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PATTERN AND PROCESS IN INSECT FEEDING BEHAVIOUR: A QUANTITATIVE ANALYSIS OF THE MEXICAN BEAN BEETLE, *EPILACHNA VARIVESTIS*

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No-choice feeding trials and automatic feeding detector studies were used in a quantitative analysis of the feeding behaviour of adult Mexican bean beetles on their host-plant. Different chemical stimuli and physiological factors gave clearly distinguishable patterns in the feeding process. Compared to controls: age had no marked effect; starvation synchronised and reduced the time of initiating feeding, increased consumption rates, increased feeding duration, but did not alter the number of meals; sucrose increased consumption rates over the same feeding duration as controls, and reduced the number of meals; phenolic compounds decreased consumption rates, feeding durations and number of meals. The methodology produces quantitative indices of specific feeding behaviours from the measurement of cumulative consumption/time data, while being sufficiently rapid for screening plant materials and chemicals. The methods are considered generally applicable to laboratory bioassays with phytophagous insects, and are being used to generate a predictive model for the Mexican bean beetle.

Behavioural components of insect feeding are of considerable importance in understanding the chemical bases of host-plant selection, preference, and resistance (Maxwell, 1977). Laboratory feeding trials measuring differential consumption at the end of a fixed time period permit investigators to rapidly screen plant materials and chemicals in a controlled situation (see Hedin *et al.*, 1974; Cook, 1976; Schreck, 1977; Smith, 1978), but provide little or no specific behavioural information, and rarely take account of individual variation in feeding behaviour (Schoonhoven, 1977a). There is an increasing need for refined bioassay techniques that elucidate the different aspects of discrimination (Maxwell, 1977; Smith, 1978).

This paper demonstrates how a generally applicable bioassay methodology can provide quantitative indices of specific feeding behaviours, while being sufficiently rapid for use in screening plants and chemicals. Our "test-case" insect was the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera; Coccinellidae). This insect is easy to rear and has been extensively studied (List, 1921; Thomas, 1924; Howard, 1941; Augustine, 1962; Lapidus *et al.*, 1963; Nayar & Fraenkel, 1963; Augustine *et al.*, 1964; Kogan, 1972, 1973; Smith *et al.*, 1979). Ageing, starvation, sucrose, and phenolics were used as examples of different physiological states and chemical stimuli whose effects can be described in detail and can be distinguished from each other, even if they have the same net effect on total consumption.

METHODS AND MATERIALS

Rearing of E. varivestis. Eggs from stock at the N.Y. State Agricultural Experiment Station, Geneva, N.Y., were reared in a cage on 10-day-old seedlings of *Phaseolus vulgaris* var. N.Y. State Light Red #1 kidney bean, replaced every 3 days. The culture was maintained in a growth room at 28 ± 2°, 70 ± 10% on a 16 hr photoperiod at 16 k lux. Beans were grown in vermiculite/potting soil (1:1) in a greenhouse.

No-choice feeding trials were based on the method of Jones & Firn (1978). Arenas were 1.5 cm × 5.0 cm diam Petri dishes with lids, lined with wet filter paper. Each arena contained a centrally placed, inverted, 1.5 cm diam control or treated bean leaf disc (26.1 ± 1.3 mg, 0.45 ± 0.05 mm thick) punched from the inter-vein areas of 10-day-old leaves. Treated discs were dipped for 0.5 sec in ethanolic solutions of test chemicals, or appropriate

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control solvents, and air-dried. Discs remained turgid for over 24 hr in the humid environment of the arena. A single adult (sexes were not distinguished in these experiments) was introduced into a minimum of 10 each of replicated control and experimental arenas. Feeding trials were carried out in the same conditions as for rearing.

No-choice trial treatments. Age trials used unstarved adults between 1 and 17 days old, 10 replicate adults per age class with untreated leaf discs. Starvation trials used 2—4-day-old adults starved from 0 to 72 hr, with 10 replicate control (unstarved) and experimental adults per starvation time class, and untreated leaf discs. Adults were removed from the host-plant at pre-determined times, and kept in ventilated plastic boxes lined with wet filter paper containing a Petri dish filled with wet cotton. This minimised insect dehydration and did permit the insects to drink from the wet cotton. Sucrose trials used 2—4-day-old unstarved adults with 39 replicates each of control and experimental arenas. Discs were dipped in 5×10^{-2}M sucrose in ethanol/water (1:1). Control discs were dipped in ethanol/water (1:1). Trials with phenolic compounds used 2—4-day-old unstarved adults, with 10 replicates of each control and experimental arena for each chemical. All experimental discs were dipped in 8×10^{-2}M ethanolic solutions of chemicals, and control discs dipped in ethanol. A large number of phenolic compounds is being used to investigate the relationship between chemical structure and feeding inhibitor activity (Jones, C. G., Hoggard, M. P., Blum, M. S.; unpubl. data). Forty-five of these compounds, 15 from each of low, medium and high activity categories, were selected for detailed data analysis. Selection was based on a gradient of C/E ratio values (see later), from over 200 compounds that have been tested. These 45 compounds included hydroxy-, methoxy-, chloro-, and bromo-substituted benzyl alcohols, benzonitriles, benzamides, benzenes, phenyl acetates, phenylacetaldehydes, phenylacetic acids, cinnamic acids and cinnamamides.

Data recording. Visual estimation of the % leaf disc area marked by feeding ridges (hereafter called area consumed) was recorded at regular time intervals of 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 22 and 24 hr using a tape recorder. Photographic enlargements of a random sample of discs measured on mm^2 grids showed that visual estimates were accurate to ± 5% when 10—90% of the disc was consumed and ± 3% when either 0—10% or 90—100% of the disc was consumed.

