do not share the same letter (Tukey–Kramer honestly significant difference test, *P* < 0.05).

the plant, namely the average length of the visit (Fig. 1a) and eggs deposited per visit (Fig. 1b), which together suggest a ranking of Young Roblin > Old Roblin > Young Key. Because both head type and the interaction between head type and visit length have significant effects on eggs per visit, it is clear that the number of eggs the female deposits is not entirely explained by how long the female stays on the seed head. Instead, the outcome of the female's visit (i.e. how many eggs she deposits) depends on the many behavioural decisions that are made before she departs from the plant.

These decisions begin when the wheat midge female first lands on the seed head. On the high-ranked Young Roblin, the first behaviour the female exhibits is almost always Probe (Fig. 2) but, on the two low-ranked types, this behaviour is almost always Sit (Fig. 2). Of the four behaviours (Table 1), Probe and Sit have the least in common. During sitting, sampling is presumably minimal, with only the tarsi making contact. By contrast, the female exhibiting Probe is constantly in motion, walking up, down and around the seed head,

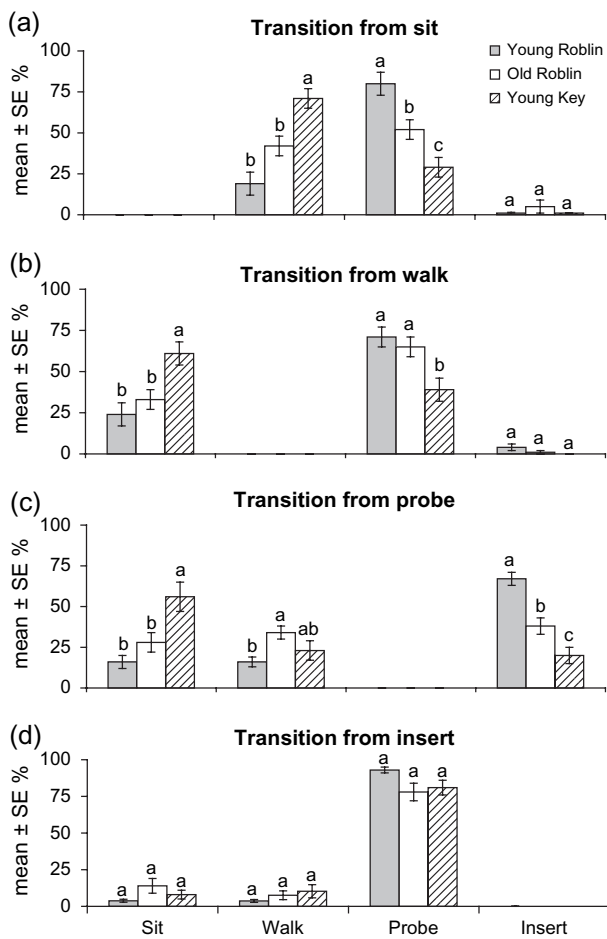


Fig. 5. Behavioural transitions exhibited by wheat midge females visiting seed heads, with transitions after Sit (a), Walk (b), Probe (c) and Insert (d), respectively. The effect of head type is shown in Table 2. Means are significantly different if they do not share the same letter (Tukey–Kramer honestly significant difference test, $P < 0.05$).

examining plant surfaces with the ovipositor and antennae (and perhaps also the tarsi). In cecidomyiids, surfaces at the tip of the antennae and ovipositor contain chemoreceptors and mechanoreceptors (Hallberg & Åhman, 1987; Solinas & Nuzzaci, 1987). The dramatic contrast in behaviour that occurs immediately after the female arrives on the seed head indicates that she is able to distinguish between high-ranked versus low-ranked seed heads in a matter of seconds.

