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Effects of geminivirus infection on population dynamics, growth and photosynthesis of *Eupatorium makinoi*

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Introduction

Plant viruses are found in many natural plant communities (e.g., MacClement and Richards 1956). Since plant viruses can cause long lasting and highly selective damage to their host plants, they, like other plant pathogens, play an important role in shaping plant communities. This has been recognized in agricultural plant communities for a long time (for a review, Matthews 1991). However, little effort has been made to examine the role of virus infection in natural plant communities.

I have been studying the physiological and ecological effects of geminivirus infection on *E. makinoi* plants. Yellow variegation is the most prominent symptom caused by the geminivirus in *E. makinoi* leaves. Since yellow variegation inevitably affects photosynthesis, I have focused on the effects of virus infection on leaf photosynthesis. In this paper, I summarise a series of my studies for the *E. makinoi* – geminivirus system to clarify the effects of virus infection on leaf photosynthesis, growth and population dynamics. Based on these data, I discuss the physiological changes to photosynthesis in virus-infected leaves and the mechanism of regulation of population dynamics by the virus infection.

Materials and methods

Study system

Eupatorium makinoi Kawahara et Yahara (Asteraceae) plants are perennial, common in open disturbed places along roadsides or forest margins in Japan. They are frequently

infected by tobacco leaf curl geminivirus (TLCV) in their natural habitats. Infected plants are distinguished by a visible symptom, yellow variegation.

Photosynthetic rate and determination of chlorophyll (Chl)

Both virus-infected and uninfected plants of *E. makinoi* were grown in vermiculite in pots in a naturally lit glasshouse. CO_2 exchange rates of attached leaves were measured at 24.5 ± 1˚C (range) using a portable gas-exchange system (LI-6400, Li-Cor, Lincoln, NE, USA). Measurements were made in a gas phase containing 350 μl l^{-1} CO_2 and ambient O_2 (21%). The light source was a 150 W halogen lamp. After the measurements of gas exchange, absorptance of the leaf was measured with an integrating sphere (Funayama et al. 1997b). Chl was extracted from leaf discs with N, N-dimethylformamide. Chl contents were determined according to Porra et al. (1989).

Determination of 5-aminolevulinate (ALA)-synthesising capacity

Plants were grown in vermiculite in pots in a controlled environment. The light source was a bank of white fluorescence tubes. Photosynthetically active photon flux density (PPFD) was 380 μmol m^{-2} s^{-1}. Day/night air temperatures were 25/20ºC, relative humidity was 60%, and day length was 12 h. ALA synthesis is one of the rate-limiting steps of Chl synthesis (von Wettstein et al. 1995). Accumulated ALA in leaves soaked in levulinic acid, an antagonistic inhibitor of ALA dehydratase, was regarded as ALA-synthesising capacity. Leaf disks (c.a. 2.4 cm^2) were incubated with 40 mM levulinic acid in 20 mM phosphate buffer (pH 7.1) in the light (120 μmol m^{-2} s^{-1}) for 2 h, and then frozen in liquid nitrogen. Incubation began from the beginning of a light period. The frozen leaf disks were homogenised in 20 mM phosphate buffer (pH 7.1), and centrifuged (20,100 x g, 10 min). Ethylacetoacetate was added to the supernatant, which was subsequently boiled for 10 min and cooled on ice for 5 min. Ehrlichs reagent was added and colour development was measured at 553 nm using a spectrophotometer. 7.45 x 10^4 M^{-1} cm^{-1} was used as the extinction coefficient for calculating amounts of ALA.

Growth analysis

Both virus-infected and uninfected plants were collected from one field population, and grown in three frames under different light conditions. Each frame contained 50 each of infected and uninfected plants at the beginning of the experiment. Light conditions of the three frames were: 70% of full sunlight for high light condition (HL), 15% for medium light condition (ML) and 5.5% for low light condition (LL). The ML and LL frames were covered with three and five layers of black shading screen, respectively.

Growth of 20 each of the infected and uninfected plants was followed in a non-destructive manner for each of the three light conditions in the early vegetative phase. The period from 4 to 14 June 1997 is termed T1 and the period from 14 to 25 June is termed T2. Ten each of both infected and uninfected plants were harvested three times from each light condition during the experimental period. From these destructive harvests, relationships between plant dry mass or leaf area and non-destructive parameters (height, basal diameter and leaf lamina length) were determined. RGR, NAR and leaf area ratio (LAR) were calculated using estimated values of dry mass and leaf area. RGR and NAR were calculated according to the method of Venus and Causton (1979). LAR was calculated as the mean of leaf area/biomass at the beginning and the end of the period under consideration.