Data analysis. Cumulative % of area consumed of each disc with time were arcsined, means and 95% intervals computed, and retransformed to %. From these data were calculated:

1. Mean cumulative area — the area under the mean consumption/time curve for the entire population of any treatment or control including insects not consuming food. Areas under the two 95% confidence interval curves were also computed (units are arbitrary and comparative).
2. C/E ratios — the ratio of control mean cumulative area divided by the experimental mean cumulative area, for a population of control and treatment. When the treatment had no effect $C/E \simeq 1$; if it increased consumption then $C/E < 1$; if it decreased consumption then $C/E > 1$. Results were considered significant when the cumulative areas of the 95% confidence intervals did not overlap (see Jones & Firn, 1978).
3. Mean total consumption after 24 hr, again including insects that did not consume food.
4. The time at which each individual insect initiated feeding was recorded from the cumulative consumption records for that individual (i.e., the time at which consumption $\geq 3\%$ was first observed), and the values from each individual were used to calculate mean time of initiating feeding, the frequency distribution of time of initiating feeding for a population of insects, and the time at which 50% of individuals had initiated feeding.
5. For the period 0—8 hr into the trial, during which measurement of consumption was made at least hourly, the cumulative % consumption/time data for each individual was broken down into observation intervals during which consumption had increased (intervals within which feeding occurred — hereafter termed intervals with feeding — IWF) or remained constant (intervals within which no feeding occurred — intervals with no feeding —IWNF). The total number and total duration of these intervals were recorded for each insect, as was the amount consumed in each IWF. Values for individuals in each treatment or control were pooled to determine the frequency distributions of these intervals, the mean consumption rates, in IWF (assuming that feeding rates were constant during an interval), and the ratio

of mean total duration of IWF to the mean total duration of IWNF. While these parameters are not an exact measure of the number, size and duration of actual meals (because each observation period could have contained a number of meals), they are useful quantitative measures of feeding events that can be determined easily and do show significant differences between treatments.

Automatic feeding detector (AFD) trials. Responses of adults to treated and untreated discs were monitored with an AFD (Jones, 1979). Movements of a leaf disc attached to a trembler were recorded as amplified signals from a linear differential transducer. Data were used to check behavioural parameters derived from no-choice trials.

RESULTS

Feeding behaviour of control insects. The mean cumulative consumption/time curve for 2—4-day-old unstarved Mexican bean beetle (MBB) adults was sigmoidal over 24 hr (Fig. 1). There was a short lag which primarily resulted from time spent in search for, orientation to, encounter with, and test-biting of the disc. Active MBB adults encountered the disc within 5—10 min, but some individuals remained relatively sessile for 2—3 hr or longer. These individuals delayed initiation of feeding, contributed markedly to the large standard deviation about the mean time of initiating feeding (Table I), and were primarily responsible for the lag in the mean cumulative consumption/time curve. Adults then moved over and around the disc a number of times before taking one or more test bites. Initiation of feeding usually took place within a few min of test-biting. Individual biting patterns and overall feeding patterns recorded with an AFD are shown in Fig. 2a and

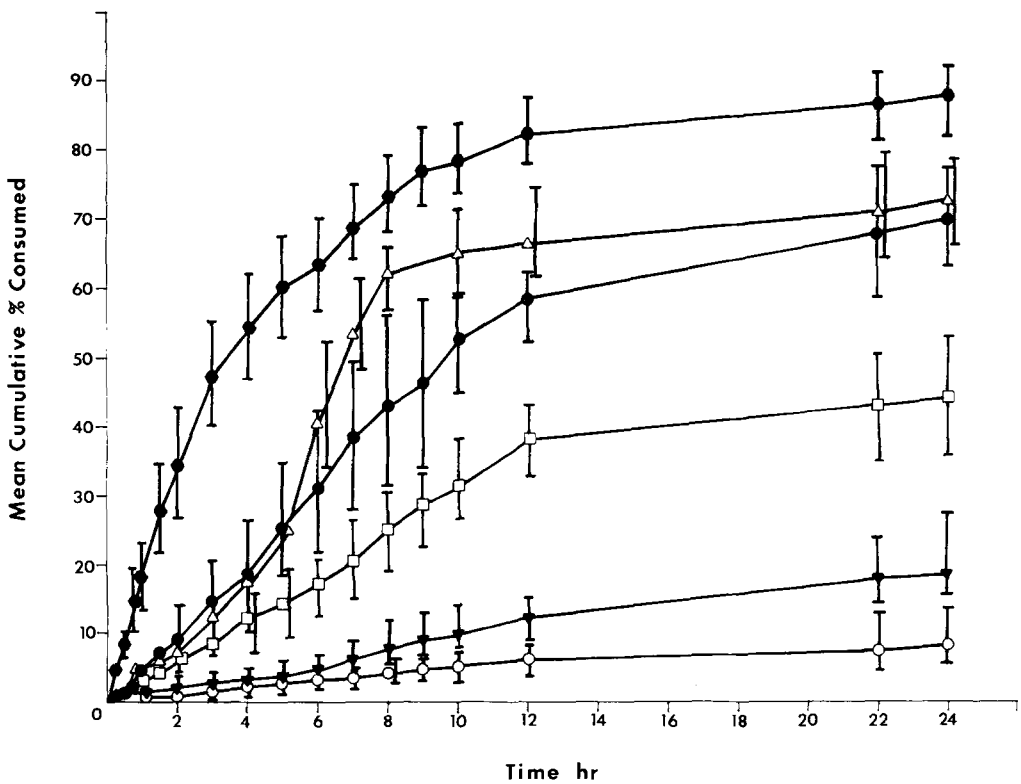

Fig. 1. Mean cumulative % leaf area consumed with time (± 95% confidence intervals) for 2—4-day-old adults of *E. varivestis*. 0.25, 0.5 and 0.75 hr values for sucrose and phenolics ≤ control values. 95% confidence intervals from 0—2 hr for all treatments except starvation omitted for clarity. ●: Controls. ●: Starved (16—64 hr). △: Sucrose (5×10^{-2}M). Pooled values for phenolic compounds (8×10^{-2}M), activity; □: Low. ▼: Medium. ○: High.

