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12 **The structure of feeding behavior in a phytophagous insect**
13 **(*Hylobius abietis*)**

14
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24
25 **Running head:** *Structure of insect feeding behavior*

26
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28 Coleoptera, Curculionidae, *Picea abies*, Pinaceae

29
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1 **Abstract**

2 Analysis of the feeding behavior of animals using such a high temporal resolution that meals
3 can be defined may improve our understanding of the mechanisms regulating feeding. Meals
4 can be distinguished in an ethologically meaningful manner by using the ‘meal criterion’, the
5 shortest non-feeding interval between feeding bouts recognized as meals. However, such a
6 criterion has only been determined for a few insect species. Applying a recent method
7 developed for assessing meal criteria for vertebrates, we determined the meal criterion for
8 *Hylobius abietis* (L.) (Coleoptera: Curculionidae) based on data from video recordings of
9 single individuals feeding on seedlings of Norway spruce, *Picea abies* (L.) Karst. (Pinaceae).
10 The pine weevil is an economically important pest insect because it feeds on the stem bark of
11 planted conifer seedlings. Weevils had 4-5 meals per day. Each meal lasted about 24 min
12 during which about 13 mm² of bark per meal were removed. Females had longer total meal
13 durations and longer non-feeding intervals within meals than males. Girdling seedlings did
14 not affect the weevils’ feeding properties. The size of meals was significantly correlated to
15 the duration of non-feeding intervals before and after them. This study is one of few
16 describing the feeding behavior of an insect at a temporal resolution that allows individual
17 meals to be distinguished. With more meal-related data from insects available, differences in
18 meal properties may be interpreted based on phylogeny, ecology, and physiology. Our results
19 may also assist in the setup and interpretation of studies of plant-insect interactions, and
20 facilitate the evaluation and development of methods to protect plants against herbivores.

21

1 **Introduction**

2 The food intake of herbivores is affected not only by their access to food, but also by the
3 handling time and the host plants' reactions to their feeding (e.g., Nuorteva, 1972; Clauss et
4 al., 2007; Zas et al., 2011). Thus knowledge about the feeding patterns of herbivores may
5 help to understand their interaction with plants, including the role of host plant resistance
6 (Mithöfer et al., 2005).

7 Studies on short-term feeding behavior, i.e., feeding patterns at the level of feeding
8 events and meals, are useful for investigating the mechanisms controlling limitations in food
9 acquisition and meal properties (Tolkamp et al., 2000; Howie et al., 2009). Feeding events,
10 such as bites into food, can be structured into discrete bouts of ingestion, so called meals,
11 which can be used as units in studies on feeding behavior (e.g., Simpson, 1981;
12 Raubenheimer & Bernays, 1993; Mitchell & Low, 1994; Bernays & Singer, 1998; Tomlin et
13 al., 1998). This facilitates comparisons of results obtained from multiple studies, as meals are
14 independent of the original unit in which they were measured (Howie et al., 2009). Generally,
15 there are numerous short non-feeding intervals during meals (intrameal intervals) and fewer
16 longer non-feeding intervals between meals (intermeal intervals) (e.g., Simpson, 1990). Thus,
17 meals can be distinguished in an ethologically meaningful manner by using the 'meal
18 criterion', the shortest non-feeding interval between feeding bouts recognized as meals
19 (Tolkamp et al., 2011), determined using the distribution of durations of non-feeding
20 intervals. This can also provide important information on pre- and post-prandial correlations,
21 i.e., the relationships between meal sizes and intermeal intervals before and after meals,
22 which are influenced by satiety and hunger, respectively (Le Magnen & Tallon, 1966;
23 Savory, 1981).

24 The short-term feeding behavior of about 10 insect species has been studied using
25 meal criteria (Bowdan, 1988a; Simpson et al., 1988, 1989; Raubenheimer & Bernays, 1993;
26 Bright et al., 1994; Mitchell & Low, 1994; Wright et al., 1999; Raubenheimer & Browne,
27 2000; Jones & Raubenheimer, 2002; Nagata & Nagasawa, 2006; Colasurdo et al., 2007).
28 These meal criteria were determined using log-survivorship and log-frequency distributions
29 of feeding events (e.g., Sibly et al., 1990; Mitchell & Low, 1994), underpinned by the
30 assumption that the events start at random times. However, it is not likely that feeding events
31 start at random times since the feeding motivation will be low directly after a meal, i.e. due to
32 satiety. Recently, a method based on normal or Weibull distributions has been developed for
33 vertebrates, which agrees better with the concept of satiety (Tolkamp et al., 1998, 2011). This

1 method has so far not been used with insects.

2 In this study, we investigated the short-term feeding behavior of the pine weevil,
3 *Hylobius abietis* (L.) (Coleoptera: Curculionidae), one of the most economically important
4 forest pests in Europe (Långström & Day, 2004). Adults feed on the tender bark of several
5 conifer species and may kill up to 90% of seedlings during the first 3 years after plantation
6 (Petersson & Örlander, 2003). Several aspects of its feeding behavior have been examined,
7 including the effects of tree species (Manlove et al., 1997; Månsson & Schlyter, 2004; Zas et
8 al., 2011, 2012), chemical composition of the bark and seedling fertilization (Wainhouse et
9 al., 2005; Zas et al., 2008; Sampedro et al., 2009), and various methods designed to reduce
10 the damage the weevil causes (Nordlander et al., 2009; Zas et al., 2014). However, the meal
11 criterion of this species has not been determined, and thus the opportunities to investigate the
12 mechanisms controlling the feeding pattern through studies of its short-term feeding behavior
13 has not been exploited. Therefore, we examined the short-term feeding behavior of the pine
14 weevil on seedlings in a controlled environment using video recordings. The aims of this
15 study were to (1) determine the meal criterion for the pine weevil using the new methodology
16 described above which takes the non-random temporal distribution of feeding initiation into
17 consideration, (2) determine fundamental parameters of feeding structure including how often
18 and how long they eat, and the amounts they eat per meal, (3) test if the sizes of pine weevil
19 meals are related (pre- and/or post-prandially) to intermeal intervals, (4) test if pine weevil
20 feeding patterns are influenced by previous injury to seedlings and/or their sex.

