

Quantitative measurement of leaf area consumption by *Epilachna vigintioctopunctata* (Fabricius) (Coleoptera: Coccinellidae) using image processing

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Abstract

The leaf area of a solanaceous weed, *Solanum carolinense*, consumed by larvae and adults of a leaf scraping coccinellid beetle, *Epilachna vigintioctopunctata*, was measured using a computer-assisted image processing system (IPS). The accuracy of this IPS was examined by measuring areas of 25 mm × 25 mm, 50 mm × 50 mm, and 100 mm × 100 mm reference plates. The relative measurement error of the IPS was < 1% for all plates. It was also demonstrated that the IPS could be effectively used to measure the leaf area consumed by a leaf-scraper insect by comparing the IPS measurements with those of an area meter. A feeding experiment of *E. vigintioctopunctata* on *S. carolinense* was carried out for 38 days at 25°C under 16L–8D, and the leaf area consumed was measured by the IPS. An average beetle consumed 1,429.5 ± 131.6 (S.D.) mm² of leaves to complete its larval development and consumed 2,510.9 ± 306.2 mm² of leaves during the first 10 days of its adult stage.

Key words: Leaf area consumption, image processing, leaf scraper, *Epilachna vigintioctopunctata*, *Solanum carolinense*

INTRODUCTION

Recently many species of weeds have been invading agricultural fields in Japan (Shimizu et al., 1994a). Horse nettle, *Solanum carolinense* L., is a perennial solanaceous herb of North American origin. *S. carolinense* is spreading widely over agricultural fields, grasslands, river beds (Shimizu et al., 1994b), roadsides, lawns and abandoned areas. The weed is difficult to control due to its deep root system and sharp prickles on the stems, leaf veins and flower stalks. Once these non-native plants have colonized, they interact with endemic herbivorous insects (Strong et al., 1984). An endemic herbivore, the phytophagous coccinellid beetle *Epilachna vigintioctopunctata* (Fabricius), was found to be a major defoliator of *S. carolinense* and appeared to have a strong interaction with the weed (Imura, unpublished).

Quantitative and accurate measurement of leaf area consumed by herbivorous insects is necessary to evaluate insect-plant interactions. Many techniques have been used to assess leaf tissue removal by insects, such as scoring or approximating percentages visually (e.g. Katakura et al., 1989), grid and dot-grid methods (Benjamin et al., 1968), a planimetric method (Pedigo et al., 1970), a photometric method (Kogan and Goeden, 1969; Pedigo et al., 1970) and an area meter method (Jensen et al., 1977). However, some of these methods are time-consuming and others are not very accurate. Furthermore, all these methods are limited to insects which gnaw holes of a simple shape.

Hargrove and Crossley (1988) and Baur et al. (1990) developed computer-assisted image analysis methods to measure leaf area loss in several tree species caused by insects which perfo-

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rated holes in the leaves. Their methods were efficient and accurate. *E. vigintioctopunctata*, however, does not perforate holes but scrapes tissue only part-way through the leaves, and the edges of the infested part are like the teeth of a comb. Nolting and Edwards (1985) measured such leaf damage by analyzing video images of back-lighted leaves. Because the veins of fresh leaves of *S. carolinense* transmit light, the back-light technique was not appropriate for the analysis of leaf area loss. Thus, we devised a method to measure leaf areas infested by leaf scrapers using an image processor system and examined the accuracy of the system and applicability of the method. Then we measured age specific leaf area consumption of *S. carolinense* by larvae and adults of *E. vigintioctopunctata* under laboratory conditions using the image processing system.