How can this assessment occur so quickly? A possible answer comes from a study of another cecidomyiid, the Hessian fly (Harris *et al.*, 1993). In this case, assessment of the plant begins when the female is 'on the wing', with the tarsi, antennae and ovipositor touching the leaf surface for a brief moment when the female is still in flight, hovering near the plant. 'On the wing' assessment is also common in butterflies where it has been shown that, by touching the leaf surface with the forelegs, the female brings chemoreceptors in contact with relevant plant cues (Calvert, 1974). A decision to accept or reject the plant is made immediately thereafter, with rejection

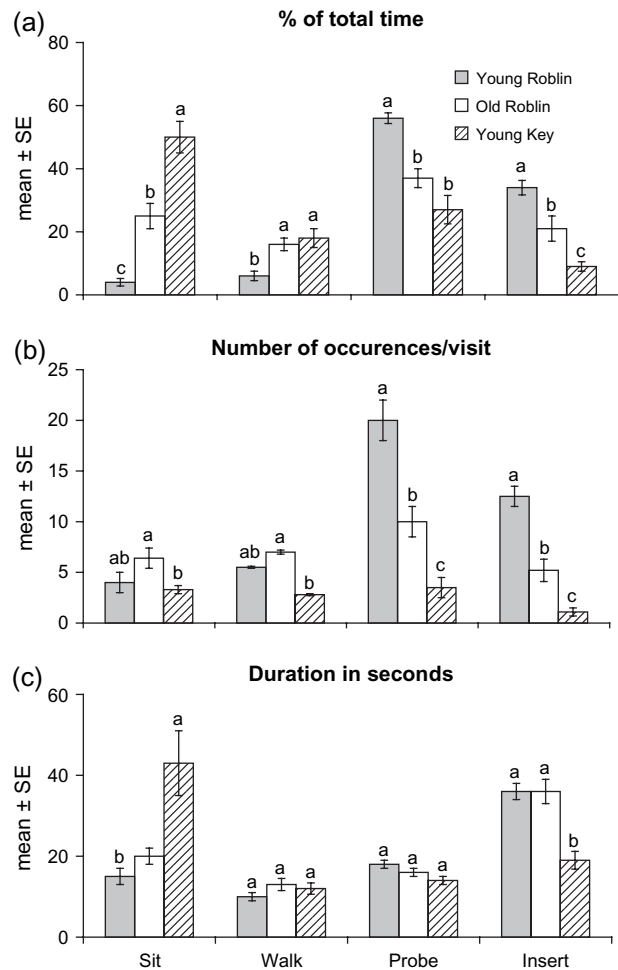


Fig. 6. For each wheat midge visit to a seed head, the proportion of time devoted to each behaviour (a), the number of occurrences of the behaviour (b), and the duration of each occurrence of the behaviour (c). The effect of head type is shown in Table 3. Means accompanied are significantly different if they do not share the same letter (Tukey–Kramer honestly significant difference test, $P < 0.05$).

signalled by rapid departure and no eggs left behind (Singer, 1982). This rejection at the early stages of plant assessment does not occur for the wheat midge. What the wheat midge female gains by visiting rather than rejecting low-ranked seed heads is presumably more information, with this occurring when the female eventually exhibits Probe approximately 30 s after arrival (Figs 3 and 4).

It is interesting that, once the female decides to exhibit Probe, the duration of the bout of Probe is not influenced by head type (Fig. 6c). This is a reminder that some aspects of host selection behaviour are stereotyped, with pattern generators in the central nervous system controlling not only how long the behaviour continues, but also the time interval between multiple bouts of the behaviour (Zupanc, 2004). Thus, when the wheat midge female remains on the seed head, bouts of examining behaviour may be triggered at regular intervals and run for a set time regardless of the information

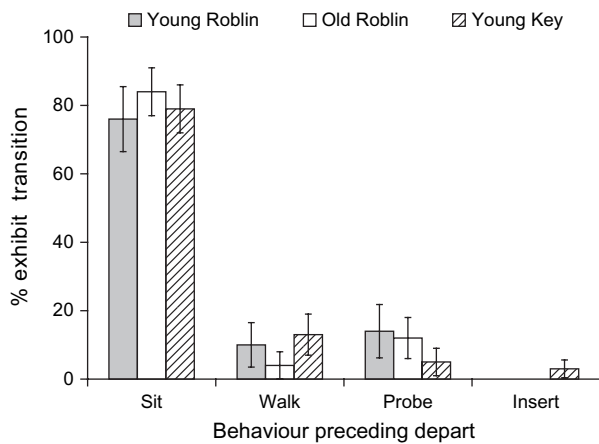


Fig. 7. Behaviour exhibited by wheat midge females immediately before departing from the seed head. The effect of head type is shown in Table 2.

that is gained during examining. Temporal patterning of host selection behaviour is documented for a number of insects (Jones, 1977; Withers & Harris, 1996) but how this impacts optimal foraging is considered only rarely (Janz *et al.*, 2005).