21

22 **Materials and methods**

23 **Insects and plant material**

24 Pine weevils were collected in central Sweden (Boda såg, Svärdsjö, 60°43' N, 015°52' E)
25 during spring migration a few weeks before the start of the experiment, then stored in
26 darkness at 10 °C and fed with branches of Scots pine [*Pinus sylvestris* L. (Pinaceae)]. Such
27 storage conditions interrupt the weevils' reproductive development and synchronize
28 oviposition to the following period, which is useful for experimental purposes because their
29 feeding increases during the oviposition period (Bylund et al., 2004). About a week before
30 their use in experiments, they were placed individually in Petri dishes (9 cm diameter)
31 together with small Scots pine twigs and a tube with water and transferred to a chamber
32 maintained at room temperature (22 °C) with an artificial light-dark cycle (L18:D6, not in
33 phase with the natural light cycle). Weevils that had eaten during this week were used in the

1 following experiments.

2 The plants used in the experiments were 1.5-year-old Norway spruce, *Picea abies* (L.)
3 Karst. (Pinaceae) seedlings obtained from a commercial nursery (Sjögränd, Bergvik Skog,
4 Falun, Sweden). They originated from seeds collected in a seed orchard and were intended
5 for planting in central Sweden. They were kept outdoors and transferred to the chamber
6 providing experimental conditions 1 day before use. They had a mean height of ca. 30 cm and
7 a lower stem diameter of ca. 0.4 cm.

8

9 **Set-up and experimental procedure**

10 The experiment was conducted at room temperature (22 °C) from June to September 2011.
11 Weevil behavior was observed in transparent plastic cylinders (8 cm high, 11 cm diameter)
12 surrounding the middle part of a seedlings' stem (Figure 1). This prevented the weevils from
13 accessing the stem bases and top shoots and thus offered a more homogeneous feeding source
14 (Kaakinen et al., 2004). To ensure high visibility of weevil activity, two video cameras
15 (Handycam HDR-XR550VE; Sony, Tokyo, Japan) were focused on the seedling with a 45°
16 angle between them. Light intensity of 59 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was used for light phase conditions
17 and 0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for dark phase conditions (measured by Sky 200 SKP; Skye Instruments,
18 Llandrindod Wells, UK). In addition, a red light was used during the dark phase to increase
19 recording visibility. The observation sessions were chosen to start 11 h after the start of the
20 light phase to temporally separate potential effects of disturbance when an observation
21 session was initiated from those of a change in light intensity. Thus, each observation session
22 started with 7 h of light phase, followed by a 6-h dark phase, and then another 11 h light
23 phase (i.e., a light-dark cycle of L18:D6).

24 To habituate the weevils to conditions in the cylinder, they were placed singly in it,
25 together with a seedling, 24 h before the start of each observation session. A tube with water
26 and a refuge made of a dark V-shaped metal plate (2.5 cm wide, 3.5 cm long, maximum
27 height 2.5 cm) were placed at the bottom of each cylinder. To avoid the effects of any feeding
28 scars, seedlings were exchanged with new ones after the habituation period.

29 Each weevil was observed in two sessions: once with a non-girdled seedling and once
30 with a seedling girdled by manually removing a 5-mm ring of outer and inner bark under the
31 first node 24 h prior to video recording. This kind of girdling mimics a type of pine weevil
32 damage that is often observed on planted seedlings (Eidmann, 1974). Half of the weevils of
33 each sex were placed in cylinders with girdled seedlings and the other half with non-girdled
34 seedlings during their first observation session. Weevil behavior was video-recorded for 24-h

1 sessions, after which the area of each final feeding scar, which could have resulted from
2 several meals, was measured. Between observation sessions, the weevils were placed for 48 h
3 in Petri dishes with pieces of Scots pine and a tube with water. In total 13 male and 12 female
4 weevils were video recorded. However, the short-term feeding behavior was only determined
5 for those six males and six females that fed from both types of seedlings and thereby a
6 balanced dataset was obtained.

7

8 **Analysis**

9 The start and end of every feeding event during each 24-h session of continuous observation
10 were noted by visual examination of the video-recordings (The Observer XT 10; Noldus
11 Information Technology, Wageningen, The Netherlands). Feeding was defined as
12 manipulation of bark or needles with the weevil's mouth parts. Video files were watched at
13 up to 16× normal speed, but during periods when the weevils were feeding the playback
14 speed was slowed, allowing recordings with a precision of 0.04 s. If an observation session
15 ended with a non-feeding period it was merged with the first non-feeding period of the same
16 session in the analysis to minimize underestimation of the length of non-feeding intervals and
17 represent the continuity between days. This minimizes a potential source of bias, as non-
18 feeding intervals are more likely to be cut off by the experimental design than feeding
19 intervals, due to their greater length and diel distribution (Fedderwitz et al., 2014). Non-
20 feeding intervals that were shorter than 1 s were excluded from all further analysis, as they
21 probably represented chewing or swallowing events that could not be detected on the video.