MATERIALS AND METHODS

The measurement system and procedure. The measurement system was composed of an image analysis processor (NEXUS Cube, Nexus Co. Ltd., Tokyo) with a display (PVM-1454Q, SONY Co. Ltd., Tokyo), desktop computer (PC9821-As2, NEC Co. Ltd., Tokyo), photo-video camera (PHV-A7, SONY Co. Ltd.), color decoder (DEC-110, Hoei Co. Ltd., Tokyo), color video monitor (PVM-20N1J, SONY Co. Ltd.), and digitizer (U4-30, OSCON Ltd., Tokyo) (Fig. 1). A program for the system to analyze leaf images was developed by S. N. based on Ninomiya et al. (1992).

Leaves infested by insects were placed on a white surface which had a ruler and were pho-

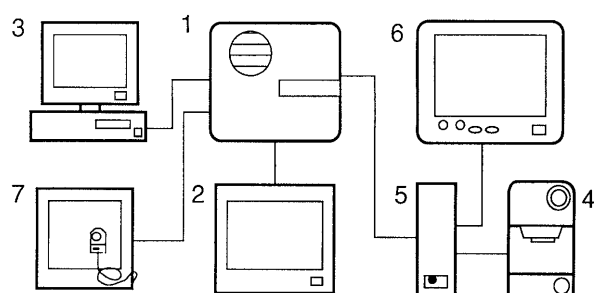


Fig. 1. Schema of the image processing system (IPS). 1: image analysis processor, 2: display, 3: desktop computer, 4: photo-video camera, 5: color decoder, 6: color video monitor, and 7: digitizer.

tographed with a camera loaded with color slide film; a strobe light was used for lighting. The image of the infested leaves on the slides was inputted into the image processor as a digital image through the photo-video camera and the decoder which converted the video signal into RGB (red, green, blue) signals. The scale of the ruler on the digital image was first read for the calculations of leaf area. Then, the leaf being analyzed was roughly cut from the background to remove the ruler and other objects in the original image. The image was binarized according to the gray level to separate the infested parts from the unaffected parts of the leaves and the leaves from the background. When necessary, the leaf image was manually retouched by adding or deleting pixels with a digitizer, referring to the video image of the leaf on a separate video monitor screen. The leaf image on the video monitor screen could be magnified independent of the image on the processor by the zoom function of the photo-video camera, enabling a detailed observation of the infested leaves for manual retouches. Finally, the pixels of the unaffected parts and the whole leaf for which the infested parts were filled out were counted and the measurement data were stored on a diskette.

Accuracy and applicability of the method. To test the accuracy of the image processing system (IPS), we measured areas of 25 mm × 25 mm, 50 mm × 50 mm and 100 mm × 100 mm square cardboard reference plates with the IPS. The plates were photographed as described above. Five independent measurements were made for each reference plate by the IPS.

We examined the applicability of the present method to the leaves infested by leaf-scraping insects by comparing the IPS measurements with those taken by an area meter (AM). The AM was model AAC-400 (HAYASHI DENKOH, Co. Ltd., Tokyo; resolving power of 1 mm² and relative measurement error of less than 1% for objects larger than 6,000 mm²) and was calibrated with a standard target before use. For the AM measurements, two photocopies of each infested leaf were made with a color copying machine. We cut the leaves out of the photocopied papers and in one of the two copies, the infested parts were carefully removed

with a knife. The areas of these two copies (the whole leaf and the leaf in which the infested parts were removed) of each leaf sample were measured by the AM and the leaf area consumed was obtained from the difference between the two measurements. The leaves were also photographed and processed for the IPS measurement.

For the sample leaves, we selected poplar (*Populus nigra* L.) leaves infested by moth larvae of the subfamily Phycitinae (species was not identified), because the moth causes a similar scraping damage to that of *E. vigintioctopunctata*, but destroys tissues surrounded by main veins. Thus the edges of the infested area were largely straight along the leaf veins, allowing us to remove the infested parts relatively easily from the copies. The infested leaves whose leaf area ranged ca. 3,500–6,000 mm² were collected in the field.

