Feeding Behavior of the Cabbage Bug, *Eurydema rugosa*
MOTSCHULSKY (Hemiptera : Pentatomidae)
on the Cruciferous Plants¹

Kôji HORI

Laboratory of Entomology, Obihiro Zootechnical University,
Obihiro, Hokkaido, Japan

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While the adult of the cabbage bug, *Eurydema rugosa* MOTSCHULSKY, feeds mainly on mesophyll and parenchymatous cells of the stem and on the pod and flower bud of the host plant, the nymph feeds not only on mesophyll but also on vascular element; the younger it is, the more it feeds on the latter.

Immediately after feeding, an irregularly round, whitish spot is manifesting itself around the point where the stylets were thrust. Among the spots inflicted by the bug of various stages, no difference could be seen in external appearance except in size. The maximum radius of the feeding spot was the same as the total length of the first and second segments of the proboscis of the bug, and the size of the spot never grew larger but remained the original size also with the lapse of time. It is suggested that the injury caused by the bug is primarily due to the mechanical destruction of cells in the tissue attacked by its stylets. The mechanisms of injury caused by the bug did not change with the progress of stage.

The bug always left many granular substances at the end of and along the feeding puncture within the attacked tissue. The tube-like salivary sheath lining the feeding puncture was not always formed, for its construction was dependent on the feeding site and the stage of the bug.

INTRODUCTION

The studies of feeding behavior of Hemiptera species are very important for analysis of transmission of virus diseases, secretion of salivary toxins and mechanisms of injury to plant tissues by them. A number of inquiries concerning the injury to the host plant caused by hemipterous insects have hitherto been reported. Based on their feeding sites (BUSGEN 1891, etc.), the species of this order are grouped into mesophyll-feeder, phloem-feeder, etc. Many workers (SMITH 1926, etc.) found that around the passage of the stylets many hemipterous insects leave a salivary sheath which forms a clearly defined tube or irregular aggregations. CARTER (1962) classified the symptoms into four categories: 1) Local lesions at the insect's feeding point. 2) Local lesions with development of secon-

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dary symptoms. 3) Tissue malformations. 4) Symptoms indicating translocation of the causal entity: a) Limited. b) Systemic.

Besides the symptoms, it is likewise of great importance to inquire into the mechanisms of injury, but so far only a few studies on this subject have been made. One of the most detailed studies was that made by KLOFT (1960) on several aphids. The present author carried out, in Hokkaido University in 1958 and in Obihiro Zootechnical University in 1966, a series of researches concerning the cabbage bug which has been already known as a pest to cruciferous crop for a long time in Hokkaido. The purpose of the study was to get some insight into their feeding habits, the injury caused to cruciferous plants, and the relation of the latter to the former.

MATERIALS AND METHODS

Observations on the feeding behavior. The hibernating adults were collected in the cruciferous crop fields from late April to middle May in 1958 at Sapporo, and were reared on four plants potted in a cage. Soon after they were released on plants, many eggs were laid on the plants, on the wire gauze, and on the frame of the cage. The eggs were then transferred to several petri-dishes over which a sheet of moist filter paper was laid. After hatching, the young nymphs were fed on plant leaves in petri-dishes or on the above-mentioned caged plants. During this time, the feeding behavior in each stage was observed.

Observations on the feeding spot and the mouth-part of the bug. The feeding spot and the mouth-part of the bugs were carefully observed, especially the radius of the spot, the length of the proboscis and the width of the stylets.

Sampling of injured plant tissue. The hibernated adults were collected from a cruciferous crop field early in May of 1966 at Obihiro. The rearing method was similar to that described above. Observations were made 1) on the changes in appearance and quality of cells in the injured tissue with the passage of time and 2) on the differences among the injured tissues in different stages of the bug. In the former case, small samples were cut off from the injured tissues so that each sample might include only one or a few feeding spots; the time of sampling was 0-1 min, 1-5 min, 10 min, 30 min, 1 hr, 2 hr, 5 hr, 1 day, and longer than one day from the start of feeding. The samples were quickly fixed in FAA fixing fluid (100 ml alcohol, 6.5 ml formalin, 2.5 ml acetic acid) for 48 hr. Then following the general preparing procedure, they were cut into sections 10 μ in thickness and stained with HANSEN's hematoxyline and safranin. In the latter case, samples with feeding spots were collected during the periods of 1st, 2nd, 3rd, 4th, 5th instar and adult. The procedure employed was the same as in the former case. The sections were observed under an microscope.