22

23 **Meal criterion**

24 The meal criterion was determined using the approach described in Tolkamp et al. (2011).
25 The durations of all non-feeding intervals were ln-transformed. Two population models with
26 combinations of Gaussian and 3-parameter Weibull distributions were used to find the best-fit
27 likelihood estimates for the model parameters (R version 2.12.1; The R Foundation for
28 Statistical Computing, Vienna, Austria). The model based on two Weibull distributions
29 provided the best fit to the data. In addition, meal criteria were determined separately for
30 girdled and non-girdled seedlings and for male and female weevils. We also performed a
31 bootstrap analysis (1 000 permutations) to obtain a 95% confidence interval for the meal
32 criterion based on the entire dataset.

33

34 **Meal properties**

1 Based on the calculated meal criterion for the entire dataset, meals were identified in the
2 observation sessions, thereby allowing the calculation of meal properties listed in Table 1.
3 Other feeding properties were also measured (listed in Table 1). For each property, linear
4 mixed-effects models were fitted using R software. Explanatory variables were weevil sex
5 and girdled/non-girdled seedlings. Weevil identity was used as a random factor to account for
6 between-individual differences. Models were reduced to only include weevil sex as a fixed
7 factor for calculating transformed mean values and standard errors.

8 9 **Prandial correlations**

10 Pre- and post-prandial Spearman's correlation coefficients were calculated using the entire
11 dataset. Then, to determine the effects of prandial correlations on the structure of feeding
12 events, the meal size and pre-prandial intervals were divided into three equally sized classes,
13 i.e., large, medium, and small meals and long, medium, and short pre-prandial intervals.

14 15 **Total feeding amount**

16 The differences between the amount of bark removed per observation session by pine weevils
17 on girdled and non-girdled seedlings were tested with a paired t-test (Minitab version 16.1.0;
18 Minitab, State College, PA, USA), as each individual fed on both a girdled and a non-girdled
19 seedling. To compare amounts removed by males and females, a two-sample t-test of
20 averages per individual was used.

21 22 **Feeding scar area**

23 It was not possible to measure the area of individual feeding scars made during most
24 individual meals because the scars were frequently connected to each other at the end of an
25 observation session. Therefore, in the following analyses, the sizes of individual meals were
26 estimated from the duration of the meals and the average feeding rate during an observation
27 session. The reliability of these estimations was supported by a non-significant Spearman's
28 correlation between feeding rate and the size of 17 individual meals for which it was possible
29 to measure the feeding scar area (median size = 15 mm², median duration = 25 min 38 s; $r_s =$
30 -0.03 , $P = 0.90$; $n = 17$; Minitab). The estimates were further supported by the non-significant
31 difference between the measured size of these 17 meals and their estimated sizes, based on
32 the duration of the meals and the average feeding rate per observation session ($t = 1.40$, d.f. =
33 16, $P = 0.18$).

34 A Spearman's correlation analysis was used to determine the correlation between

1 feeding scar area and meal duration based on the 17 meals for which it was possible to
2 measure the feeding scar area of individual meals.

3

4 **Movement after meals**

5 To evaluate the frequency and duration of time periods that weevils spent on the seedling in
6 relation to feeding we also recorded the duration of each interval between a pine weevil
7 climbing on and off the seedling without feeding, the time it spent on the seedling before
8 each meal, and the time it spent on the seedling after each meal until it either left the seedling
9 or started a new meal. The intervals between a meal and the weevil leaving the seedling or
10 starting to feed again, as well as the intervals between consecutive meals when weevils did
11 and did not leave the seedling, were compared using Mann-Whitney tests (Minitab).

12

13 **Results**

14 This study focused on the analysis of feeding patterns at such a high temporal resolution that
15 individual meals can be distinguished. However, results of feeding properties per 24-h
16 observation session are also presented but due to the low sample size the power to detect
17 differences is limited (Table 2).

18

19 **Meal criterion**

20 The meal criterion calculated using the entire dataset was 4 min and 8 s (95% CI: 2 min 8 s -
21 6 min 33 s; n = 1 205; Figure 2). The meal criteria for weevils on girdled seedlings (5 min 31
22 s; n = 621) and non-girdled seedlings (3 min 13 s; n = 584), as well as for males (3 min 54 s;
23 n = 362) and females (4 min 20 s; n = 843) all have overlapping 95% confidence intervals. It
24 was therefore concluded that the meal criterion based on the entire dataset was representative
25 for all subsets.

26

27 **Meal properties**

28 Girdling of the seedlings did not affect any of the measured meal or other feeding properties
29 and neither did the interaction of girdled/non-girdled seedlings and weevil sex ($P > 0.05$ for all
30 properties listed in Table 1). However, feeding patterns differed between males and females
31 (Figure 3). The total meal duration during an observation session was significantly shorter for
32 males than for females, and accordingly the total intermeal duration was longer (Table 2).

33 Furthermore, fewer feeding occurrences within meals were observed for males, and both the

1 average and total durations of their intrameal intervals were significantly shorter (Table 3).
2 No other meal or feeding properties significantly differed between the sexes (Tables 2 and 3).

3 Male weevils had about four meals per day, each lasting about 25 min (median
4 values; Tables 2 and 3). The intermeal intervals were about 6.5 h. During a meal by males,
5 seven intrameal intervals with a duration of 9 s each were observed and feeding occurrences
6 lasted slightly more than 2 min (all medians). Female weevils had about five meals per day,
7 each lasting about 22 min, with an intermeal interval of approximately 4 h (all medians).
8 There were 11 intrameal intervals, each lasting 13 s and the duration of their feeding
9 occurrence was slightly less than 2 min (all medians).

