Physiological Characteristics of Systemic Toxemia in Soybean

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ABSTRACT

Systemic toxemia in young trifoliolate leaves of soybean is caused by toxin(s) produced in older leaves infected with *Pseudomonas glycinea*. Symptoms were most severe 6-8 days after inoculation, and affected leaves could partially recover. Systemic toxemia could be prevented by steam-killing petioles of inoculated leaves, suggesting that the toxin was translocated in the phloem. Trifoliolate leaves affected by toxemia had a reduced rate of photosynthesis 6-10 days after inoculation, but by the 12th day, their rate of photosynthesis was equal to that of healthy leaves. Although leaves recovered in chlorophyll concentration 10 days after inoculation, stunting was permanent.

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Characteristic toxemia symptoms, stunting and chlorosis, are frequently observed in young trifoliolate leaves of soybean (*Glycine max* L.) when lower leaves are heavily infected with *Pseudomonas glycinea* Coerper (8). Affected leaves become a normal green color as they mature, and stunting becomes less apparent. Pathogenic bacteria have not been isolated from the systemically affected leaves (8), but a toxin capable of causing symptoms of toxemia has been isolated from infected leaves as well as from culture filtrates of the pathogen (5). Symptoms are therefore attributed to a translocatable toxin produced in infected leaves. No information is available on translocation of bacterial toxins in plants. Although most material translocated from soybean leaves is transported in the phloem, the possibility of xylem transport is suggested by the fact that sugars may move through a steam-killed segment of the stem (10).

Toxemia of soybean provides a unique opportunity to study the effect on the host plant of toxin(s) produced in vivo because the symptoms produced are spatially separated from the site of primary infection by the pathogen, and affected tissue partially recovers. Both are unusual features among plant disease symptoms caused by

toxin-producing pathogens. Our objectives were to study translocation of the toxin(s) and to examine the possibility that it impaired the photosynthetic efficiency of soybean. Finally, experiments were conducted to determine whether increases in photosynthetic rate accompanied the apparent recovery associated with increases in chlorophyll concentration and area of leaves.

MATERIALS AND METHODS.—One unifoliolate leaf of 10-day-old greenhouse-grown soybean plants (cultivar Chippewa 64) was inoculated with a 1×10^8 cell/ml suspension of a 24- to 48-hr-old culture of *P. glycinea* by means of an airbrush at 15 psi. When the first trifoliolate leaf unfolded, it showed symptoms of toxemia. Three of five isolates of *P. glycinea* freshly obtained from soybean plants in the field caused toxemia symptoms in all Chippewa 64 plants inoculated. One of these isolates was selected for subsequent tests.

Translocation was studied by interrupting phloem transport. A small segment of petioles of inoculated unifoliolate leaves was treated with steam directed through a No. 19 hypodermic syringe needle (Fig. 1-D). Transverse sections of petioles indicated that steaming destroyed all living cells, including those of the epidermis, cortex, and phloem, but did not disrupt the tracheary elements of the xylem (Fig. 1-B, C). Sets of 10-12 inoculated plants were treated in this manner and compared with controls in each of three experiments.

Photosynthetic rates of lateral leaflets showing toxemia were measured by methods modified from those used by Brun & Cooper (2). By this procedure, individual leaflets were inserted into a 10-cm^2 plexiglass chamber, and fluorescent lights were placed above and below the chamber to illuminate both leaf surfaces. An air mixture with 300-320 ppm CO_2 was passed over the leaflets at 250 ml/min. Change in CO_2 concentration due to apparent photosynthesis was determined with an infrared gas analyser (Beckman Model IR250). Approximately 2 hr prior to each reading, plants were moved from the greenhouse to a well-illuminated growth chamber, and later placed under a bell jar under high light intensity in the laboratory for 15 min to help open stomata before each individual reading. We obtained leaf areas at each reading by making a tracing of the leaf and determining area with a planimeter. A treatment consisted of 10 plants; i.e., one healthy and one diseased plant were grown in each of ten 4-inch clay pots. Initial readings were taken 6 days after inoculation, and four additional readings were taken on the same group of plants and leaves on alternate days thereafter.

Total chlorophyll concentration of leaves in a comparable group of plants was determined on each day in which a photosynthetic reading was taken. A sample consisted of five 8.5-mm-diam discs cut with a cork borer from each of three leaflets from different plants. Three samples were analyzed for both healthy and affected leaves at each reading. Leaf discs were homogenized in 80% acetone in a tissue grinder, and chlorophyll concentration was determined spectrophotometrically as outlined by Strain & Svec (9). Leaf areas were determined as in the photosynthetic study. All data were analyzed by the split plot in time design.

