Papaya Ringspot Virus Influences Net Gas Exchange of Papaya Leaves

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Abstract. Leaves of container-grown papaya (*Carica papaya* L.) plants were inoculated with papaya ringspot virus (PRV) to determine its influence on dark respiration and photosynthesis. Photosynthetic capacity, apparent quantum yield, ratio of variable to maximum fluorescence from dark-adapted leaves, and photosynthetic CO₂-use efficiency were reduced by PRV infection. Internal CO₂ partial pressure at ambient external CO₂ was not affected, but leaf dark respiration was increased by PRV infection. These results suggest that reduced growth, yield, and fruit quality common in PRV-infected papaya plants is caused, at least partially, by reduced photosynthesis and increased respiration.

Papaya is a popular fruit crop in the tropics and subtropics. It is an important agricultural commodity in Guam, and the current market makes producing papaya profitable. Papaya production in Guam, however, is limited severely by papaya ringspot virus (PRV), partially due to an abundance of aphid vectors and PRV-infected papaya plants in natural settings (Wall, 1989). A planting is almost always infected extensively before harvest. Although plants typically are removed soon after symp-

toms appear, it also is common for growers to leave infected plants in the hope that some of the fruit will be marketable.

PRV reduces papaya growth, fruit set, and fruit quality and causes leaf mottling and distortion (Pohronezny and Litz, 1987; Purcifull, 1972). Plant viruses, such as leaf mosaic (Decker and Tio, 1958) or leaf reduction viruses (Singh et al., 1979), reduce photosynthesis in the host plant (Agrios, 1978). The photosynthetic capacity of a papaya genotype also influences papaya fruit quality (Salazar, 1978).

The objective of this study was to determine the effects of PRV on papaya leaf physiology. These data may explain the physiological mechanism by which PRV affects papaya growth and development and improve management of PRV-infected papaya plantings.

Plant material. 'Kapoho' papaya seeds were planted in flats in early Sept. 1991 and transplanted to 2.6-liter containers on 16 Oct. 1991. Sunshine Mix 4 (Fisons Horticulture, Vancouver, Canada) was used as the container medium. The plants were fertilized with a solution of 0.68 g HydroSol (W.R. Grace & Co., Fogelsville, Pa.) and 0.45 g calcium nitrate/liter. The solution's nutrient concentration approximated one-half strength Hoagland's solution (Hoagland and Amon, 1950). Each container received 125 ml of solution once a week until 2 Dec. 1991, and twice a week thereafter. The plants received rainfall or irrigation to maintain H₂O content at near-container capacity (determined by tensiometry). All plants were grown in full sun under insect-protective lucite screening (BioQuip Products, Santa Monica, Calif.). Maximum photosynthetic photon flux (PPF) under these conditions was $\approx 1250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the top of the plant canopy.

On 13 Nov. 1991, when ≈ 15 cm high, one-half of the plants was inoculated with PRV. The inoculum was made by grinding 1 g of virus-infected leaf tissue with 10 ml potassium phosphate buffer (Purcifull, 1972). Two newly expanded leaves per plant were abraded with carborundum powder and the inoculum. Two leaves of each of the control plants were abraded similarly with carborundum powder and potassium phosphate buffer. The mottling symptoms characteristic of PRV on papaya (Purcifull, 1972) were visible on the inoculated leaves in < 2 weeks following inoculation, and vein clearing and leaf deformation were apparent on systemically infected leaves by week 3.

Leaf gas-exchange measurements. Leaf gas exchange was measured 7 to 8 weeks after

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inoculation (5-8 Jan. 1992), when characteristic PRV leaf mottling and distortion were evident on all inoculated plants. All control plants were symptomless.

A survey of gas exchange of leaves showing PRV symptoms revealed that leaf areas with various degrees of mottling but no distortion had measurable gas exchange, whereas areas with distortion had negligible net gas exchange. As a result, leaves with mottling but no distortion were chosen for study. Recently expanded leaves of the same age were used for all measurements.