10

11 **Prandial correlations**

12 The pre- and post-prandial correlations were both significant ($r_s = 0.33$, $P < 0.001$ and $r_s =$
13 0.23 , $P = 0.01$, respectively; both $n = 117$). When the meals were grouped into three equally
14 sized classes according to the durations of the pre-prandial interval, the meals following
15 medium pre-prandial intervals were more similar in size to those following long pre-prandial
16 intervals than those following short pre-prandial intervals (16, 18, and 9 mm², respectively;
17 medians) (Figure 4A). When the post-prandial intervals were grouped into three equally sized
18 classes according to meal size, the post-prandial intervals following medium-sized meals
19 were more similar in duration to those following small meals than to those following large
20 meals (87, 64, and 160 min, respectively; medians) (Figure 4B).

21

22 **Total feeding amount**

23 Weevils removed 61 mm² of bark per observation session (median values based on means for
24 individuals; $n = 12$), corresponding to about 6% of the bark available in the cylinders. The
25 minimum amount of bark removed was 6 mm² and the maximum 114 mm². There was no
26 significant difference in the total area of feeding scars between girdled and non-girdled
27 seedlings (55.9 ± 8.6 and 59.5 ± 7.4 mm², respectively; $t = -0.32$, d.f. = 11, $P = 0.76$) nor
28 between male and female weevils (49.2 ± 5.8 vs. 66.3 ± 8.7 mm², respectively; $t = 1.64$, d.f. =
29 8, $P = 0.14$).

30

31 **Feeding scar area**

32 The feeding of a pine weevil typically resulted in separate feeding scars that were produced
33 during one or several meals. By the end of the observation sessions the median area of
34 feeding scars made by males was 23 mm², very similar to the median area of scars known to

1 be the result of a single male meal (21 mm^2) and the calculated median male meal size
2 (median meal duration \times median feeding rate = 22 mm^2). In contrast, the median feeding scar
3 made by females by the end of an observation session (31 mm^2) was substantially larger than
4 the median area of scars known to be the result of a single female meal (14 mm^2) and the
5 calculated median female meal size (median meal duration \times median feeding rate = 14 mm^2).
6 There was a strong positive correlation between feeding scar area and meal duration (based
7 on data from scars known to be the result of a single meal by the end of an observation
8 period: $r_s = 0.63$, $P = 0.005$; $n = 17$).

9

10 **Movement after meals**

11 After 17% of the meals, the weevils did not leave the seedling before starting another meal.
12 The time between meals was shorter when weevils did not leave the seedling before eating
13 another meal than when they did [median: 32 (n = 19) vs. 119 min (n = 74); $W = 3\,818$,
14 $P < 0.01$]. Weevils spent 32 min on seedlings after a meal when they did not leave the seedling
15 before taking another meal and only 49 s when they left the seedling before the next meal (W
16 = 4 553, $P < 0.01$; medians). Four meals were excluded from this analysis, because the weevils
17 stayed on the seedling after these meals until the observation session ended (median: 32 min
18 29 s).

19

20 **Discussion**

21 In most studies of short-term feeding behavior, log-survivorship or log-frequency analysis
22 has been used to determine meal criteria (e.g., Sibly et al., 1990; Mitchell & Low, 1994;
23 Tolkamp et al., 1998; Tolkamp et al., 2011). However, in recent years an approach taking the
24 non-random initiation of feeding times into consideration has been developed, thereby
25 providing a more biologically meaningful definition of a meal (Tolkamp et al., 2011). This
26 may facilitate comparisons between distantly related chewing herbivores, as the underlying
27 triggers of hunger and satiety are the same when food access is unlimited. Previously this
28 method has only been used to analyze the feeding behavior of vertebrates (Tolkamp et al.,
29 2011). Here we extend the methodology by applying it to calculate the meal criterion for a
30 phytophagous chewing insect, the pine weevil.

31 The estimated meal criterion for the pine weevil (4 min 8 s) is in the lower range of
32 meal criteria reported for other phytophagous insects, which include approximately 2 min for
33 the fifth instars of the tobacco hornworm caterpillar [*Manduca sexta* (L.)] and the larvae of

1 the silkworm (*Bombyx mori* L.), 3 min for the German cockroach [*Blattella germanica* (L.)],
2 4 min for the caterpillar of the cotton bollworm [*Helicoverpa armigera* (Hübner)], 5 min for
3 the Colorado potato beetle [*Leptinotarsa decemlineata* (Say)], over 6 min for the migratory
4 locust [*Locusta migratoria* (L.)], 8 min for the western horse lubber grasshopper [*Taeniopoda*
5 *eques* (Burmeister)], 2-9 min for the first instar of the American bird grasshopper
6 [*Schistocerca americana* (Drury)], about 12 min for the plains lubber grasshopper
7 [*Brachystola magna* (Girard)], and up to about 16 min for the forest tent caterpillar
8 (*Malacosoma disstria* Hübner) (Simpson, 1982; Reynolds et al., 1986; Chapman & Beerling,
9 1990; Raubenheimer & Bernays, 1993; Bright et al., 1994; Mitchell & Low, 1994;
10 Raubenheimer & Browne, 2000; Jones & Raubenheimer, 2002; Nagata & Nagasawa, 2006;
11 Colasurdo et al., 2007). However, all these meal criteria were estimated from log-
12 survivorship analyses, which generally result in shorter estimated meal criteria, e.g., the meal
13 criterion for cows increases from about 8 min to 35 min when estimated with a log-normal
14 distribution instead of a log-survivorship analysis (Tolkamp & Kyriazakis, 1999).

