EFFECT OF INSECT CLIP CAGES ON PHOTOSYNTHESIS OF COTTON AND CANTALOUPES

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Abstract

In insect-plant interaction studies, small clip cages are frequently used to confine the target insects on attached leaves. Little information is available on the clip cage effects on the leaf physiology that may confound experimental results. Objective of the study was to quantify the clip cage effects on photosynthesis of cotton (*Gossypium hirsutum* L) and cantaloupes (*Cucumis melo* L). Results showed that 24 h after the enclosure of clip cages (11.3 cm^2 area) leaf chlorophyll content of the sampled cotton leaves was significantly increased compared to the non-enclosed areas. Three days after attaching clip cages to cotton leaves, Rubisco activity and carbon dioxide exchange rate (CER) were significantly decreased. Effects of clip cages on cantaloupe photosynthesis were similar to that found for cotton leaves. We suggest that clip cage effects on leaf physiology and micro-environment should be considered when interpreting results for insect-plant interactions.

Introduction

The use of cages to contain insects on plants is common and necessary to facilitate many types of experiments conducted by entomologists and crop scientists. Many types of cages have been designed, ranging from large cages for whole plants to small clip cages that can be used to confine small insects on individual leaves (Simmons and Yeargan, 1990; Mowry, 1993; McAuslane, 1996). Although the potential for confounding effects of insect cages on experimental results has been recognized (Simmons and Yeargan, 1990), there is little documentation of specific effects on leaf physiology. Our objective was to determine the effect of small clip cages, commonly used for whiteflies, aphids and mites, on photosynthesis of cotton (*Gossypium hirsutum* L. cy Coker 100A- glandless) and cantaloupe (*Cucumis melo* L. cv Imperial 45) leaves.

Materials and Methods

Seeds of cotton cultivar 'Coker 100A-glandless' and cantaloupe cultivar 'Imperial 45' were germinated in pans containing commercial potting mixture (Grow More, Inc., Gardena, CA) or on filter paper in petri dishes, respectively. After one week, uniform seedlings were transplanted into 15 x 15-cm pots containing potting mixture. Plants were cultured in a greenhouse under natural light with day:night temperatures of 28:24 °C. Light intensity peak daily at approximately 1900 mmole/m^2/s photosynthetically active radiation (PAR). There were two cotton plants or one cantaloupe plant in a pot. Beginning 10 days after transplanting, the plants were fertilized two times per week with 750 mL of a solution containing 2 g/L of 20-20-20 (Grow More, Inc., Gardena, CA) supplemented with 0.5 mL/L of a micronutrient solution. Clip cage treatments were started at approximately four weeks after planting.

The fourth and third leaf above the cotyledon for cotton and cantaloupes, respectively, were used as experimental material. The leaves were recently fully expanded when experiments were initiated on 11 May and 11 June 1998, for cotton and cantaloupes, respectively. At this time clip cages, similar in design to those described earlier (Mowry, 1993) were attached to one half of the leaf. The clip cages were made from small petri dishes that were 3.8 cm in diameter and 1.8 cm high (Falcon 3001, Becton Dickinson Labware, Lincoln Park, NJ). The bottom of the petri dish, used for the bottom of the clip cage, was replaced with 52-mesh nylon net for air circulation. The top of the petri dish, used for the top of the clip cage, had six holes, each 3 mm in diameter, in order to facillitate air circulation. Foam rubber was glued to the rim of each cylinder that contacted the leaf surface. A large hair clip was glued to each half of the cage and hinged to allow minimal pressure on the leaf surface. The clip cages weighed an average of 7 g. Clip cages were attached to leaves and supported with metal wire extending from the soil to the leaf such that normal leaf orientation was maintained and the pressure of the cage on the leaf was minimal.