RESULTS.—The first trifoliolate leaf above the inoculated unifoliolate leaf unfolded 4-5 days after inoculation and developed the typical toxemia symptoms of stunting and chlorosis. When only one unifoliolate leaf was inoculated, one lateral leaflet and half of the middle leaflet of the trifoliolate leaf showed symptoms (Fig. 1-A). However, some of this specificity in localization of effects was lost at high inoculum concentrations. Systemic symptoms were not initiated in trifoliolate leaves when they were fully expanded before inoculation of the unifoliolate leaf.

When petioles were steamed to interrupt phloem transport, the leaves remained turgid and survived up to 2 weeks, suggesting that the xylem was functioning normally. Bacterial blight was more severe on inoculated leaves with steamed petioles than on leaves with intact petioles, and leaves inoculated 4 days after petioles were steamed still became readily infected.

When petioles of unifoliolate leaves were steamed immediately before or up to 2 days after inoculation, no toxemia developed in the trifoliolate leaves. The steaming of unifoliolate petioles 3 or more days after inoculation did not stop symptom expression in the trifoliolate leaves, but symptoms did not develop to maximum severity if petioles were steamed prior to the 6th or 7th day after inoculation. It was noted that if phloem transport was not interrupted in plants with an infected unifoliolate leaf, toxemia symptoms developed in three trifoliolate leaves successively as they unfolded. Toxin translocation apparently began about the 3rd day after inoculation, and continued until infected leaves became senescent.

Toxemia-affected leaflets had a photosynthetic rate 70% that of healthy leaflets when the first reading was taken 6 days after inoculation (Fig. 2). From the 6th day onward, rates of photosynthesis of affected leaflets increased through the 12th day after inoculation, when rates were equal to those of healthy plants, whereas in healthy trifoliolate leaflets there was no further increase in rate of photosynthesis after the 8th day. A general decrease of photosynthetic rate was evident in both diseased and healthy plants on the 14th day, although leaves were not noticeably senescent at that time. Leaves of plants in both treatments increased rapidly in area through the 10th day, and thereafter continued to grow slowly (Fig. 3). However, areas of toxemia-affected leaflets were significantly smaller (1% level) for all 5 days on which they were measured (Fig. 3). Recovery in area of affected leaflets occurred from 49% of control on the 6th day to 65% of control on the 10th day, after which no further recovery occurred.

Chlorophyll concentration of toxemia-affected leaflets showed a nearly linear increase from the 6th day through the 14th day after inoculation (Fig. 4). A smaller increase in chlorophyll concentration was