RESULTS

The Feeding Behavior

At feeding, the bug set the straightened proboscis vertical to the plant surface and then bent it at the first and second articulations in directions reverse to each other. The bug then moved its head up and down so that the space between the

head and the plant surface changed recurrently. As a consequence of this, the stylets which are enclosed in and have the same length as the proboscis could move back and forth in the tissue. Since the stylets consist of very flexible and elastic chitin, they can be bent in any direction. The bug could thus stay on the original point where the stylets were thrust in and imbibe plant juice of the tissue around that point by bending the stylets here and there. It can thus be estimated that the maximum distance for the stylets to be able to reach from the thrust point is equal to the total length of the first and second segments of the proboscis (Fig. 1).

Fig. 1. Movement of proboscis and stylets within leaf tissue during the feeding of *Eurydema rugosa* MOTSCHULSKY. Af: First articulation. As: Second articulation. Lr: Labrum. S: Stylets. l: Length of the first segment. m: Length of the second segment. n: $l+m$ ($dx=0$). dx : Distance for the stylets to reach from its thrust point. $dx=(l+m)-n$. dm : Maximum distance for the stylets to be able to reach from its thrust point. $dm=l+m$ ($n=0$).

The adults fed actively on the host plant, particularly in hot weather. They sometimes remained with their stylets inserted in the same tissue for a few hours, though they seemed not to have fed continuously all the time. The feeding sites on the host plant were not always uniformly distributed; it appears that they feed more preferably on the leaves than on the stems. When there were flower buds or pods, they fed on nothing else but these. It has been observed that most of the 1st-instar nymphs feed very seldom although during the latter period of the instar, some are found to feed only slightly on leaves without leaving any trace of feeding spots. The nymphs of succeeding instars fed very actively on flower buds, peduncles, and pods of cruciferous crops that had just then grown to maturity (except that they take rest in the moulting time).

Symptoms of the Injury

Just after feeding, a more or less roundish and whitish spot manifested itself around the point on the plant where the stylets had been thrust. The spot was an assemblage of radial or branch-like stripes starting from its center. The spaces between neighboring stripes and a small restricted portion about the center remained generally green; but when attacked more heavily, the spot showed no green space but turned into a uniformly whitish spot. In most cases, a number of spots

were formed close to each other or one on top of the another until the damaged spot looked like a large, evenly whitish figure (Fig. 2, A). As time elapsed, the spot lost the green space entirely, if any had existed, and came out dry about 20 days after feeding. The spot on a leaf was then easily breakable.

The spots inflicted by bugs of various stages showed no essential external difference in appearance except in size. As will be seen from Table 1, the increase in mean and maximum radius of the spot is roughly proportional to the stage number. The fact that the mean radius of the adult's spots came out smaller than that of the 5th instar is probably due to the limited number of observed adults. We could not find any spot inflicted by the 1st instar nymphs even at the place where they were obviously feeding.

Table 1. SIZE OF FEEDING SPOTS IN EACH STAGE OF *Eurydema rugosa* MOTSCHULSKY

Stage		2 nd	3 rd	4 th	5 th	Adult
Radius	Mean	0.36 ^a	0.66	0.90	1.11	1.10
	Max.	0.50	0.81	1.00	1.25	1.50
	Min.	0.13	0.45	0.75	0.85	0.50
Number observed		30	29	20	20	9

^a Radius in mm.

A young leaf concentratively attacked by the bugs withered very soon, and a stem from the attacked area upwards died sooner or later on account of the destruction of the vascular bundle.

In early spring, according to our observation, younger cruciferous crops in the field were only sparsely populated with the bugs so that crops did not suffer any serious damage; but in June a large number of the bugs appeared on the seed crops and fed actively on the flower buds and young pods. When the buds are attacked by the bugs once and only once, they wither away with the result that the seed yield diminishes.

External Structure of the Mouth Parts

The description of the mouth parts of Hemiptera was given by BORROR and DELONG (1954). The structure of the mouth parts of the cabbage bug proved to be similar to that mentioned by them. The tip of the mandibular stylet has many regularly arranged, sharp sawteeth and the maxillary stylet has a few irregular jags. The proboscis has such a structure that it can be bent easily at the first and second articulations in directions reverse to each other. The length of the proboscis and the width of the stylets remain constant within the period of each stage. But they increase stepwise with stages as does the body length, although in the adult stage there is some difference in length between male and female (see Table 2).