TABLE I

Feeding behaviour, 0—24 hr, of adults of E. varivestis. *Mean values are ± 1 SD, ranges in parentheses. Means significantly different from controls,* T-*test:* *, $p \leq 0.05$; **, $p < 0.1 > 0.05$. *C/E ratios significantly different from controls:* ***.

% initiating feeding, hr	Controls n = 180	Starved 16—64 hr n = 50	Sucrose 5×10^{-2}M n = 39	Phenolic compounds Low activity n = 150	Medium activity n = 150	High activity n = 150
0— 1	30.0	90.0	25.6	17.3	5.3	2.0
0— 2	53.9	98.0	46.2	42.0	12.0	9.3
0— 3	62.8	100.0	64.0	53.3	17.3	12.0
0— 4	71.1	—	76.9	62.7	26.7	16.0
0— 6	84.4	—	87.2	80.0	44.0	28.7
0— 8	95.0	—	97.4	87.3	61.3	44.7
0—24	98.9	—	100.0	98.7	87.3	74.0
% not feeding	1.1	0.0	0.0	1.3	12.7	26.0
Mean time initiating feeding, hr - adults feeding within 8 hr	2.9 ± 2.3 (0.25-8)	0.5 ± 0.4* (0.25-3)	3.0 ± 1.9 (0.25-7)	3.2 ± 2.1 (0.25-8)	4.8 ± 2.3* (0.5-8)	5.1 ± 2.4* (1-8)
Time for 50% all adults to initiate feeding, hr	1.9	0.2	2.0	2.8	6.7	10.9
Mean cumulative area, units	1086 ± 201	1638* ± 195	1264** ± 189	651* ± 118	244* ± 98	124* ± 25
C/E ratio	1.0 (0.84 −1.22)	0.66*** (0.48 −0.83)	0.86 (0.61 −1.19)	1.67*** (1.23 −2.41)	4.45*** (2.59 −8.82)	8.75*** (5.94 −13.00)
Mean total consumption at 24 hr, %	69.7 ± 12.2	87.2* ± 10.5	72.5 ± 12.6	44.3* ± 8.6	18.7* ± 8.6	8.2* ± 4.7

2b. Feeding motions have been previously described for larvae by Howard (1941) and Kogan (1973) and are essentially the same for adults.

Following the lag, there was a linear period of consumption of circa 12 hr, the slope corresponding to a population mean consumption rate of 5.3 ± 1.3% hr^{-1} (1.40 ± 0.34 mg tissue with feeding ridges hr^{-1}). The linearity indicated that the consumption rate was relatively constant for the population as a whole. A study of the feeding patterns of individuals over the first 8 hr (i.e., a theoretical maximum of 8 IWF and/or IWNF) showed that adults had between 1—4 IWF or IWNF (Table II). The mean number of IWF and IWNF was similar for the population, but each individual did not necessarily have the same number of IWF as IWNF. The mean total duration of IWF and IWNF was also similar, irrespective of whether individuals had 1, 2, 3, or 4 IWF or IWNF. The total amount consumed in all IWF was relatively independent of the number of IWF (linear regression for number of IWF, x, consumption, y:$y = 1.24x + 48.17$; $n = 4$; coefficient of determination, $r^2 = 0.38$). On the other hand, there was a high degree of correlation between total consumption and the total duration of IWF (linear regression for duration of IWF, x, consumption, $y = 13.13x - 2.56$; $n = 10$; $r^2 = 0.93$). Thus consumption was more a function of time spent feeding than the number of times feeding took place. When the value for consumption rate in feeding periods was averaged out over IWF and IWNF, the resultant rate (5.79% hr^{-1}) fell within the range determined for the slope of the linear phase of the mean cumulative consumption time curve (5.3 ± 1.3% hr^{-1}).

The linear phase was followed by a consumption rate decline or stationary phase lasting from 12—24 hr. Populational consumption at 12 hr (58.5 ± 6.9%) continued at a reduced rate until approximately 70% of the leaf disc was consumed after 24 hr (absence of data recording during 12—22 hr was not likely to markedly affect the overall shape of the cumulative curve since most values at 12 hr were < 10% lower than values at 22 hr, see Fig. 1). The same lag, linear and stationary phases