15 We observed that some of the pine weevil meals did not have any intrameal intervals
16 (16 out of 117 meals). Wright et al. (1999) observed no intrameal intervals in meals of the
17 Costa Rican weevil [*Exopthalmus jekelianus* (White)]. The Colorado potato beetle did not
18 have any intrameal intervals in approximately half of its meals (Mitchell & Low, 1994),
19 whereas Chapman & Beerling (1990) observed intrameal intervals in all recorded meals of
20 the first instar of the American bird grasshopper. These differences in intrameal structures are
21 interesting, but more research is needed before this variation can be understood.

22 The pine weevil had about four meals per day, relatively few compared to other
23 frequencies recorded for insect species: about 1.4 meals in 5 h for the German cockroach
24 (Jones & Raubenheimer, 2002), five in 10 h for the Costa Rican weevil (Wright et al., 1999),
25 10 per day for the adult migratory locust (Simpson, 1982), 7-9 per day for advanced first
26 instars of the American bird grasshopper (Chapman & Beerling, 1990), about six in 7 h for
27 cotton bollworm caterpillars based on meal and intermeal durations (Raubenheimer &
28 Browne, 2000), nine in 7 h for the Colorado potato beetle (Mitchell & Low, 1994), two per
29 hour for tobacco hornworm caterpillars based on meal and intermeal durations (Reynolds et
30 al., 1986), and up to 163 meals in 48 h for the forest tent caterpillar (Colasurdo et al., 2007).

31 The meals of the pine weevil lasted about 24 min, on average, close to the 20 min
32 recorded for the Colorado potato beetle (Mitchell & Low, 1994) and the 33 min for the forest
33 tent caterpillar on a balanced artificial diet (Colasurdo et al., 2007), but substantially longer
34 than the recorded durations (2-18 min) for meals of Costa Rican weevil, the first instar of

1 American bird grasshopper, tobacco hornworm caterpillar, the caterpillar of the cotton
2 bollworm, the larvae of the silkworm, German cockroach, and fifth instar migratory locusts
3 (Simpson, 1982; Reynolds et al., 1986; Chapman & Beerling, 1990; Wright et al., 1999;
4 Raubenheimer & Browne, 2000; Jones & Raubenheimer, 2002; Nagata & Nagasawa, 2006).
5 In total, female pine weevils spent more time in meals than male pine weevils, which is in
6 accordance with previous studies (Merivee et al., 1998; Bylund et al., 2004; Toivonen &
7 Viiri, 2006). No such differences between the sexes were observed in the Colorado potato
8 beetle (Szentesi & Jermy, 1993).

9 Both feeding rate and meal duration can influence meal size. Whether the main
10 factors governing the termination of meals are the amount ingested or the time spent feeding
11 (in the absence of interruptions by external agents, e.g., predators) can be determined from
12 analyses of variations in feeding rates and meal sizes. For example, meals of the migratory
13 locust are terminated after a certain time rather than after a certain amount has been ingested
14 (Simpson, 1982). We calculated the meal sizes of pine weevils from their durations, which
15 makes it more difficult to draw any such conclusions, but it was possible to determine the
16 feeding rate directly for 17 out of 117 meals (both groups with similar median duration). Data
17 for these 17 meals indicate that meal size is correlated linearly to feeding duration, but meal
18 size is not correlated to feeding rate. Previous studies have found that female weevils have a
19 higher feeding rate (Wainhouse et al., 2004) and make smaller feeding scars than males
20 (Bylund et al., 2004; Toivonen & Viiri, 2006). However, neither was observed in the present
21 study, indeed there were indications of the opposite. Like the pine weevil, feeding rate of the
22 Colorado potato beetle appears to be roughly constant for small meals, but for larger meals it
23 is substantially more variable (Mitchell & Low, 1994). The migratory locust, first instars of
24 the American bird grasshopper, and the Australian sheep blowfly [*Lucilia cuprina*
25 (Wiedemann)] also have variable feeding rates and quite constant meal durations (Simpson et
26 al., 1988; Simpson et al., 1989; Chapman & Beerling, 1990). The feeding rates of these three
27 insects per feeding occurrence do not increase with meal size – except when the migratory
28 locust feeds very large meals –, but the overall feeding rate per meal increases, due to
29 increases in the proportion of time spent feeding per meal and therewith a decrease of the
30 time spent in intrameals (Simpson et al., 1988; Chapman & Beerling, 1990). In the Australian
31 sheep blowfly, the feeding rate on food with a better quality is increased due to more time
32 spent feeding within a meal (Simpson et al., 1989). Similarly, forest tent caterpillars increase
33 the time spent feeding within a meal on protein-rich food (Colasurdo et al., 2007). Thus,
34 several factors can influence interactions between feeding rate, meal duration, and the

1 resulting meal size. For example, the insect's physiology influences how fast it can obtain
2 and process food, and the composition of available food, such as deterrent or toxin contents,
3 can profoundly affect meal durations or feeding rate (Szentesi & Bernays, 1984; Usher et al.,
4 1988; Glendinning & Slansky, 1995; Jones & Raubenheimer, 2002).