Measurement of leaf area consumption by *E. vigintioctopunctata*. Seven egg-masses of *E. vigintioctopunctata*, whose size ranged from 10 to 34 eggs, were collected from mass rearing cages maintained at 25±0.5°C and a long-day condition of 16L–8D. Each egg-mass was placed in a glass petri dish (10 cm in diameter and 2 cm high) and kept under the same rearing conditions as the mass rearing insects. After egg hatching, a fresh leaf of *S. carolinense*, whose petiole was covered with moistened cotton to prevent desiccation, was placed in the petri dish. The leaves were collected from *S. carolinense* plants growing in a greenhouse. Small young leaves and old large leaves which were yellowish in color were not used for the experiment. The infested leaf was replaced with a new one every other day until the 14th day from egg laying, after which it was replaced daily until the end of the experiment (38th day). When the 2nd and 4th (the last) stage larvae appeared, the number of individuals in each petri dish was reduced to 10 and 5, respectively. Because we confined female and male adults in the same petri dishes to allow mating and egg-laying, the leaf consumption was not examined separately by sex; the average sex ratio ($\sigma/(\sigma + \text{♀})$) in the seven replications was 0.4. The infested leaves were photographed upon collection. Later, the leaf area consumption was analyzed for each

leaf sample by the IPS.

RESULTS

Accuracy of the IPS and applicability of the method

The mean areas of the five independent measurements of the reference plates, 25 mm × 25 mm (625 mm²), 50 mm × 50 mm (2,500 mm²) and 100 mm × 100 mm (10,000 mm²), were 622.6±2.0 (S. E.) mm², 2,493.4±2.7 mm², and 10,088.6±22.6 mm², respectively. The relative measurement error of the mean was less than 1% for all the plates.

Table 1 shows the consumption area in mm² by the moth larvae in five leaf samples measured by the IPS and the AM methods. The mean difference between the two measurements was -12.4±71.1 (95% C.L.) mm² (Table 1). The narrow 95% confidence interval of the mean difference including zero indicated that the measurements of leaf consumption by the two methods were almost equivalent.

Leaf area consumption by *E. vigintioctopunctata*

Figure 2 (upper panel) shows the transition of life stages of the beetle, for which the seven cohorts examined were combined. The larvae of the beetle had four stages whose mean periods (in sequence) were 2.95, 2.38, 3.00, and 4.99 days, respectively.

The leaf area consumption by the first and second stage larvae was less than 50 mm²/individual/day (Fig. 2, lower panel). However, after the 9th day from egg laying when the third stage larvae emerged, the consumption in-

Table 1. Comparison of consumption areas measured by the image processing system (IPS) and the area meter method (AM) in poplar leaves infested by moth larvae

Leaf no.	Leaf consumption area (mm ²)		
	IPS	AM	Difference (IPS-AM)
1	799	716	83
2	1,264	1,286	-22
3	539	611	-72
4	1,264	1,285	-21
5	772	802	-30
Mean			-12.4
95% C.L.			±71.1