Table 2. SIZE OF MOUTH PARTS OF *Eurydema rugosa* MOTSCHULSKY

Stage	Observed Nr.	Body length Mean (mm)	Length of proboscis Mean (mm)	Length of each segment of proboscis (mm)					
				1 st segment			2 nd segment		
				Max.	Min.	Mean	Max.	Min.	Mean
1 st	30	1.28	0.69	0.18	0.13	0.15	0.20	0.15	0.17
2 nd	13	1.57	0.91	0.25	0.20	0.21	0.33	0.25	0.30
3 rd	10	3.07	1.20	0.33	0.28	0.30	0.42	0.38	0.40
4 th	10	4.08	1.57	0.44	0.39	0.41	0.52	0.53	0.54
5 th	8	5.53	2.02	0.55	0.48	0.53	0.75	0.69	0.71
Adult	10	7.61	2.41	0.73	0.60	0.65	0.90	0.80	0.86
♀	5	8.19	2.53	0.73	0.63	0.69	0.90	0.85	0.88
♂	5	7.03	2.29	0.65	0.60	0.62	0.85	0.80	0.83

Stage	Length of each segment of proboscis (mm)			Width of stylets (μ)		
	1 st seg. (max.)	3 rd seg.	4 th seg.	Max.	Min.	Mean
	2 nd seg. (max.)	Mean	Mean			
1 st	0.38	0.12	0.22	11	6	7.6
2 nd	0.58	0.16	0.24	14	12	12.8
3 rd	0.75	0.22	0.29	17	15	15.6
4 th	1.00	0.28	0.34	21	19	20.5
5 th	1.30	0.35	0.43	27	24	25.3
Adult	1.63	0.41	0.49	34	29	30.8
♀	1.63	0.43	0.52	34	30	32.4
♂	1.50	0.39	0.46	30	29	29.2

Observation of the Injured Plant Tissue

1). *General symptoms of the injured tissues.* When the bug fed on mesophyll, the symptoms were as follows (Fig. 2, B and C): The place in the tissue pierced directly by the stylets, the so-called "feeding puncture" (FP), lost all of the cells and became empty. The wrecks of the cells were pushed to the side or to the end of the FP. The cells adjacent to the FP, however, underwent little or no change except that some of them could be more or less strongly stained. In an extreme case it was observed that even a single layer of cells left between two FP's or a cell bordering on broken cells remained normal for a few days after the feeding (Fig. 2, D). The width of the FP made by a single thrust remained unchanged for a few days at least. All along, from the entrance to the end of the FP, the radius of the area of broken tissue never exceeded that of the feeding spot externally measured (Table 3 and 4).

The bug fed within a certain area around the thrust point; but in the earlier stage of feeding process, it left a narrow green zone surrounding the center. The only remaining were a few paths through which the stylets had passed as far as

the feeding sites. From the end of each path and within the feeding site numerous branches spread out which had been produced by the bending, elongation, or contraction of the stylets. The green zone remaining in the feeding sites seems to be of no little importance. If there had been only a single path for each act of feeding, that is, if there had been no branching of the path, the bug would have been compelled to bring back its stylets to the original thrust point after each individual thrust. Thus it would have had to raise its head each time sufficiently distant from the plant surface and expend a rather wasteful amount of time and energy.

A tube-like salivary sheath forms a lining of the FP and was rarely found in the mesophyll although a number of hematoxyline-safranin-stainable granular substances were always left in the broken empty cells and intercellular space on the wall or at the end of the FP. The tube-like sheath was shaped only around the thrust point of the stylets, in some cases on the leaf and in all cases on the stem. The granular substances, when formed in the mesophyll, were mostly of irregular form with sharp protrusions. Those formed in the elongate parenchymatous cells of the stem all had a regular tip-round, arrow-head-like form (Fig. 2, E).