Net CO₂ assimilation (A) as a function of incidental PPF was determined in six plants per treatment. The measurements were made on the first four plants per treatment on 5 Jan. 1992 and the remaining two plants per treatment on 6 Jan. The plants were transferred to a laboratory the morning they were measured. Leaf gas exchange was measured using a portable photosynthesis system (model LI-6200; LI-COR, Lincoln, Neb.) with a gas analyzer (model LI-6250). One leaf on each plant was inserted into a 1-liter chamber (model LI-6200-12) fitted with inserts to enclose 10 cm² of leaf surface. Light was supplied by three 300-W quartz halogen lamps suspended above a thermal-filtering 2.3-cm-deep circulating water bath. Light level was lowered between each measurement by placing nylon screening in layers over the cuvette. This procedure allowed up to nine measurements at various light levels between the light compensation point and 1115 μmol·m⁻²·s⁻¹. Ambient temperature (33.4 ± 0.2C) and CO₂ concentration (358 ± 10 μbar) were maintained by opening the exhaust port between each measurement. Three 10-sec CO₂ depletion periods were recorded at each light level, and appropriate software was used to calculate A. Since all leaves were saturated at ≈800 μmol·m⁻²·s⁻¹, A at the highest PPF level was considered photosynthetic capacity (A_{max}).

The relationship between A and external CO₂ concentration was determined using six randomly chosen plants per treatment. A and CO₂ were measured on the first four plants per treatment on 7 Jan. 1992 and the two remaining plants per treatment on 8 Jan.. The laboratory conditions were the same as those described previously. The chamber was positioned to obtain a PPF of 1115 μmol·m⁻²·s⁻¹. The procedure described by Davis et al. (1987) was used to determine A at various external CO₂ concentrations from the compensation point to ≈700 μbar CO₂. Steady-state relative humidity was maintained in the closed system by monitoring and continuously adjusting a mass-flow valve that controlled the air stream passing through a magnesium perchlorate H₂O scrubber. Ambient air during measurement was 35.4 ± 0.3C.

Dark respiration (R_d) was measured on one leaf on each of six randomly selected plants per treatment on 5 Jan. 1992. The chamber was enclosed in black plastic to exclude light after a leaf was inserted in the cuvette. Ambient air (29.8 ± 0.2C) and CO₂ (369 ± 4 μbar) were maintained in the cuvette during dark adaptation by keeping the exhaust port on the analyzer open until measurements were made. Three 10-sec CO₂ efflux periods were recorded after 10 min of dark adaptation.

Chlorophyll fluorescence was measured on one leaf on each of seven randomly selected plants per treatment on 7 Jan. 1992. Fluorescence was measured at 1400 HR with a chlorophyll fluorescence measurement system (model CF-1000; P.K. Morgan Instruments, Andover, Mass.) on the adaxial surface of each leaf. Before fluorescence was measured, 30 min of dark adaptation was provided using the system cuvettes. Induced fluorescence was measured for 60 sec using 475-μmol·m⁻²·s⁻¹ excitation light.

Data analysis. R_d, A_{max}, internal CO₂ concentration (C_{int}) at A_{max}, and chlorophyll fluo-

Table 1. Influence of papaya ringspot virus on net CO₂ assimilation at saturating light and ambient CO₂ (A_{max}), internal CO₂ partial pressure at A_{max} (C_{int}), CO₂ use efficiency, apparent quantum yield (ϕ), the ratio of variable to maximal fluorescence (F_v/F_{max}), and dark respiration (R_d) of 'Kapoho' papaya plants. Measurements were made on systemically infected leaves, 7 to 8 weeks after inoculation (n = 7 for F_v and 6 for others).

Variable	Control	Diseased
A _{max} (μmol CO ₂ /m ² per sec)	15.7	8.9**
C _{int} (μbar CO ₂)	273	267 ^{ns}
CO ₂ use efficiency (μmol CO ₂ /μbar CO ₂)	0.053	0.030***
ϕ (μmol CO ₂ /μmol photons)	0.039	0.016***
F _v /F _{max}	0.80	0.76*
R _d (μmol CO ₂ /m ² per sec)	1.79	2.94**

^{ns},***,****Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively.

rescence data were subjected to analysis of variance. The response curves of A, as influenced by light or CO₂, were determined by nonlinear regression analysis. Apparent quantum yield (ϕ) was calculated using linear regression analysis as the slope of the initial part of the light response curve (<150 μmol·m⁻²·s⁻¹). Similarly, photosynthetic CO₂-use efficiency was defined as the rate of increase in A with increase in ambient CO₂ concentration, and was calculated as the slope of the linear regression, with A as the dependent and CO₂ concentration as the independent variable. The slopes for all replications in the control or PRV-infected groups were homogeneous based on analysis of covariance. Replications then were pooled to test if treatment differences were significant using the same test.