Demography of a E. makinoi *population under a virus epidemic in the field*

A plot (6 x 20 m) was set up in 1991 in Gora-dani (Yahara and Oyama 1993), where is located in Fukuoka Prefecture in Japan (33º33' N, 130º34' E, 265 m above sea level). The Gora-dani population was on the floor of a plantation of Japanese cedar, *Cryptomeria japonica* (L. fil.) D. Don, and the average relative PPFD measured in September at the height of 1.5 m above the ground was 16% of full sunlight.

Demography of the Gora-dani population was studied from 1991 to 1998. A field census was taken every year in late September or early October. The locations of all shoots of *E. makinoi*, including newly established plants, were mapped, and their infection status was assessed by the visible symptom. Plant height was measured with a scale in 1991 and 1992. Most plants had only one shoot. Relative stem growth rate (RSGR) was calculated as:

$$RSGR = (H_2 - H_1)/(\Delta T \times H_1) \qquad (cm\ cm^{-1}\ yr^{-1}),$$

where H_1 and H_2 refer to plant heights in 1991 and 1992, respectively. ΔT is one yr in these calculations.

Statistical analyses

Data was analysed with Stat View ver. 5.0 (SAS institute, Cary, NC, USA).

Results

Effects of virus infection on leaf photosynthesis

Fig. 1 shows typical light-response curves of fully developed *E. makinoi* leaves infected and uninfected by the geminivirus. The initial slope of light-response curves of photosynthesis (photosynthetic rate under ambient CO_2 condition on a leaf area basis) measured on an incident quantum basis ($\phi_{CO_2, inc}$) was significantly lower in infected leaves than that in uninfected leaves (Mann-Whitney U-test, $P < 0.05$) (Fig. 1a). On the other hand, the light-saturated photosynthetic rate at ambient CO_2 (P_{max}) in infected leaves was comparable to that in uninfected leaves (Mann-Whitney U-test, $P = 0.275$) (Fig. 1). The difference in the initial slopes between infected and uninfected leaves was negligible and insignificant when the photosynthetic rate was plotted against an absorbed quantum (Mann-Whitney U-test, $P = 0.513$) (Fig. 1b). Chl content (mmol m^{-2}) and Chl *a*/*b* in leaves in Fig. 1 were 0.10 ± 0.02, 3.91 ± 0.28 in infected leaves, and 0.28 ± 0.04, 3.10 ± 0.01 in uninfected leaves (Mean ± SD, $n = 3$). Leaf Chl level was significantly lower (Mann-Whitney U-test, $P < 0.05$), and Chl *a*/*b* was significantly higher (Mann-Whitney U-test, $P < 0.05$) in infected leaves than in uninfected leaves. This suggested that preferential loss of Chl *b* occurred in infected leaves.

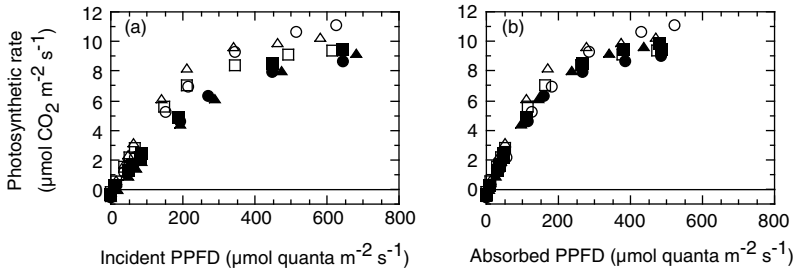

Fig. 1 Light response curves of net photosynthesis (a) on an incident quantum basis and (b) on an absorbed quantum basis in virus-infected and uninfected *E. makinoi* leaves. Each type of symbol indicates one leaf. Closed symbols, infected leaves; open symbols, uninfected leaves (Redrawn from Funayama-Noguchi et al. 1998)

Fig. 2 shows Chl content, $\phi_{CO_2, inc}$ and P_{max} in leaves of various sizes. Leaf size is considered as an index of leaf age. In uninfected leaves, Chl content, $\phi_{CO_2, inc}$ and P_{max} increased with lamina length and reached steady-state levels (Fig. 2). In infected leaves, P_{max} increased with the increase of lamina length as uninfected leaves (Fig. 2c), while Chl content and $\phi_{CO_2, inc}$ remained at low levels (Fig. 2a, b). These results suggested that the decrease in photosynthetic rate under low irradiance was closely related with less Chl accumulation in infected leaves during leaf development.