5 Prandial correlations provide indications of the mechanisms underlying the initiation
6 and termination of meals and predictions of both meal sizes (from durations of preceding
7 non-feeding intervals) and durations of following non-feeding intervals (from meal sizes).
8 Both pre-prandial and post-prandial correlations were significant for the pine weevil. Meals
9 were much smaller after short pre-prandial intervals than after medium or long pre-prandial
10 intervals. Similarly, post-prandial intervals were much shorter after small and medium-sized
11 meals than after large meals. Both types of correlation are also significant for Colorado
12 potato beetle, migratory locust, and cotton bollworm (Simpson, 1982; Simpson & Ludlow,
13 1986; Mitchell & Low, 1994; Raubenheimer & Browne, 2000). In contrast, Costa Rican
14 weevils have a significant post-prandial correlation, but no pre-prandial correlation (Wright et
15 al., 1999), whereas the opposite pattern has been observed for Australian sheep blowfly,
16 tobacco hornworm, and American bird grasshopper (Reynolds et al., 1986; Bowdan, 1988a,b
17 Simpson et al., 1989; Chapman & Beerling, 1990).

18 The pine weevils created one or several new feeding scars and sometimes returned to
19 old scars during a meal. To estimate how many meals were needed to make a median-sized
20 feeding scar, we compared the areas of feeding scars known to be the result of a single meal,
21 the areas of feeding scars measured at the end of an observation session, and the areas of
22 feeding scars calculated from the median meal duration and median feeding rate. There was
23 good agreement between these estimates for scars made by males, thus most feeding scars
24 made by males had a similar area as those known to be the result of a single meal. Female
25 weevils, however, required 2.2 meals to create a median sized feeding scar. Consequently, the
26 number of feeding scars made by a male weevil after 24 h could be used as a proxy for the
27 number of meals, whereas it is more complicated for female weevils.

28 Most pine weevils that did not stay on the seedling to eat another meal left the
29 seedling shortly after each meal was finished. Additionally, weevils that stayed on the
30 seedling fed again sooner than those that left. These differences in behaviors may reflect a
31 conflict between increased opportunities to meet a mate when staying on the seedling and the
32 higher energy use when leaving the seedling.

33 None of the examined meal or other feeding properties significantly differed between
34 weevils on girdled vs. non-girdled seedlings. Girdling of a seedling may affect food quality

1 and therewith feeding patterns. Accordingly, the food quality (glucose concentration)
2 negatively affects the size and frequency of meals, and intrameal durations in the Australian
3 sheep blowfly (Simpson et al., 1989). The time interval between the first and second
4 wounding chosen in the present study might not have been long enough to reach peak levels
5 of induced defenses (Lewinsohn et al., 1991), but represents the naturally occurring situation
6 for seedlings planted on clear cuts (Nordlander, 1991; Björklund et al., 2005), which might
7 explain why no differences in feeding patterns were found.

8 Establishing meal criteria provides opportunities to explore short-term feeding patterns at
9 high resolution, rather than merely examining the end results of feeding. Meal criteria have
10 only been determined for a few insect species as yet, but the accumulation of data for more
11 species should facilitate conclusions about differences in meal properties based on
12 phylogeny, ecology, and physiology. In this study, we determined the meal criterion for the
13 pine weevil with a method originally developed for vertebrates, thereby validating its use for
14 invertebrates. Basic knowledge about feeding patterns could also assist in the setup and
15 interpretation of studies of herbivore-plant interactions, and it may facilitate the evaluation
16 and development of methods to protect plants against herbivores.

17

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25

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4

5

6

1 **Figure captions**

2 **Figure 1** Experimental setup. A small tube with water and a dark-colored refuge for the pine
3 weevils inside the cylinder are not shown.

4

5 **Figure 2** Distribution of non-feeding intervals ($n = 1\,205$) in all pine weevil observation
6 sessions and fitted model for two Weibull distributions. The arrow indicates the intersection
7 and thus the location of the meal criterion.

8

9 **Figure 3** Schematic feeding pattern of male and female pine weevils during 24 h; medians
10 based on mean values for individuals ($*P < 0.05$ between sexes; linear mixed-effects models)
11 (see also Tables 2 and 3).

12

13 **Figure 4** (A) Pre-prandial and (B) post-prandial correlations. Pre-prandial correlation based
14 on the distribution of pine weevils' meal size (bin width 5 mm^2 , $n = 117$) and post-prandial
15 correlation based on the distribution of intermeal intervals after a meal (bin width 30 min, $n =$
16 117). Data were split into groups of equal sizes based on intermeal intervals before the meal
17 for pre-prandial correlations and based on meal size for post-prandial correlations. Dashed
18 lines represent median values.

19

1 **Table 1** Definition of meal and other feeding properties

Feeding/meal property	Unit	Definition
No. meals		No. all meals during one observation session
Total meal duration	h	Summed durations of all meals during one observation session
No. intermeal intervals		No. all intermeal intervals (i.e., non-feeding intervals between meals) during one observation session
Total duration of intermeal intervals	h	Summed durations of all intermeal intervals during one observation session
Total area eaten	mm ²	Area of bark removed during one observation session
Feeding scar area	mm ²	Area of individual feeding scars after an observation session
Feeding rate	mm ² s ⁻¹	Amount of bark removed per unit time (see text for details)
Meal duration	s	Duration of a single meal including feeding and non-feeding intrameal intervals
Intermeal interval duration	min	Duration of a single intermeal interval
Feeding amount	mm ²	Area of bark removed during one meal (meal duration × feeding rate)
Feeding percentage	%	Percentage of time spent feeding within a meal
No. feeding occurrences		No. single feeding occurrences per meal separated by intrameals
Total feeding within a meal	min	Duration of all feeding within one meal
Duration of feeding occurrence	min	Duration of a single feeding occurrence
No. intrameals		No. intrameal intervals during one meal
Total duration of intrameals	min	Duration of all intrameal intervals during one meal
Duration of intrameal	min	Duration of one intrameal interval

2

3

1 **Table 2** Meal and other feeding properties for male and female pine weevils (per 24-h
 2 observation session). Medians are based on untransformed means per individual. Grand
 3 means are based on transformed data (Mean^T)