In the cases where the bug fed on the vascular element, the injured symptoms in the tissue were as follows (there was no symptom which could be observed externally). As the bug pierced the vascular element directly with its stylets, only the limited number of cells through which the stylets passed from the thrust point to the object and only a few pierced cells in the vascular element were broken. The malformation of the cells lining the FP was similar to the case of the mesophyll. The tube-like salivary sheath was formed along the whole length of the FP whenever the stylets had passed through a vascular element from the open to the closed end, but only seldom was this the case when it had passed through both mesophyll and vascular element (Fig. 2, F). But even here, it was always the case that the sheath was molded at the thrust point of the stylets (Fig. 2, G) and along the FP within the vascular element. Sometimes the bug was observed to change its feeding site from vascular element to mesophyll after feeding on the former for a while; and also in this case, the tube-like salivary sheath was not formed in the mesophyll.

2). *Changes in appearance of the cells in the injured tissue with the progress of time.* The above-mentioned symptoms in the injured tissue appeared immediately after feeding, and showed no appreciable change at least within a few days thereafter. The width of the FP and the radius of broken tissue in the feeding spot never grew larger with time after the 1-5 minutes feeding (Table 3). The cells in the feeding spot which remained normal in external appearance, withered in about 20 days, and the spot became wholly white. However, neither the size of the spot nor the radius of broken tissue showed any tendency to increase.

3) *The injured tissue in each stage.* The range of broken tissue came out different from stage to stage; it was larger in the older than in the younger stage except for the fifth instar. The anomaly appearing in the fifth instar may be only apparent and may be accounted for by the fact that the measurement was made for only one bug. The radius of broken tissue in each stage never exceeded the maximum radius of the corresponding feeding spot (Table 1 and 4).

Fig. 2. Photomicrographs of the injured tissue caused by *Eurydema rugosa* MOTSCHULSKY. A : Feeding spots on a leaf. B : Vertical section of the feeding spot ($\times 168$). C : Horizontal section of the feeding spot ($\times 154$). FP; feeding puncture. D : A layer of cells left between two FP's or a few cells bordering on broken cells remained normal for a few days after the feeding ($\times 644$). Ec; empty cell, Nc; normal cell. E : Granular substance taken a regular tip-round, arrow-head-like form in the elongate parenchymatous cell of the stem ($\times 1750$). F : Tube-like salivary sheath ($\times 175$). G : Tube-like salivary sheath molded at the thrust point of the stylets ($\times 700$).

Table 3. VARIATION IN FP WIDTH AND IN RADIUS OF BROKEN TISSUE WITH TIME AFTER FEEDING OF *Eurydema rugosa* MOTSCHULSKY

Time	0-1 min	1-5 min	10 min	30 min	1 hr	2 hr	5 hr	1 day
Minimum width(μ)	50	30	— ^a	30	30	30	35	36
Radius (mm)	0.47	1.35	— ^a	0.86	— ^a	0.99	1.20	1.17

^a The injured tissue had no feeding spot that could be measured accurately.

Table 4. VARIATION IN FP WIDTH AND IN RADIUS OF BROKEN TISSUE IN EACH STAGE OF *Eurydema rugosa* MOTSCHULSKY

Stage	1 st	2 nd	3 rd	4 th	5 th	adult
Minimum width (μ)	— ^a	— ^b	20	24	23	36
Radius (mm)	— ^a	0.50	0.73	0.89	0.85	1.17

^a No measurable feeding spot can be found for the 1 st instar.

^b The injured tissue has no appreciable FP.

The feeding site of the bug varied with the stage (Table 5). The adult usually fed on mesophyll, while the nymphs fed on both mesophyll and vascular element; especially in the second instar, as many as 90% of nymphs were found to prefer the latter. The result that the nymphs of the third instar fed only on mesophyll is probably an accidental one. Too few observations of the injured tissue were responsible for it. As for the first-instar nymphs, it may be assumed that they fed on vascular element since no feeding spot could be found even where it was evident that they were feeding.

Table 5. FEEDING SITE AND FORMATION OF THE TUBE-LIKE SALIVARY SHEATH IN EACH STAGE OF *Eurydema rugosa* MOTSCHULSKY

Feeding site on the leaf (%) ^a						
		Mesophyll			Vascular element	
Tube-like sheath	Present	Present only at the thrust point of the stylets	Absent	Present	Present only at the thrust point of the stylets	Absent
Stage						
2 nd	0	11.1	0	88.9	0	0
3 rd	0	50.0	50.0	0	0	0
4 th	0	42.9	7.1	50.0	0	0
5 th	18.2	18.2	9.1	54.5	0	0
Adult	0	10.3 ^b	75.9	10.3	3.5	0

^a The values are the ratio in % to the total number of the observed feeding sites.