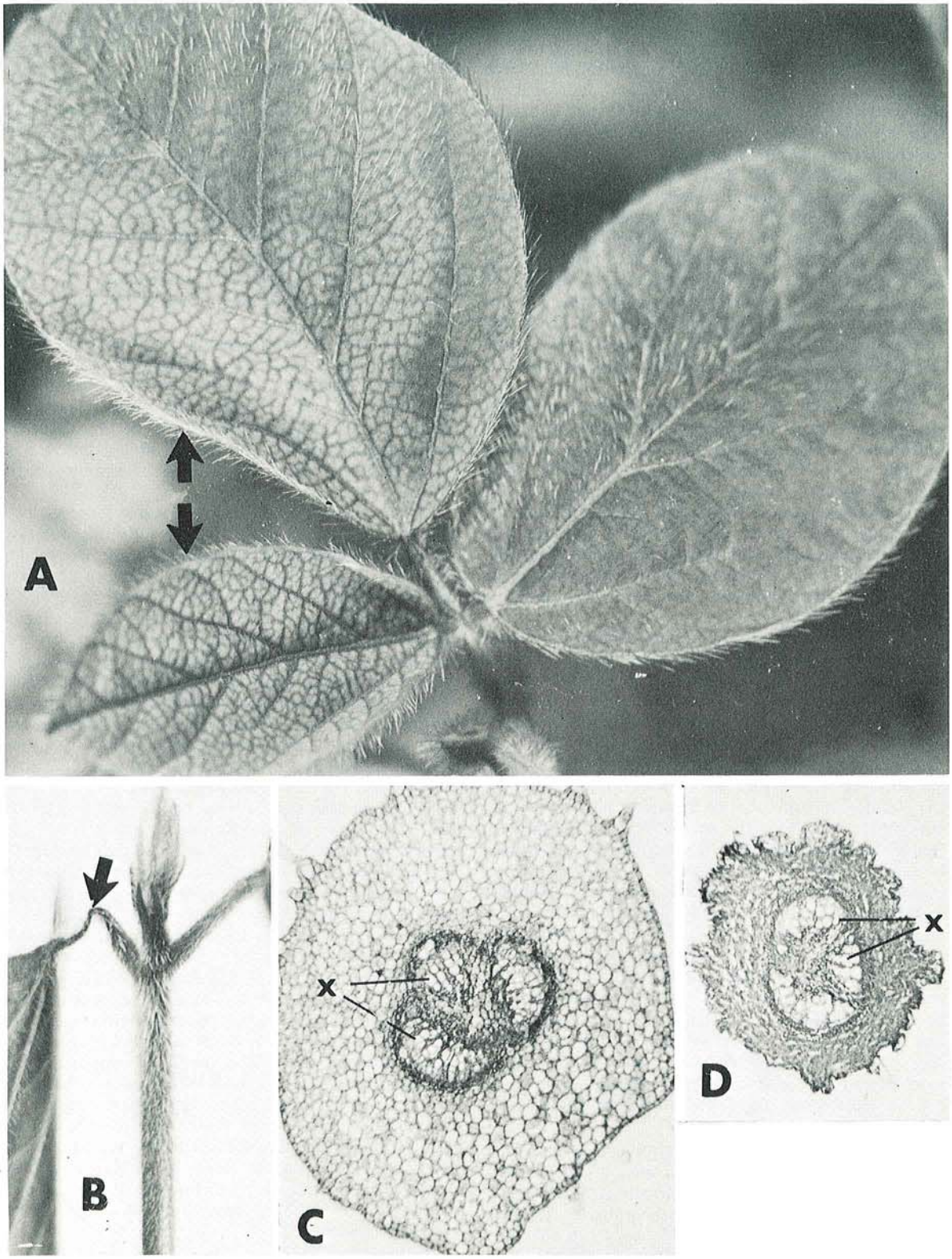


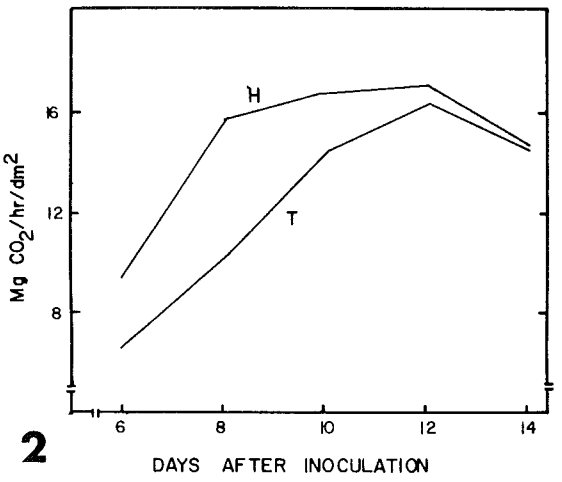
Fig. 1. A) Ten-day-old soybean seedling with symptoms of systemic toxemia resulting from inoculation of one unifoliolate leaf with *Pseudomonas glycinea*. Note stunting and chlorosis predominating on the left half of the trifoliolate leaf (arrows). B) Appearance of steam-treated petiole is indicated. C) Transverse section of a healthy petiole; and D) a steam-treated petiole showing destroyed living tissue but intact tracheary elements.

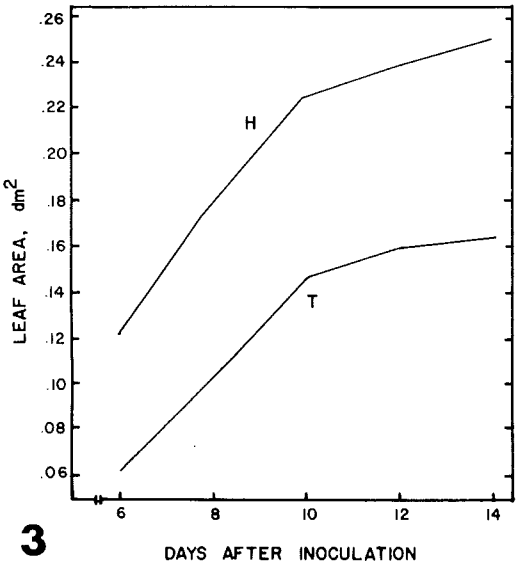
observed in healthy soybean leaves as they matured. The slight decline in chlorophyll concentration of healthy leaves between the 8th and 10th days may be attributed to sampling different groups of plants for each reading. When the first reading was taken, diseased leaves had 77% as much chlorophyll per unit area as control plants. The affected leaves recovered from their chlorotic condition, and on the 10th day no difference in chlorophyll concentration was observed between healthy and diseased leaflets. This result agreed with visual comparison of leaves. Leaflet areas of plants used for chlorophyll extraction were similar to those used for photosynthetic readings.