Feeding/meal property	Median		Mean ^T ± SE		t (d.f. = 10) ¹	P
	Male	Female	Male	Female		
No. meals	3.50	5.25	1.32 ± 0.20 ²	1.78 ± 0.17 ²	1.75	0.11
Total meal duration (min)	64.59	102.78	7.73 ± 0.84 ³	10.84 ± 0.84 ³	2.62	0.03
No. intermeal intervals ⁴	3.50	5.25	1.56 ± 0.16 ²	1.93 ± 0.14 ²	1.73	0.12
Total duration of intermeal intervals (h)	22.70	21.93	22.66 ± 0.26	21.64 ± 0.26	2.74	0.02
Total area eaten (mm ²)	41.75	66.25	49.17 ± 7.61	66.25 ± 7.61	1.59	0.14
Feeding scar area (mm ²)	23.18	31.33	2.96 ± 0.19 ²	3.27 ± 0.19 ²	1.16	0.27
Feeding rate ⁵ (mm ² /min)	0.90	0.62	1.15 ± 0.19 ³	0.75 ± 0.19 ³	1.46	0.18

4 ¹Linear mixed-effects models.

5 ²ln-transformation was used to meet normality and homoscedasticity requirements.

6 ³Square-root transformation was used to meet normality and homoscedasticity requirements.

7 ⁴The first and last intermeal per observation were combined to minimize underestimation of
 8 the length of non-feeding intervals and represent continuity between days.

9 ⁵One observation session was excluded because the calculated feeding rate (16.33 mm²/min)
 10 was considered to be an outlier.

11

1 **Table 3** Meal properties for male and female pine weevils (per meal). Medians are based on
 2 untransformed means per individual. Grand means are based on transformed data data
 3 (Mean^T)

Meal property	Median		Mean ^T ± SE		t (d.f. = 10) ¹	P
	Male	Female	Male	Female		
Meal duration (min)	24.58	22.42	3.97 ± 0.43 ³	4.21 ± 0.39 ³	0.42	0.69
Intermeal interval duration ⁴ (min)	393.51	258.49	5.23 ± 0.31 ²	4.75 ± 0.28 ²	1.15	0.28
Feeding amount (mm ²)	13.81	11.75	3.44 ± 0.38 ³	3.15 ± 0.36 ³	0.55	0.60
Feeding percentage (%)	94.51	89.01	7.54 ± 0.28 ³	7.01 ± 0.23 ³	1.44	0.18
No. feeding occurrences	8.04	12.33	2.00 ± 0.11 ²	2.33 ± 0.09 ²	2.39	0.04
Total feeding within a meal (min)	23.17	19.48	3.81 ± 0.44 ³	3.96 ± 0.41 ³	0.24	0.82
Duration of feeding occurrence (min)	2.21	1.71	0.09 ± 0.17 ²	-0.22 ± 0.16 ²	1.34	0.21
No. intrameals	7.04	11.35	1.80 ± 0.13 ²	2.17 ± 0.11 ²	2.14	0.06
Total duration of intrameals (min)	1.27	2.75	0.92 ± 0.11 ³	1.33 ± 0.09 ³	2.80	0.02
Duration of intrameal (s)	9.23	13.31	1.67 ± 0.08 ^b	1.93 ± 0.08 ^b	2.56	0.03

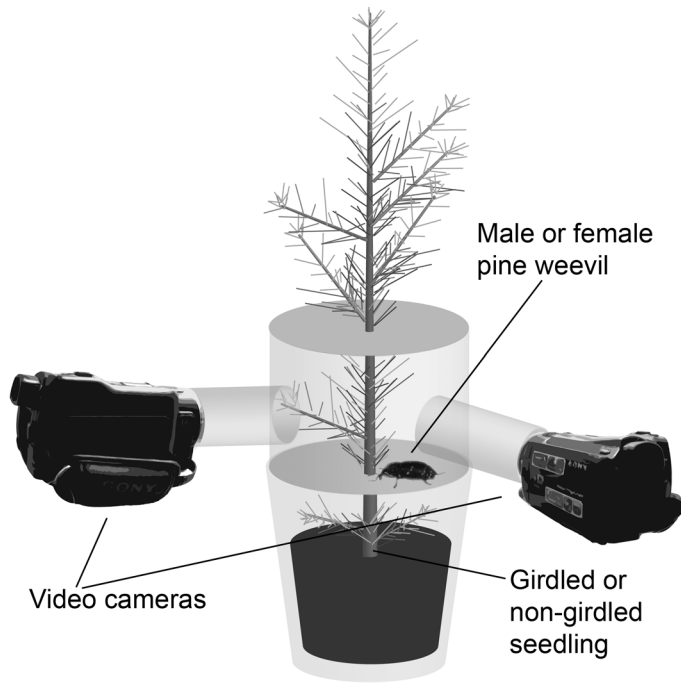
4 ¹Linear mixed-effects models.

5 ²ln-transformation was used to meet normality and homoscedasticity requirements.

6 ³Square-root transformation was used to meet normality and homoscedasticity requirements.