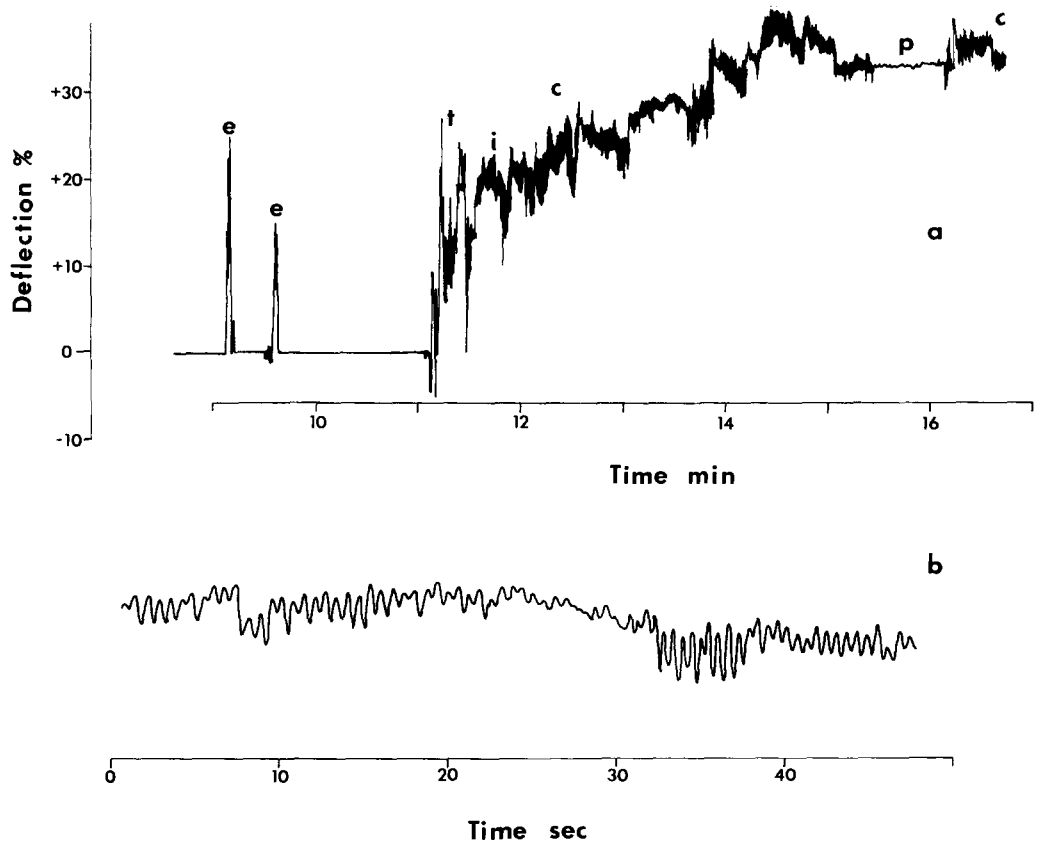

Fig. 2. Portions of AFD traces of control adults of *E. varivestis*: a. showing encounter (e), test biting (t), initiation of feeding (i), continuous feeding (c) and pause in feeding (p); b. showing feeding motions. Deflection scale in b is same as a.

were also obtained when partially consumed discs were replaced by fresh material during the trial, when up to 3 times as much leaf disc was initially presented in the trial, or when the trial was repeated for another 24 hr using the same individuals.

Effects of adult age. Mean (± 1 SD) cumulative area (1100 ± 199), C/E ratio [0.99 (range 0.98—1.0)], and mean (± 1 SD) total consumption after 24 hr (72.8 ± 11.7%) were not significantly different from 2—4-day-old unstarved MBB adult controls (adults aged 1—8, 10, 12, 15, 17 days; n = 150; t-test, $p > 0.25$). There was some fluctuation on a daily basis with 2—4-day-old adults being the most uniform, and for this reason this age class was chosen as the standard age for other trials. A detailed analysis of 0—8 hr IWF and IWNF periods was not carried out for these trials.

Effects of starvation. Although the mean cumulative consumption/time curve for 2—4-day-old, MBB adults starved 16—64 hr was of a similar shape to that of controls, there was no extensive lag phase, the linear phase initially had a greater slope and then decreased to control values, and total consumption was greater (Fig. 1). Mean cumulative area was significantly greater than controls with a C/E ratio significantly less than unity, demonstrating increased consumption (Table I). Mean cumulative consumption/time curves, mean cumulative areas and C/E ratios for adults starved 16, 24, 39, 48 and 64 hr were not significantly different from each other (t-test, $p > 0.5$) and therefore these results were pooled. Values for adults starved 72 hr were not significantly different from controls (t-test, $p > 0.25$). Starved adults initiated feeding more rapidly than con-

TABLE II

Feeding behaviour, 0—8 hr, of adults of E. varivestis. *Mean values are ± 1 SD, ranges in parentheses. IWF: intervals with feeding. IWNF: intervals with no feeding. Means significantly different from controls, T-test: *, $p \leq 0.05$; **, $p < 0.1 > 0.05$.*

					Phenolic compounds		
		Controls n = 180	Starved 16—64 hr n = 50	Sucrose 5×10^{-2}M n = 39	Low activity n = 150	Medium activity n = 150	High activity n = 150
% adults with	1 IWF	30.5	26.0	53.8	29.3	31.3	24.0
	2 IWF	43.9	54.0	35.9	42.0	20.0	19.3
	3 IWF	17.8	20.0	7.7	15.3	8.7	1.3
	4 IWF	2.8	0.0	2.6	0.7	1.3	0.0
	≥1 IWF	95.0	100.0	100.0	87.3	61.3	44.6
Mean #IWF - all adults		1.8 ± 0.9(0-4)	1.9 ± 0.7(1-3)	1.6 ± 0.8(1-4)*	1.6 ± 0.9(1-4)**	1.0 ± 0.9(0-4)*	0.7 ± 0.6(0-3)*
Mean #IWF - adults with ≥ IWF		1.9 ± 0.8(1-4)	1.9 ± 0.7(1-3)	1.6 ± 0.8(1-4)*	1.9 ± 0.7(0-4)	1.7 ± 0.8(1-4)*	1.5 ± 0.6(1-3)*
Mean total duration of IWF, hr, # adults	1 IWF	3.9 ± 1.8(1-8)	5.7 ± 1.3(4-7)*	4.1 ± 1.6(2-8)	2.3 ± 1.4(1-7)*	1.5 ± 0.9(1-4)*	1.4 ± 0.6(1-3)*
	2 IWF	4.4 ± 1.3(2-7)	4.6 ± 1.2(2-6)	4.7 ± 1.1(2-6)	3.7 ± 1.5(2-7)*	2.8 ± 1.0(2-6)*	2.3 ± 0.5(2-4)*
	3 IWF	4.4 ± 0.9(3-6)	4.5 ± 0.7(3-5)	4.3 ± 0.6(4-5)	4.3 ± 0.8(3-6)	3.3 ± 0.5(3-4)*	3.5 ± 0.7(3-4)**
	4 IWF	4.8 ± 0.8(4-6)	—	5.0	4.0	4.5 ± 0.7(4-5)	—
	≥ 1 IWF	4.3 ± 1.4(1-8)	4.9 ± 1.2(2-7)*	4.4 ± 1.4(2-8)	3.4 ± 1.5(1-7)*	2.3 ± 1.2(1-6)*	1.9 ± 0.8(1-4)*
Mean consumption in IWF %, adults with ≥ IWF		51.2 ± 19.6 (3-80)	67.4 ± 15.1* (30-90)	63.2 ± 7.9* (40-80)	31.7 ± 17.1* (3-65)	13.9 ± 9.0* (3-40)	9.0 ± 6.1* (3-30)
Mean consumption rate in IWF, % hr^{-1}, adults with ≥ IWF		11.8 ± 2.3 (7.0-16.7)	13.8 ± 3.1** (8.6-17.3)	14.3 ± 3.9* (5.0-16.5)	8.8 ± 2.0* (5.0-11.0)	5.2 ± 1.0* (3.0-6.9)	4.4 ± 0.5* (3.7-5.5)
% adults with	1 IWNF	29.4	40.0	33.3	34.0	54.7	65.3
	2 IWNF	46.1	50.0	48.7	48.0	28.7	25.3
	3 IWNF	21.7	0.0	15.4	16.6	16.0	8.7
	4 IWNF	2.8	0.0	0.0	1.4	0.6	0.7
	≥ 1 IWNF	100.0	90.0	97.4	100.0	100.0	100.0
Mean # IWNF - all adults		2.0 ± 0.8(1-4)	1.4 ± 0.7(0-2)*	1.7 ± 0.7(0-4)**	1.9 ± 0.7(1-4)**	1.6 ± 0.8(1-4)*	1.5 ± 0.7(1-4)*
Mean # IWNF - adults with ≥ IWNF		2.0 ± 0.8(1-4)	1.6 ± 0.5(1-2)*	1.8 ± 0.6(1-4)**	1.9 ± 0.7(1-4)**	1.6 ± 0.8(1-4)*	1.5 ± 0.7(1-4)*
Mean duration of IWNF, hr, adults with	1 IWNF	4.5 ± 2.5(1-8)	1.9 ± 1.1(1-5)*	4.0 ± 1.5(1-6)	5.7 ± 2.4(1-8)*	7.5 ± 1.1(2-8)*	7.8 ± 0.7(5-8)*
	2 IWNF	3.9 ± 1.6(2-8)	2.7 ± 0.8(3-5)*	3.6 ± 1.2(2-6)	4.8 ± 1.4(2-7)*	5.9 ± 1.1(3-7)*	6.4 ± 1.0(4-8)*
	3 IWNF	4.4 ± 1.1(3-7)	—	3.5 ± 0.8(3-5)*	4.6 ± 1.0(3-6)	5.0 ± 0.9(3-6)*	5.9 ± 0.7(5-7)*
	4 IWNF	5.2 ± 1.1(4-7)	—	—	4.0	5.0	5.0
	≥ 1 IWNF	4.2 ± 1.8(1-8)	2.4 ± 1.0(1-5)*	3.7 ± 1.3(1-6)**	5.1 ± 1.8(1-8)*	6.6 ± 1.4(2-8)*	7.3 ± 1.1(4-8)*
Ratio duration IWF/IWNF, adults with ≥ 1 IWF & IWNF		1.02	2.04	1.19	0.67	0.35	0.26