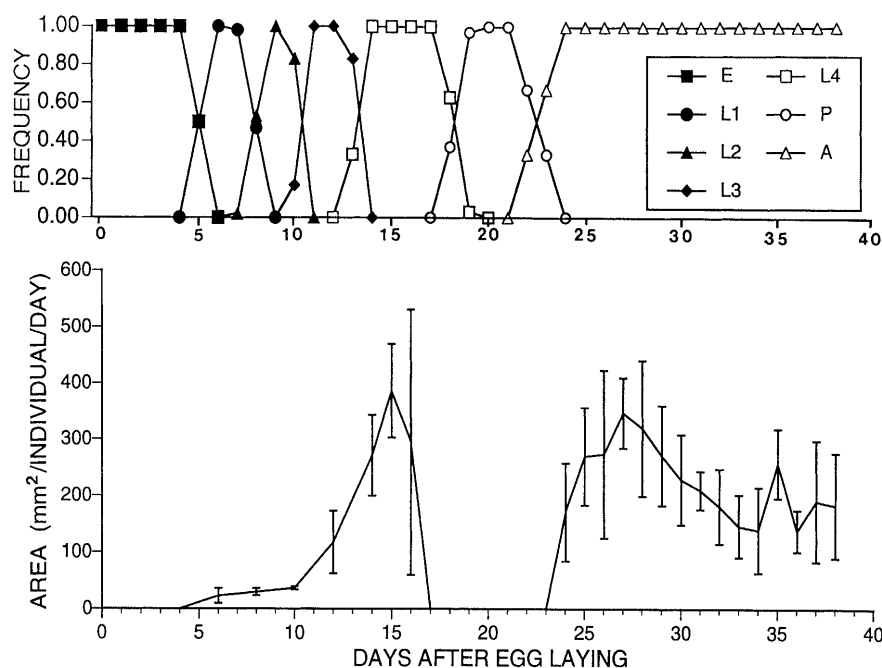


Fig. 2. Upper panel: Transition of life stages of *E. vigintioctopunctata* experimental cohort populations reared on *S. carolinense* leaves. E: egg; L1: 1st stage larva; L2: 2nd stage larva; L3: 3rd stage larva; L4: 4th stage larva; P: pupa and A: adult stages. Lower panel: Consumption of *S. carolinense* leaf in mm²/individual/day by the *E. vigintioctopunctata* populations. Vertical lines indicate S.D. The experiment was carried out at 25°C and 16L-8D.

creased markedly, peaking on day 15 (386.1 ± 83.5 (mean \pm S. D.) mm²/individual/day) when all individuals were in the fourth stage (Fig. 2). In the late period of the fourth stage, consumption decreased, and before pupation the larvae stopped feeding. Newly-emerged adults took about 1 day to initiate feeding, and their maximum consumption of 346.9 ± 62.5 mm²/individual/day was attained on day 27, after which it decreased gradually to around 150–200 mm². An average larva consumed $1,429.5 \pm 131.6$ mm² of *S. carolinense* leaves to complete larval development and an adult consumed $2,510.9 \pm 306.2$ mm² during the first 10 days after the initiation of feeding. Although, the sex ratio of adults varied from 0.2 to 0.8 across the cohorts, sex of the adults did not seem to make a noticeable difference on the leaf consumption.

DISCUSSION

The results of the accuracy test indicate that the IPS was sufficiently accurate for the present purpose. Further, we compared the consumption rates measured by the IPS with those by the AM method to determine whether

the method devised here could be applied to measure the consumption by leaf-scraper insects. The small difference between the two measurements indicated that our method could accurately measure leaf consumption, although we examined using the leaves with a rather simple feeding trace.

We measured more than 150 infested *S. carolinense* leaves by the IPS for the leaf consumption experiment. It was not so economical to store many color digital image data which demanded a large memory space in the storage device of the computer. Thus we visually recorded the infested leaves with color slide film upon collection. The film gave high quality leaf images which were needed to separate delicate infested parts from the sound parts of the leaves by gray levels. Additionally, once the pictures were taken, the images could be analyzed at any time.

It took from 2 min 30 s to 3 min to process one leaf sample; the system was not as efficient as that of Nolting and Edwards (1985). In our system, there were several manual processes such as determination of gray levels, because the condition of the infested leaves varied

from sample to sample. Careful determination of gray levels was particularly important to obtain precise measurements in our system. Although we measured detached leaves in this study, we may be able to improve the method for nondestructive measurement of consumption rates in the field.

The pattern of temporal change in food consumption of *E. vigintioctopunctata* larvae measured in terms of leaf area (Fig. 2) was essentially the same as that of other phytophagous insects measured in fresh or dry weight (Waldbauer, 1968). In *E. vigintioctopunctata*, the last two stages contributed to 86.6% of the total consumption in the larval stage.