^b The existence of the sheath was not so obvious.

The nymphs, in most cases, formed the tube-like salivary sheath lining the FP from the open to the closed end if the passage had lain in vascular element. But in case it had lain in mesophyll, they formed the sheath only at the thrust point of the stylets. The adults scarcely formed the sheath in mesophyll.

DISCUSSION

Local lesions at the feeding point of plant leaves are considered to be the simplest

manifestation of the toxic effect of insect feeding. There are no apparent systemic effects; the cellular disturbance is limited to a small welldefined area (CARTER 1962). There are many indications of local lesions arising from various causes. The symptoms in the case of the cabbage bug are similar to the local lesions caused by *Empoasca* (SMITH and POOS 1931).

Fig. 3. Relation between the maximum radius of the feeding spot and the sum of the maximum lengths of the first and second segments of the proboscis in each stage of *Eurydema rugosa* MOTSCHULSKY.

Figure 3 shows that the maximum radius of the feeding spot in each stage agrees very well with the sum of the maximum lengths of the first and second segments of the proboscis, that is, the maximum distance that the stylets are able to reach from their thrust point. This fact suggests that the feeding spot is made by the stylets which pass through the tissue and destroy the cells in their passage. Furthermore, the injury caused by the bug is primarily due to the mechanical destruction of the cells and the withdrawal of the cell contents, but seems not due to the toxin in the salivary secretion. The observation was made that the intact cells within the feeding spot which were isolated from those around the spot, remained normal for a few days, but they hardly lived as long as for twenty days or so after feeding. This may be considered to indicate that these cells were cut off from the supply of water. Besides this secondary mechanical effect, there may be a physiological effect. Since it is known that some aphids inject salivary secretion into plant tissue at the time of their feeding and the secretion can spread in it with time (CARTER 1945, KLOFT 1960), it is also conceivable that the cabbage bug may likewise inject salivary secretion and may affect physiologically some of the cells around or within the feeding spot.

The feeding sites of the hemipterous insects vary with the species. The cabbage bug gradually alters its feeding site from the vascular element to mesophyll with the advance of stage. The adult is usually a mesophyll feeder. This alteration of the feeding site in ontogenesis is very interesting in relation to the variation of feeding habits in phylogeny. Inasmuch as the food content differs among

different tissues, the digestive function of bugs each stage may differ also. The inquiry into the salivary secretion in each stage is therefore of particular importance. There are descriptions concerning the salivary sheath of various species of the Hemiptera. Their sheath materials are largely of insect origin, do not contain plant substance (SMITH 1933, FIFE 1937, BENNETT 1934, NICKEL and SYLVESTER 1959), and coagulate rapidly (MILES 1959, 1959a). We can not tell whether a lot of granular substances which were observed along and at the end of the FP produced by the feeding of cabbage bug are of insect origin or of plant origin. However, provided the former be the case, we may perhaps say as follows: "Some of the salivary secretion (probably the sheath material) injected into the tissue coagulates in the intercellular space and within the empty cells whose content has been imbibed, so that they can be cast into variant shapes. This phenomenon is observed most distinctly in the elongate parenchymatous cells of the stem in which the substances are found to be of regular tip-round, arrow-head-like form. The observation that the corners of granular substances are sharply protruding seems to indicate that the sheath material of the bug has high viscosity. If it be justified to consider that the sheath is not indispensable in mesophyll at the time of feeding of the bug, the granular substances must be something disused or superfluous." POLLARD (1959) reported that the lace-bug, *Urentius aegyptiacus* BERGEVIN, which fed on the palisade layers and spongy mesophyll produced no salivary sheath. It seems therefore apparent that the mesophyll feeder of this type hardly needs the salivary sheath for its feeding, and the sheath materials of the adult of cabbage bug which is usually a mesophyll feeder are disused substances except when it feeds on vascular element. Of course, for the nymph which feeds actively on the vascular element, they are indispensable.

It is highly desirable to perform the further comparative studies on the mechanism of the injury, the feeding site and the occurrence of the salivary sheath for various species of the Hemiptera.

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