trols with much greater synchronisation (Table I), and this eliminated the lag phase (Fig. 1).

The differences between controls and 16—64 hr starved MBB adults can be more clearly seen from an examination of IWF and IWNF data. Starved insects showed a significant reduction and greater uniformity in the number of IWNF, but no increase in the number of IWF; all insects fed; the total duration of IWF was significantly longer with a concomitant,

significant reduction in the total duration of IWNF; the consumption rate was significantly increased; and the ratio of IWF to IWNF doubled (Table II).

Effects of sucrose. These adults showed a lag and 2—5 hr linear period similar to control insects (Fig. 1). The times of initiating feeding and the frequency distribution of % adults initiating feeding with time was also similar (Table I). There was an increase in the slope of the linear phase at 5 hr — the slope being greater than that of starved adults — but this phase was of shorter duration than controls. The final amount consumed was not significantly different from controls, but the mean cumulative area was significantly different. The reduced C/E ratio arose from the differential areas over 5—12 hr. Analysis of IWF and IWNF data showed that the % adults having only one IWF had increased markedly from controls, with a corresponding decrease in the % adults having 2 or 3 IWF; in fact, 2.6% adults fed during each of 8 successive hr. Although the frequency distribution of adults with IWNF was not markedly different from the controls, the durations were significantly reduced. The consumption rate was greater than controls or starved adults (Table II). The combination of a significant reduction in the number of IWF and IWNF, significant decrease in duration of IWNF, and significantly increased consumption rate were responsible for the reduced C/E ratio.

Effects of phenolic compounds. The selection of 15 phenolics in each of 3 arbitrary activity classes was based on the C/E ratio only (since we could not analyse all 200 tested compounds at this stage), and provided a gradient of activity. Therefore C/E ratios, overall consumption and mean cumulative areas are all inevitably significantly different from controls (Table I). However, our selection criteria did not tell us what changes in other parameters (such as consumption curves, IWF and IWNF data) would be associated with different C/E ratios.

The mean cumulative consumption/time curve for discs treated with phenolics of low activity was of a similar shape to controls, but was depressed in both the linear phase slope and the amount consumed after 24 hr. Medium and high phenolic activity curves were almost linear (Fig. 1). There was a considerable delay in initiation of feeding which increased with activity level. This was accompanied by an increase in the number of insects not initiating feeding over 24 hr, the mean time of initiating feeding and the time for 50% of adults to initiate feeding (Table I). The distribution of the number of IWF did not change markedly from the control distribution. However, the mean number of IWF for all adults decreased because fewer adults fed. For those adults that did feed, there was a marked reduction in the duration of IWF and consumption rates, with a concomitant increase in the duration of IWNF, although the mean number of IWNF decreased (Table II).

DISCUSSION

Continuous monitoring procedures have been developed for a number of insects (e.g., Kogan, 1973; Bernays, 1979; Jones, 1979 and references therein). Discriminatory bioassays have also been used (Jermy, 1971; Kennedy, 1977a; Higgins & Pedigo, 1979), and the advantages and disadvantages of these systems have been extensively reviewed (Barton Browne, 1974; Cook, 1976; Schreck, 1977; Smith, 1978). Therefore, we will concentrate on showing how cumulative consumption/time data can be used to derive quantitative indices that distinguish effects of different stimuli. The phytophagous insect host-plant selection and feeding processes can be divided into orientation and recognition, initiation, maintenance and cessation of feeding (Thorsteinson, 1960; Beck, 1965). The mean cumulative consumption/time curves can be considered as a summation of these processes and the analysis and comparison of curves can be used to identify behavioural modifications.