Although a small number of features of the behaviour of wheat midge female may be stereotyped, others depend on the type of seed head that the female visits. Here, the greatest contrast is for females on the high-ranked type versus females on the two low-ranked types. On high-ranked heads, much of the female's time is devoted to Probe and Insert (Fig. 6a), most behavioural transitions lead to Probe (Fig. 5) and Walk is the behaviour that is observed relatively late in the visit (Fig. 4). By contrast, on the two low-ranked types, much of the female's time is devoted to Walk and Sit (Fig. 6a), most behavioural transitions lead to Sit (Fig. 5) and the first occurrence of Probe occurs relatively late in the visit (Fig. 4). The female's decision to devote a lot of time to Sit is important because this is the behaviour that is most closely associated with rejection of the seed head (i.e. Sit precedes 75–80% of all departures by flight) (Fig. 7).

The contrast between females visiting the two low-ranked types is more subtle, with differences emerging slowly over the 5–10 min that females remain on the seed head. Based on visit length and eggs per visit (Fig. 1), Old Roblin ranks higher than Young Key, yet visits begin in the same way, with the female exhibiting Sit rather than Probe immediately after landing (Fig. 2). Differences emerge after this, however, with females on Young Key showing a more extreme form of inactivity, being less likely to exhibit Probe and Insert (Fig. 3) and more likely to exhibit transitions from active to inactive behaviours (Fig. 5). On Young Key, many females fail to exhibit Insert (Fig. 3). Those that do exhibit Insert require more time to do so (Fig. 4) and then only exhibit Insert for half as long as females on Old Roblin (Fig. 6c). This pattern of many small differences suggests that there is no one point in the behavioural sequence when all females detect a difference between Old Roblin and Young Key. Some

females detect a problem with Young Key relatively early on and never exhibit Probe (Fig. 3), whereas others reject Young Key only after they have exhibited Insert or oviposited a few eggs.

With respect to decision-making, behavioural transitions provide valuable insights and have the advantage of being relatively easy to score (Singer, 1986; Harris & Miller, 1991; Parr *et al.*, 1998; Hora & Roessingh, 1999). Each transition is based on both external inputs (e.g. sensory information about the plant) and internal inputs (e.g. egg load and the biological clock) (Harris & Foster, 1995) and therefore can be viewed as a 'mini-decision'. Each decision that keeps the female on the plant and actively examining surfaces contributes to the two ultimate decisions (i.e. whether to deposit eggs and how many eggs to deposit). A preponderance of transitions from high to low levels of activity (i.e. 'regressive' transitions) signals that the female will probably fly away from the plant before ovipositing, whereas a preponderance of transitions from low to high levels of activity (i.e. 'progressive' transitions) signals that at least some eggs will be deposited. Several other behavioural measures also signal the likely outcome of a visit (e.g. latency and duration and the proportion of time devoted to the behaviour). However, the tedious time-keeping required for these measures probably causes many scientists to avoid behavioural observations and instead choose egg counts, a measure from which behaviour can only be inferred.

The conclusion that the behaviour of the time-limited wheat midge is designed to thoroughly examine suboptimal resource patches is not consistent with the idea that such females should be less 'fussy' than longer-lived females (Abrahamson & Weis, 1997) but is consistent with optimal foraging theory (Krebs & Davies, 1996). The latter predicts that time-limited animals will thoroughly search a suboptimal resource because there may not be enough time to find better resources. The need to thoroughly consider the use of suboptimal hosts is particularly strong for the wheat midge and gall midges in general. The adult gall midge does not feed and therefore has limited resources to fuel flight (Gagné, 1989). Moreover, moving between plants or patches is not always easy. Females commonly fall prey to spiders during flight (Barnes, 1956) and controlled flight is only possible in essentially windless conditions (Withers & Harris, 1997). Given these problems, a low-level investment of time and eggs in suboptimal hosts by gall midges may be a more sensible decision than outright rejection.

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