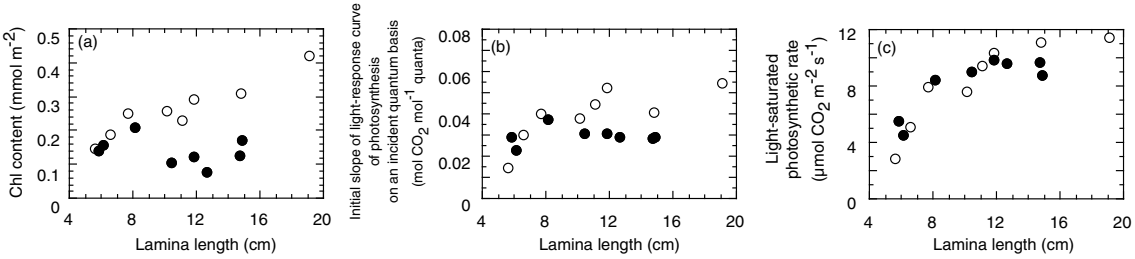

Fig. 2 (a) Chl content, (b) the initial slope of light-response curve of photosynthesis on an incident quantum basis, (c) light-saturated photosynthetic rate in the leaves of various sizes. Each point indicates one leaf. Closed symbols, infected leaves; Open symbols, uninfected leaves (Data of Funayama-Noguchi et al. 1998)

Fig. 3 shows ALA-synthesising capacity in virus-infected and uninfected leaves of *E. makinoi*. ALA was accumulated significantly less in infected leaves than in uninfected leaves (Fig. 3, two-way ANOVA, infection, $P < 0.0001$; leaf size, $P < 0.001$), which suggested that the activity of Chl synthesis decreased in infected leaves.

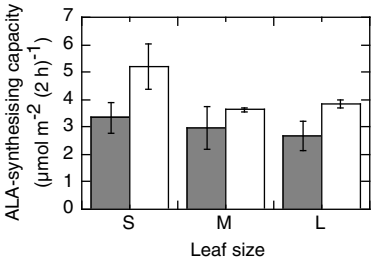

Fig. 3 ALA-synthesising capacity in expanding (S, M) and fully developed (L) leaves of *E. makinoi* infected and uninfected by virus. S: lamina length of leaves was shorter than 4 cm; M: lamina from 4 to 8 cm; L: lamina longer than 8 cm. Means ± SD (n = 4-7) are shown. Shaded columns, infected plants; open columns, uninfected plants. (Data of Funayama-Noguchi 2001)

Effects of virus infection on growth of individual plants

Fig. 4 shows the basic parameters of growth analysis (RGR, NAR, LAR) at T1 and T2.

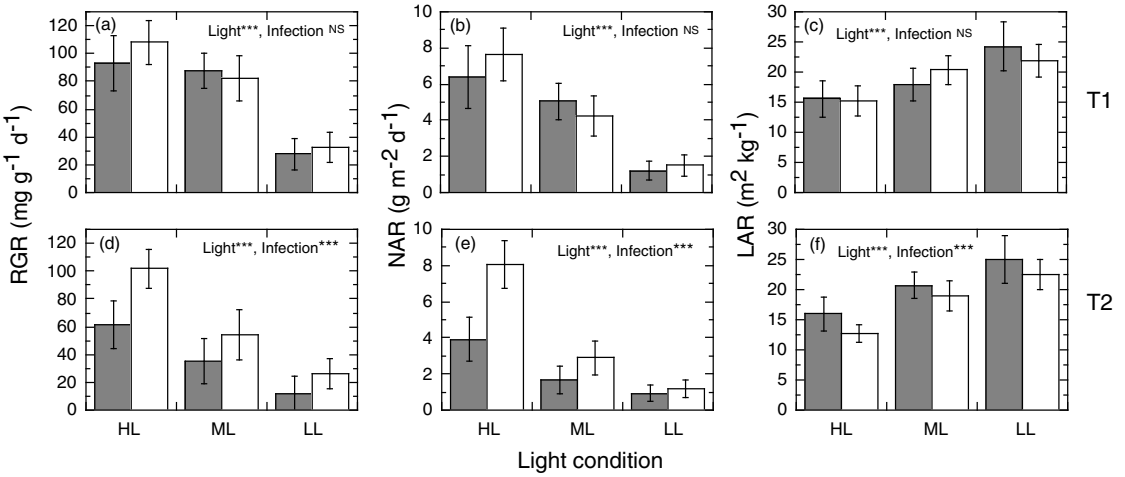

Fig. 4 (a) (d) RGR, (b) (e) NAR, (c) (f) LAR of virus-infected and uninfected *E. makinoi* plants under three light conditions (HL, ML, LL) in two growth periods (T1 T2). Means ± SD (n = 20) are shown. Shaded columns, infected plants; open columns, uninfected plants. *** $P < 0.001$; NS not significant (two-way ANOVA). (Data of Funayama and Terashima 1999)

Significant effects of light were found in all of RGR, NAR and LAR at both T1 and T2.