Orientation, recognition and initiation of feeding. The no-choice bioassay does not measure long- or medium-range orientation and recognition, because movement is confined by the arena. Factors such as attractants and repellents (e.g., Hedin *et al.*, 1974; Augustine, 1962; Augustine *et al.*, 1964) can be independently evaluated using the AFD (see Fig. 2). Alternatively, the arena/food area ratio in no-choice trials (10% basal surface area, 3% total surface area in our system) could be increased, and movement and time before contact with food measured. Since we did not measure these parameters, the time before initiating feeding should be considered as a quantitative index of all short-range orientation, recognition and feeding initiation processes. Obvious-

ly then, this index could be affected by extrinsic chemical stimuli such as attractants, repellents (Kennedy, 1977b), deterrents and excitants (Kogan, 1976; Städler, 1976) as well as intrinsic physiological factors such as age, sex and food deprivation (Barton Browne, 1975; Schoonhoven, 1977b; Smith, 1978). Some changes in response may also result from induction of preferences (e.g., Jermy *et al.*, 1968) or food aversion learning (Dethier, 1980).

All unstarved insects showed considerable asynchrony in the time of initiating feeding (i.e., SD and range values were large) despite marked differences in the mean time of initiation in different treatments (see Table I). However, starvation reduced the asynchrony as well as the mean value so that food deprivation was likely to have been the major factor influencing initiation synchrony. Age had no obvious effects, although it may be an important factor for many insects (e.g., Smith, 1978). MBB adults that are not of the overwintering generation can survive up to 50 days (Thomas, 1924) and age effects would not necessarily be expected to have a marked effect over 17 days. Starvation-induced synchrony in the MBB is compatible with that found in other insects (see Cook, 1976; Jones, 1977). The reasons why food deprivation increases population synchrony, as well as reducing the mean time of initiating feeding, could be the result of a combination of factors such as increase in locomotory activity, absence of food in the gut, or the generation of an excitatory state by initial contact with the food (Gelperin, 1971; Bernays & Chapman, 1974; Barton Browne, 1975; Dethier, 1976).

Differences in the mean time of initiating feeding resulting from different chemical treatments can also be clearly seen (Table I). Sucrose had no significant effect on this parameter, but did affect consumption rates (Table II). Sucrose has been reported as a "phagostimulant" or "feeding excitant" for the MBB (Augustine *et al.*, 1964) and a number of other insects (see Kogan, 1976), and sugar receptors have been reported in numerous cases (e.g., Schoonhoven, 1969). Since these compounds are non-volatile, effects on feeding behaviour would be expected to occur after contact.

Phenolic compounds did significantly reduce the mean time of initiating feeding as well as consumption rates. While the pooling of the in-

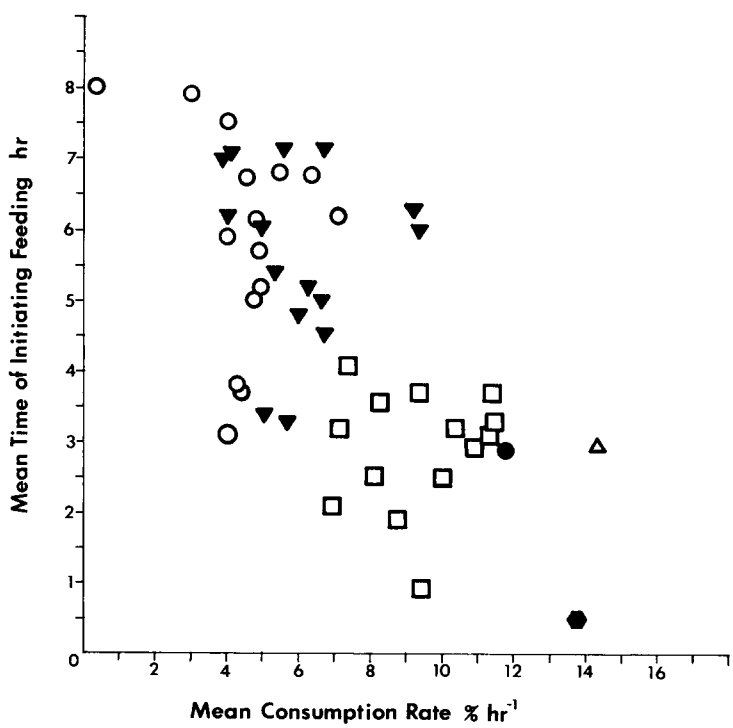

Fig. 3. Scatter plot of mean time of initiating feeding (hr) plotted against mean consumption rate during intervals with feeding (% hr^{-1}) for adults of *E. varivestis*. ●: Controls.●: Starved (16—64 hr). △: Sucrose (5×10^{-2}M). Individual values for phenolics (8×10^{-2}M), activity: □: Low (C/E 1.23—2.41). ▼: Medium (C/E 2.59—8.82). ○: High (C/E 5.94—13.00).

dividual compounds in each activity class does show distinct trends (Tables I and II), a study of individual compounds clearly demonstrates that different effects are distinguishable (Fig. 3). At the same concentrations (8×10^{-2} M), some compounds increased the mean time of initiating feeding to a greater extent than they decreased consumption rates; for other compounds the reverse was true. We suggest that the former primarily affect behaviours prior to and during initiation, while the latter affect behaviours after initiation. The advantages of being able to distinguish these effects are of obvious value. Further studies will be necessary to establish the reasons (e.g., volatility) for these differences.