Papaya leaves from PRV-infected plants exhibited an A_{max} 57% of that of leaves from healthy plants (Fig. 1, Table 1). The light dependence of A in the light levels used in this study followed a logarithmic pattern (Fig. 1). Apparent ϕ of diseased leaves decreased to 41% of that of control leaves (Table 1). These results indicate that diseased plant A may be reduced compared to that of healthy plants, even during the initial and ending phases of each photoperiod and on severely cloudy days that are common during the rainy season of tropical environments.

PRV reduced photosynthetic CO₂-use efficiency relative to that of control plants (Table 1). The change in A per unit change in external CO₂ was 1.8 times greater for control plants than diseased plants. Calculations from the highest light level in the light curves revealed that the reduced A_{max} from PRV infection was not accompanied by a change in internal CO₂ partial pressure (Table 1). As a result, CO₂ availability as a substrate was most likely not a factor in the reduced A_{max} by PRV at ambient CO₂ concentration.

Fig. 1. Net CO₂ assimilation (A) of leaves from control (■) and papaya ringspot virus-infected (▲) *Carica papaya* plants as a function of incident photosynthetic photon flux (PPF). Each point represents the mean of six replications ±SE determined on 5–6 Jan. 1992. Equations for the response curves are A = (–21.67) + 5.32 ln PPF, r² = 0.98 (control); and A = (–15.77) + 3.37 ln PPF, r² = 0.98 (diseased); SE was smaller than symbols where no bars are visible.

The ratio of variable to maximal fluorescence (F_v/F_{max}), taken from the fluorescence induction curves obtained from dark-adapted leaves, was lower for diseased than control papaya plants (Table 1). Fluorescence is a sensitive indicator of damage to the photosynthetic apparatus caused by environmental stress. A decline in F_v/F_{max} indicates photoinhibitory damage, and, when measured at ambient temperature, the interpretation of results is restricted to the pigments associated with photosystem II (Bolhar-Nordenkamp et al., 1989). The decline in F_v/F_{max} in the diseased papaya plants was due exclusively to reduced F_v (data not shown). Environmental stress that causes thylakoid damage usually lowers the variable fluorescence yield (Krause and Weis, 1984), and reduced F_v indicates weakened electron flow through photosystem II (Lloyd et al., 1986). This impaired photosynthetic electron transport may render PRV-infected papaya plants more susceptible to photoinhibitory damage by the high incidental PPF that is common in the tropics. Further, these data indicate that fluorescence may help in studying the influence of biotic and abiotic stresses on photosynthesis.

R_d in papaya leaves was increased by PRV infection. Mean R_d was 1.6 times higher in the diseased plants than in the healthy plants (Table 1). This increased respiratory activity, coupled with the reduced photosynthetic capacity of an individual leaf, would severely reduce the extent to which each leaf could function as a carbon source for the diseased papaya plant.

Various degrees of symptoms appeared on PRV-infected plant leaves, a characteristic typical of diseased plants in the field. Under

the conditions of this study, photosynthetic capacity depended directly on the symptoms in the immediate area of gas-exchange measurements. For example, the measured CO_2 flux of symptomless areas of leaf tissue on PRV-infected plants was not different from that of the control plants (data not shown). As a result, the overall canopy photosynthesis of PRV-infected papaya plants may depend on the percentage of leaves with symptoms and the severity of those symptoms.

Knowing the physiology of diseased papaya plants may help in making rational crop management decisions, since an environmental or genetic factor that influences growth can do so only by way of its effects on physiological plant processes (Kramer, 1980). These results suggest that reduced photosynthesis and increased respiration, combined with reduced leaf area, may allow PRV to reduce growth, yield, and fruit quality of infected papaya plants. Wind, drought, and edaphic conditions in heavy soils during the rainy season commonly reduce papaya productivity in Guam and other tropical regions. Producers who choose to continue to grow rather than rogue PRV-infected papaya plants should try to reduce these and other environmental stresses that may further decrease photosynthesis.

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