Pots were arranged in a completely randomized designed with eight replications sampled at 4 and 3 times for cotton and cantaloupes, respectively. For each replicate, the leaf tissue enclosed within the clip cage and comparable leaf tissue from the other half of the same leaf were sampled at 1000 h. Clip cages were removed and steady state CER was determined, after which a 0.8-cm^2 leaf disc was removed and frozen in liquid N_2 for subsequent enzyme assay. Another two leaf discs (each 0.4 cm^2) were removed and kept on ice prior to soluble protein and chlorophyll extraction. Controls were sampled in the same manner as described for the clip cage treatment.

Steady state CER and stomatal conductance to CO_2 were determined using a LI-COR 6400 (LI-COR Inc., Lincoln, NE) portable photosynthesis system. The PAR within the sample chamber was 1800 mmol photons/m^2/sec and partial pressure of CO_2 was maintained at a constant 350 mL/L. Chlorophyll fluorescence yield was determined using a WALZ PAM-2000 fluorometer (Walz, Effeltrich, Germany) as described in Feller et al. (1998). The chlorophyll concentration was determined by overnight extraction in dark in 1 mL of methanol, as described by Holden (1976). Rubisco activity was extracted and assayed from light-

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saturated leaf tissue in 1.5 mL of buffer as described in Feller et al. (1998).

Results and Discussion

Attaching clip cages to cotton and cantaloupe leaves significantly increased chlorophyll content for both species after 24 h, from 350 to 400 mg/m^2, probably attributable to a shading effect created by the clip cage (Zhao and Oosterhuis, 1998). For cotton, clip cage treatment differences in chlorophyll content disappeared by 7 days as a result of increased chlorophyll content of the control leaf tissue. For cantaloupes the leaf tissue enclosed within the clip cage maintained higher chlorophyll content compared to controls over a 7-day period. Throughout the experimental time course, the ring of tissue bounded by the edges of the cages became progressively more chlorotic for both plant species.

Contrary to chlorophyll content, steady state CER was decreased by the clip cage treatment within one and three days, from 34 to 29 and 35 to 30 mmole/m^2/sec for cotton and cantaloupes, respectively, and remained significantly lower for the duration of the experiment. It appears that the major effect of the clip cages could be described as an enhancement of senescence, with characteristic declines in CER and Rubisco activity (Feller and Fischer, 1994).

Clip cage inhibition of CER was not associated with stomatal closure as stomatal conductance to CO_2 was similar for both treatments in cotton whereas for cantaloupes the clip cage treatment let to significantly higher stomatal conductance relative to controls.

The initial activity of Rubisco extracted from cotton leaves that were sampled immediately after measuring CER was decreased by 9% and 40% due to the clip cage treatment from 1 and 11 days, respectively (Table 1). The relative decrease in initial Rubisco activity for the clip cage treatment was in proportion to the decrease in CER for both plant species. In addition to the clip cage effect on initial Rubisco activity, the results for controls provided further evidence that the cantaloupes, but not cotton, leaves were undergoing senescence during the experimental time course.

We conclude that the impact of clip cages on leaf development and insect biology may be dependent on the plant species. More importantly, our results indicate that the clip cage had a rapid and significant impact on the physiology of the leaf tissue that is enclosed within the cage. Therefore, the carbon and nitrogen nutrition available to insects would be altered by the clip cage treatment, especially for insects caged for three or more days. Based on the senescence-like symptoms induced by the clip cages, it is possible that the contents of mineral nutrients other than nitrogen were altered. In addition to the perturbations of leaf physiology caused by the clip cages, altered microenvironment within the clip cage, such as increased leaf temperature (personal communication, Eric Erickson, USDA-ARS, Tuscon, AZ) may also impact insect behavior/physiology.

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Table 1. The effect of clip cages on the initial activity of Rubisco on cotton and cantaloupes.

		Non-caged area	Caged-area	
Crop Species	Days after treatment	Initial activity	Initial activity	% increase (+) or reduction (-)
		-------mmol/m^2/sec------		
Cotton1	1	111	101	-9
	11	116	70	-40
Cantaloupes	1	88	91	+3
	11	53	35	-34