At T1, no significant differences were found between infected and uninfected plants for any of RGR, NAR and LAR (Fig. 4a, b, c), probably because symptoms in infected leaves had not fully developed by T1. On the other hand, the effects of infection were significant for all the parameters at T2. RGR and NAR were significantly decreased by virus infection (Fig. 4d, e), whereas LAR of infected plants was significantly greater than that of uninfected plants (Fig. 4f). These results suggested a close relationship between RGR and NAR. The increase in LAR could compensate for the impaired assimilation in infected leaves. However, in this case, the compensation by the increased LAR was much smaller than the decrease in NAR by infection. Thus, it can be concluded that the decreased NAR was the predominant factor explaining the decrease in RGR in virus-infected plants.

Effects of virus infection on population dynamics in the field

Fig. 5 shows the population dynamics of *E. makinoi* in the Gora-dani plot. The epidemic of virus infection occurred in 1992, and numbers of plants rapidly declined from 119 individuals in 1991 to a single individual in 1998 (Fig. 5).

Fig. 6 shows RSGR between 1991 and 1992 in Gora-dani. Positive and negative RSGRs mean increase and decrease in height, respectively. Infection significantly decreased the RSGR (Fig. 6). Mortality of infected plants significantly increased with decreasing plant height, but the relationship between mortality and plant height was not significant for uninfected plants (Funayama et al. 2001).

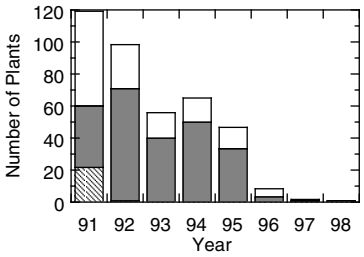

Fig. 5 Year changes in the numbers of *E. makinoi*, both infected and uninfected by virus in Gora-dani. Shaded columns, infected plants; open columns, uninfected plants; hatched columns, plants in which infection status was unknown due to withered leaves. (Data of Funayama et al. 2001)

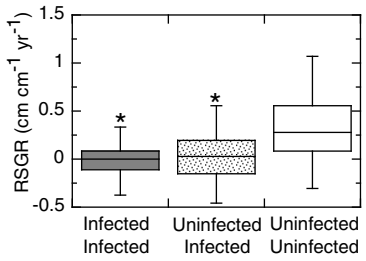

Fig. 6 Relative stem growth rate in *E. makinoi* plants in the Gora-dani population. Infected-infected: plants infected both in 1991 and 1992 (n = 28); uninfected-infected: plants uninfected in 1991 and infected 1992 (n = 27); uninfected-uninfected: plants uninfected both in 1991 and 1992 (n = 20). The asterisk denotes the significant difference from uninfected plants (Dunnett's method, $P < 0.05$). (Data of Funayama et al. 2001)

Discussion

Physiological changes of photosynthesis in virus-infected E. makinoi *leaves*

In virus-infected *E. makinoi* leaves, $\phi_{CO_2,\,inc}$ decreased (Fig. 1). The decrease in $\phi_{CO_2,\,inc}$ was caused by the decrease in light absorptance (Fig. 2). The low light absorptance was attributed to the small amount of Chl proteins, particularly light-harvesting Chl *a/b* protein complex associated with photosystem II (LHCII) (Funayama et al. 1997a, b). The amount of LHCII is regulated by Chl *b* biosynthesis (Flachmann and Kühlbrandt 1995, Tanaka et al. 2001). A plant preferentially makes more Chl *a* and less Chl *b*, when the Chl supply is limited by a bottleneck in Chl synthesis (e.g., Falbel and Staehelin 1994). In infected leaves, ALA-synthesising capacity was lowered, and less Chl accumulated during leaf development, compared with uninfected leaves (Fig. 2, 3). These would cause the low amount of LHCII. In summary, this study demonstrated that

the decrease in the activity of Chl synthesis lowered the photosynthetic rate through the decrease in light absorptance in virus-infected leaves. Further studies of both plants and viruses at the molecular level may clarify the relationship between virus replication and the inhibition of Chl synthesis.

The mechanism of regulation of E. makinoi *population by virus infection*

Virus infection exerted adverse effects on plant growth in both the field (Fig. 6) and the glasshouse (Fig. 4). The growth analysis in the glasshouse showed that the decrease in RGR caused by virus infection could be attributed to the decrease in NAR, which was determined primarily by leaf photosynthesis. These suggested that the impairment of photosynthesis by virus infection would cause the inferior performance of infected plants not only in the glasshouse, but also in the field.

The population of *E. makinoi* rapidly declined to a single individual during the virus epidemic in the Gora-dani plot. After the virus epidemic in 1992, most of the plants became infected and many infected plants died (Fig. 5). The adverse effects on RSGR (Fig. 6) made infected plants small, which resulted in death of infected plants through size-dependent mortality. This study showed the mechanism of the population decline caused by virus infection and the importance of virus infection as a factor that determines the dynamics of *E. makinoi* population in the field.

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