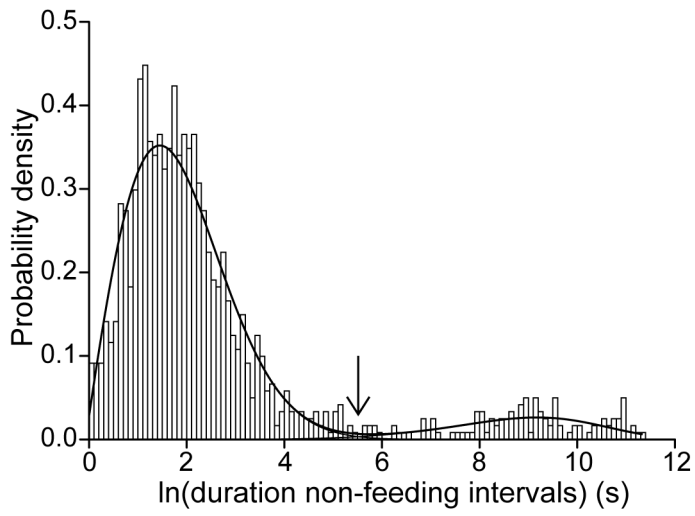
7 ⁴The first and last intermeal per observation were combined to minimize underestimation of
 8 the length of non-feeding intervals and represent continuity between days.

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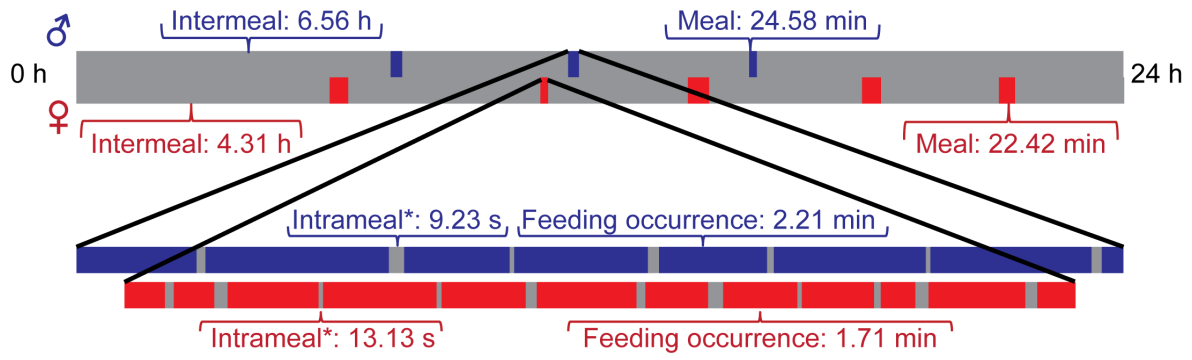
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Figure 1

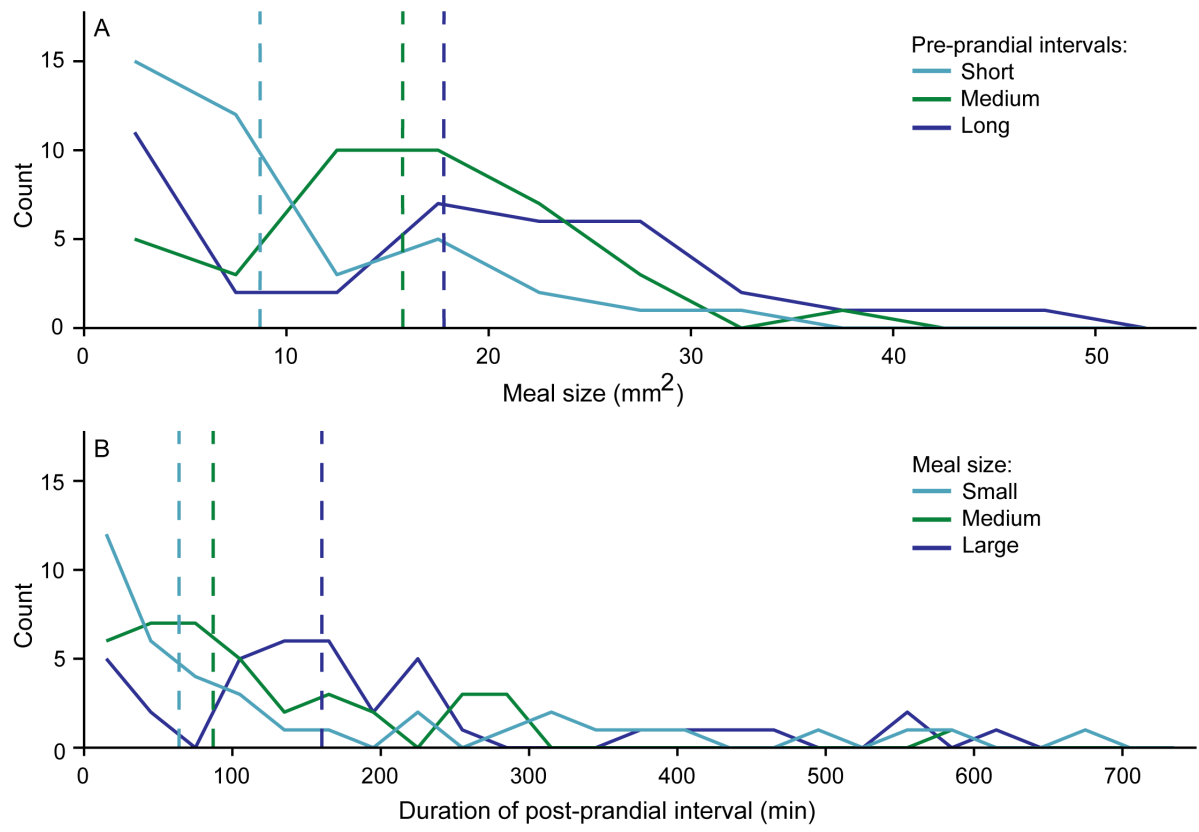


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Figure 2



1
2 Figure 3



5
6 Figure 4