The abdomen of newly-emerged adults was flat and the females did not have mature eggs. The first egg mass was laid between 8 and 12 days after emergence. The sexual maturity of the adults was likely to have been responsible for the high feeding activity after adult emergence (Kono, 1982).

It is not possible to measure food consumption in fresh or dry weight directly, because water content or dry weight of the leaves at the start of insect feeding must be estimated by taking samples from the leaves (Waldbauer, 1968; van Loon, 1991). Thus leaf area removal can be a convenient measure of food consumption by herbivorous insects. It is also a better measure of herbivore impact on the host plants than the fresh or dry weight measure in terms of photosynthesis reduction.

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REFERENCES

- Baur, R., A. Fritschy, R. Camenzind and G. Benz (1990) Quantitative analysis of leaf area loss caused by insects: A method combining photography and electronic image. *J. Appl. Ent.* 109: 182-188.
- Benjamin, D. M., G. H. Freeman and E. S. Brown (1968) The determination of irregularly-shaped areas of leaves destroyed by chewing insects. *Ann. Appl. Biol.* 61: 13-17.
- Hargrove, W. W. and D. A. Crossley, Jr. (1988) Video digitizer for the rapid measurement of leaf area lost to herbivorous insects. *Ann. Entomol. Soc. Am.* 81: 593-598.
- Jensen, R. L., L. D. Newsom, D. C. Herzog, J. W. Thomas, Jr., B. R. Farthing and F. A. Martin (1977) A method of estimating insect defoliation of soybean. *J. Econ. Entomol.* 70: 240-242.
- Katakura, H., M. Shioi and Y. Kira (1989) Reproductive isolation by host specificity in a pair of phytophagous ladybird beetles. *Evolution* 43: 1045-1053.
- Kogan, M. and R. D. Goeden (1969) A photometric technique for quantitative evaluation of feeding preferences of phytophagous insects. *Ann. Entomol. Soc. Am.* 62: 319-322.
- Kono, Y. (1982) Change of photoperiodic sensitivity with fat body development during prediapause period in the twenty-eight-spotted lady beetle, *Henosepilachna vigintioctopunctata* Fabricius. *Appl. Entomol. Zool.* 17: 92-101.
- Ninomiya, S., Y. Ohishi and K. Saio (1992) Image database with enhanced analyzing system. *IDEALS. Agr. Res.* 1: 23-45 (in Japanese with English summary).
- Nolting, S. P. and C. R. Edwards (1985) Defoliation assessment using video image and microcomputer. *Bull. Entomol. Soc. Am.* 31: 38-42.
- Pedigo, L. P., J. D. Stone and R. B. Clemen (1970) Photometric device for measuring foliage loss caused by insects. *Ann. Entomol. Soc. Am.* 63: 815-818.
- Shimizu, N., T. Harashima and S. Yagi (eds.) (1994a) *Pictorial Guide of Exotic Weeds*. Chikusan Gijutsu Shinkokai, Tokyo. 44 pp. (in Japanese).
- Shimizu, N., J. Uozumi, T. Nishida, T. Harashima, K. Matoba, Y. Tamura, M. Tachibana, K. Ito, K. Ogino, S. Sato, R. Kobayashi and H. Tateno (1994b) Recent situation of emergence of alien weeds invading grasslands and forage crop fields. *J. Weed Sci. Soc., Jpn.* 39 (special issue 1): 228-229 (in Japanese).
- Strong, D. R., Jr., J. H. Lawton and R. Southwood (1984) *Insects on Plants*. Blackwell Sci. Pub., Oxford. 313 pp.
- van Loon, J. J. A. (1991) Measuring food utilization in plant-feeding insects - toward a metabolic and dynamic approach. In *Insect-Plant Interactions*. Vol. III (E. A. Bernays ed.). CRC Press, Boca Raton, pp. 79-124.
- Waldbauer, G. P. (1968) The consumption and utilization of food by insects. *Adv. Insect Physiol.* 5: 229-288.