Maintenance and cessation of feeding. Once the insect has initiated feeding, the measurement of duration and number of intervals with feeding, with no feeding, and consumption rates are probably the most useful quantitative parameters. Distinct differences between treatments in these values are apparent. Overall, in comparison to controls, starved insects consumed food slightly faster over longer periods, but had the same number of IWF. IWNF decreased both in number and duration. Insects on sucrose-treated discs consumed food more rapidly over the same period of time as controls, but had less IWF. Again, as with the starvation, IWNF decreased in number and duration. Insects on some phenolic compound-treated discs fed more slowly, over shorter periods of time, and had less IWF. There was a corresponding increase in the duration of IWNF, but since these were longer, there were less IWNF.

It should be noted that factors affecting initiation of feeding and the first meal will also have effects in subsequent feeding during the trial. Other factors, such as photoperiod, may well be partially responsible for the stationary phases in control cumulative consumption/time curves that occur at the onset of the scotophase. While it is known that MBB larvae do feed in the dark (Kogan, 1972), further studies on this and any of the other factors affecting maintenance and cessation of feeding would have to be carried out to establish their effects.

Evaluation and utilization of indices. Tables I and II show that a large number of quantitative indices of the chain of events in the feeding process can be derived from the simple measurement of cumulative consumption with time for individual insects. This dissection of the feeding process indicates how different and similar final consumption values can arise by different mechanisms, as well as assessing individual variation. For example, starvation and sucrose treatments both increased the mean total consumption value at the end of the experiment (87.2 ± 10.5 and 72.5 ± 12.6, respectively). However, sucrose significantly decreased the number of IWF but not their duration, while starvation significantly increased the duration of IWF but did not affect the number of IWF. Because the number and duration of IWNF significantly decreased in both cases, this led to quite different ratios in the duration of IWF to IWNF (Table II).

Our studies have made no attempt to examine all factors affecting feeding behaviour, or their causes, but our methods do quantify and distinguish certain aspects of feeding behaviour, while being sufficiently rapid to use in screening plant materials and chemicals. Further dissection of feeding events can be achieved with the AFD. These data can be used to confirm consumption rates and feeding periodicities, and measure time before first contact with food. The use of high speed traces permits measurement of biting frequency and amplitude of biting (also see Kogan, 1973).

These data are currently being used to develop a predictive model of feeding behaviour in the bioassay system using regression and correlation values for the quantitative indices. The model will then be tested for independent data sets. Previous studies, using some of these methods for *Pieris brassicae* L, *Chilo partellus* Swinhoe, *Spodoptera littoralis* Boisduval, *Yponomeuta evonymella* L. (Lepidoptera), *Schistocerca gregaria* Forskal (Orthoptera), *Phyllobius argentatus* L. and *P. pyri* L. (Coleoptera) (Jones, 1977, 1979; Jones & Firn, 1978, 1979a, 1979b), suggest that the methods are of general applicability, although the numerical values would have to be derived independently for each species. Such modelling is by no means a total representation of selection and food intake, but may help in the use and understanding of bioassays: they may later be extended to field situations and thereby help to increase our understanding of the complex insect/plant relationship.

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RÉSUMÉ

*Caractéristiques et déroulement du comportement alimentaire des insectes: une analyse quantitative d'*Epilachna varivestis

Des expériences sans choix et des études avec un enregistreur automatique de l'alimentation ont été utilisées pour une analyse quantitative du comportement alimentaire des adultes d'*Epilachna varivestis* sur leur plante hôte.

La consommation cumulée à partir de données temporelles d'insectes isolés a permis de déterminer les courbes temporellement cumulées de la consommation moyenne, les surfaces recouvertes par ces courbes, un rapport entre les aires des courbes expérimentales et témoin, le moment du début du repas, le nombre et la durée des périodes de jeûne et de repas et les taux de consommation pendant les repas. Les témoins ont été comparés aux résultats avec des stimuli physiologiques et chimiques variés.

L'âge n'a pas d'effet sensible, peut être parce qu'il s'agit d'un insecte à longévité élevée. La privation d'aliment synchronise et réduit le temps de latence avant le repas, accroît les taux de consommation et les périodes de repas, mais augmente les taux de consommation pour les périodes d'alimentation identiques par rapport aux témoins; le nombre de périodes de repas et le nombre et la durée des périodes de jeûne diminuent. Quelques composés phénologes augmentent le temps avant le repas tandis que d'autres ne le font pas. Beaucoup de composés phénologes diminuent les taux de consommation, les durées et le nombre de repas, tandis qu'ils augmentent la durée des périodes de jeûne et diminuent leur nombre.

Cette méthodologie fournit des indices quantitatifs des comportements alimentaires spécifiques et est suffisamment rapide pour tester des végétaux et des substances chimiques. Ces méthodes paraissent applicables à la majorité des essais en laboratoire avec des insectes phytophages, et ont été utilisées pour construire un modèle prédictif sur *Epilachna varivestis*.

REFERENCES

Augustine, G. M. (1962). Studies on host-plant selection by the Mexican bean beetle, *Epilachna varivestis* Muls. Ph.D. dissertation, Ohio State University, Columbus.

Augustine, G. M., Fisk, F. W., Davidson, R. H., Lapidus, J. B. & Cleary, R. W. (1964). Host-plant selection by the Mexican bean beetle, *Epilachna varivestis*. *Ann. Ent. Soc. Amer.* **57** : 127—134.

Barton Browne, L. (1974). Ed. *Experimental analysis of insect behaviour*. Berlin, Springer-Verlag.

Barton Browne, L. (1975). Regulatory mechanisms in insect feeding. *Adv. Insect Physiol.* **11** : 1—116.

Beck, S. D. (1965). Resistance of plants to insects. *Annu. Rev. Ent.* **10** : 207—232.

Bernays, E. A. (1979). The use of doppler actographs to measure locomotor activity in locust nymphs. *Ent. exp. & appl.* **26** : 136—141.

Bernays, E. A. & Chapman, R. F. (1974). The regulation of food intake by acridids. In: *Experimental analysis of insect behaviour*. (L. Barton Browne, Ed.): 48—59. Berlin, Springer-Verlag.