DISCUSSION.—Failure of systemic symptom development after steam killing of petioles of inoculated unifoliolate leaves, despite the apparently normal development of bacterial lesions on these leaves, suggests that the toxin was translocated in the phloem. Movement of toxin through the parenchyma tissue of the cortex cannot be precluded, but it is doubtful that effective quantities of toxin could be translocated in this manner. The xylem does not appear to be involved in translocation of the toxin.

The ability of toxemia-affected leaves to recover in chlorophyll concentration and rate of photosynthesis may be a reflection of the relatively mild symptoms expressed. Chlorosis caused by other toxin producing organisms; e.g., *P. tabaci, P. coronofaciens* (7), or *Alternaria tenuis* (4), where irreversible damage to plant tissue occurs, is more severe than that caused by *P. glycinea*. Soybean leaves with the severest toxemia symptoms had 22% less chlorophyll than did healthy leaves of the same age, whereas Halloin et al. (4) reported a 90% reduction of chlorophyll concentration in cucumber seedlings treated with tentoxin from *Alternaria tenuis* cultures.

There is lack of agreement on the relationship between loss of chlorophyll in diseased tissue and occurrence of changes in the rate of photosynthesis. Changes in the photosynthetic pattern have been reported to occur before any visible chlorosis for several host-pathogen combinations (1, 3, 6). However, chloroplasts isolated from cotton plants infected with *Verticillium albo-atrum* had a decreased efficiency in carrying out the Hill reaction, suggesting a direct effect on the photosynthetic mechanism (6). On the other hand, Beckman et al. (1) concluded that alterations in the photosynthetic pattern of banana infected with *P. solanacearum* were due to a water





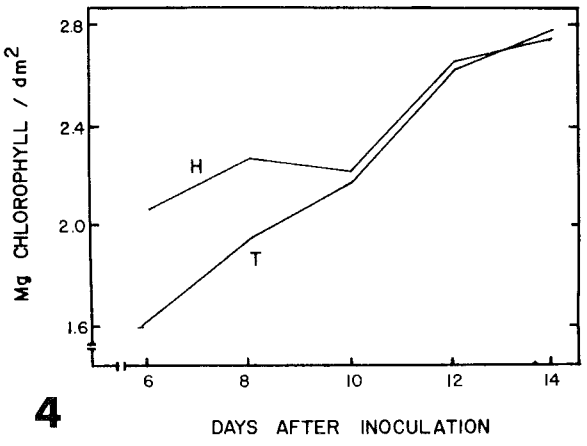


Fig. 2-4. 2) Rates of apparent photosynthesis of healthy (H) and toxemia affected (T) lateral soybean leaflets. Differences 6 and 8 days after inoculation are significant at the 1% level; 10 days after inoculation, at the 5% level; and not significant for the 12th and 14th days. **3)** Areas of lateral leaflets from trifoliolate leaves of healthy (H) and toxemia affected (T) soybean. Differences in area for all 5 days are significant at the 1% level. **4)** Chlorophyll concentration of healthy (H) and toxemia-affected (T) leaflets of soybean. Differences in concentration 6 and 8 days after inoculation are significant at the 1% level, and differences for other days are not significant.

shortage in the leaves rather than to an impairment of the photosynthetic mechanism by the pathogen. In toxemia of soybean, the trifoliolate leaves were chlorotic when the bud unfolded and had an impaired rate of photosynthesis. Recovery in chlorophyll concentration indicated that the diseased leaves were recovering in photosynthetic ability. Apparently, leaves affected by toxin produced by *P. glycinea* in vivo recovered from chlorosis slightly in advance of restoration of photosynthetic activity. Ultrastructural work done in our laboratory indicated a general retardation of cellular development as well as development of chloroplasts in toxemia-affected leaves. The mechanism of action of the toxin may therefore not specifically involve inhibition of the photosynthetic mechanism.

The diseased leaves did not fully recover in area, thus in spite of their recovery in rate of photosynthesis per unit area, these plants did not have the equivalent total capacity of healthy plants to synthesize carbohydrates.

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