Cook, A. G. (1976). A critical review of the methodology and interpretation of experiments designed to assay the phagostimulatory activity of chemicals to phytophagous insects. In: *The Host-Plant in Relation to Insect Behaviour and Reproduction*. (T. Jermy, Ed.): 47—54. New York, Plenum Press.

Dethier, V. G. (1976). The importance of stimulus patterns for host-plant recognition and acceptance. *Ibid*: 67—70.

Dethier, V. G. (1980). Food-aversion learning in two polyphagous caterpillars, *Diacrisia virginica* and *Estigmene congrua*. *Physiol. Ent.* **5** : 321—325.

Gelperin, A. (1971). Regulation of feeding. *Annu. Rev. Ent.* **16** : 365—378.

Hedin, P. A., Maxwell, F. G. & Jenkins, J. N. (1974). Insect plant attractants, feeding stimulants, repellents, deterrents, and other related factors affecting insect behaviour. In: *Proceedings of the Summer Institute for Biological Control of Plant Insects and Diseases* (F. G. Maxwell & F. A. Harris, Eds.): 494—527. Jackson, Univ. Mississippi Press.

Higgins, R. A. & Pedigo, L. P. (1979). A laboratory antifeedant simulation bioassay for phytophagous insects. *J. econ. Ent.* **72** : 238—244.

Howard, N. F. (1941). Feeding of the Mexican bean beetle larva. *Ann. Ent. Soc. Amer.* **34** : 766—769.

Jermy, T. (1971). Biological background and outlook of the antifeedant approach to insect control. *Acta Phytopathol. Acad. Sci. Hung.* **6** : 253—260.

Jermy, T., Hanson, F. E. & Dethier, V. G. (1968). Induction of specific food preference in lepidopterous larvae. *Ent. exp. & appl.* **11** : 211—230.

Jones, C. G. (1977). Chemical content and insect resistance of bracken fern. D. Phil. thesis, University of York.

Jones, C. G. (1979). An automatic feeding detector (AFD) for use in insect behaviour studies. *Ent. exp. & appl.* **25** : 112—115.

Jones, C. G. & Firn, R. D. (1978). The role of phytoecdysteroids in bracken fern, *Pteridium aquilinum* (L.) Kuhn as a defense against phytophagous insect attack. *J. Chem. Ecol.* **4** : 117—138.

Jones, C. G. & Firn, R. D. (1979a). Resistance of

Pteridium aquilinum to attack by non-adapted phytophagous insects. *Biochem. System. Ecol.* **7** : 95—101.

Jones, C. G. & Firn, R. D. (1979b). Some allelochemicals of *Pteridium aquilinum* and their involvement in resistance to *Pieris brassicae*. *Biochem. System. Ecol.* **7** : 187—192.

Kennedy, J. S. (1977a). Behaviourally discriminating asays of attractants and repellents. In: *Chemical Control of Insect Behavior*. (H. H. Shorey & J. J. McKelvey, Jr., Eds.): 215—229. New York, John Wiley & Sons.

Kennedy, J. S. (1977b). Olfactory responses to distant plants and other odor sources. *Ibid*: 67—91.

Kogan, M. (1972). Intake and utilization of natural diets by the Mexican bean beetle, *Epilachna varivestis* — a multivariate analysis. In: *Insect and Mite Nutrition*. (J. G. Rodriguez, Ed.): 107—126. Amsterdam, North-Holland.

Kogan, M. (1973). Automatic recordings of masticatory motions of leaf-chewing insects. *Ann. Ent. Soc. Amer.* **66** : 66—69.

Kogan, M. (1976). The role of chemical factors in insect/plant relationships. *Proc. Int. Congr. Ent.* **15** : 211—227.

Lapidus, J. B., Cleary, R. W., Davidson, R. H., Fisk, F. W. & Augustine, G. M. (1963). Chemical factors influencing host selection by the Mexican bean beetle, *Epilachna varivestis* Muls. *J. Agric. Food Chem.* **11** : 462—463.

List, G. M. 1921. The Mexican bean beetle. *Colorado Agric. Exp. Stat. Bull.* **271** : 1—58.

Maxwell, F. G. (1977). Host-plant resistance to insects-Chemical relationships. In: *Chemical Control of Insect Behavior*. (H. H. Shorey & J. J. McKelvey, Jr., Eds.): 299—304. New York, John Wiley & Sons.

Nayar, J. K. & Fraenkel, G. S. (1963). The chemical basis of the host selection in the Mexican bean beetle *Epilachna varivestis* (Coleoptera, Coccinellidae). *Ann. Ent. Soc. Amer.* **56** : 174—178.

Schoonhoven, L. M. (1969). Gustation and food plant selection in some lepidopterous larvae. *Ent. exp. & appl.* **12** : 555—564.

Schoonhoven, L. M. (1977a). On the individuality of insect feeding behavior. *Proc. Koninklijke Nederlandse Akad. Series C.* **80** : 341—350.

Schoonhoven, L. M. (1977b). Insect chemosensory responses to plant and animal hosts. In: *Chemical Control of Insect Behavior* (H. H. Shorey & J. J. McKelvey, Jr., Eds): 7—14. New York, John Wiley & Sons.

Schreck, C. E. (1977). Techniques for the evaluation of insect repellents: A critical review. *Annu. Rev. Ent.* **22** : 101—119.

Smith, C. M. (1978). Factors for consideration in designing short-term insect-host plant bioassays. *Bull. Ent. Soc. Amer.* **24** : 393—395.

Smith, C. M., Wilson, R. F. & Brim, C. A. (1979). Feeding behavior of Mexican bean beetle on leaf extracts of resistant and susceptible soybean genotypes. *J. econ. Ent.* **72** : 374—377.

Städler, E. (1976). Sensory aspects of insect plant interactions. *Proc. Int. Congr. Ent.* **15** : 228—248.

Thomas, F. L. (1924). Life history and control of the Mexican bean beetle. *Bull. Alabama Agric. Exp. Stat.* **221** : 1—99.

Thorsteinson, A. J. (1960). Host selection in phytophagous insects. *Annu. Rev. Ent.* **5** : 193—198.

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