Fine Structure Analyses of Stem Nematode-Induced White Flagging in Medicago sativa[1]

W. F. CAMPBELL and G. D. GRIFFIN[2]

Abstract: White flagging of alfalfa, *Medicago sativa* 'Ranger', found associated with *Ditylenchus dipsaci* in the Columbia River Basin was observed in northern Utah during 1971. This is a report on chloroplast changes, induced by *D. dipsaci* in alfalfa leaves, as observed with an electron microscope. Leaves from alfalfa plants infected with *D. dipsaci* were either devoid of normal pigmentation or displayed various shades of yellow-green. Cells of leaf tissue from noninfected plants exhibited normal chloroplast structure. By contrast, the chloroplast structure in cells of leaf tissue from infected plants showed progressive degradation as normal pigmentation decreased. *Key Words:* Alfalfa, electron microscope, injury, pigmentation.

The stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, is reported to induce a condition in alfalfa (*Medicago sativa* L.) known as "white flagging" and has been reported in at least two other locations in the intermountain region (6). These symptoms included partial to complete loss of normal green pigmentation of the leaf and stem tissues. The morphological symptoms we observed in northern Utah agree with those reported previously. These symptoms appeared under conditions of high light intensity, high temperature (35 C and above), and high relative humidity caused by recent over-irrigation. Examination of the weather records for the past 10 years in northern Utah, however, indicated no unusual deviations from the normal. Plants exhibiting the white flagging represented about 1% of the plant population as compared to 2.2% reported by Evans et al. (6). Chang (3) and Chang et al. (4) have observed chloroplast abnormalities in alfalfa hypocotyl tissue infected with this same nematode. This paper reports on the fine structural changes observed in infected alfalfa leaves.

MATERIALS AND METHODS

Alfalfa, *Medicago sativa* L. 'Ranger', infected with stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, and exhibiting a condition known as white flagging was found in a field near Logan, Utah. White, yellow and pale green leaf tissue from infected plants and green leaf tissue from noninfected plants were harvested and fixed 4 hr at 25 C in a mixture of paraformaldehyde:glutaraldehyde buffered to pH 7.2 with 0.2 M cacodylate (8). Tissue was rinsed twice in cacodylate buffer and post-fixed 1 hr at 4 C with cacodylate buffered 2% osmium tetroxide, rinsed twice in same buffer, dehydrated with an ethanol-propylene oxide series and embedded in BEEM capsules in Spurr's medium (12). The resin was polymerized at 40 and 60 C for 24 hr each. Resin blocks were trimmed and thin sections were cut with glass knives on a Sorvall MT-2 Porter-Blum ultramicrotome. Sections were mounted on acetone washed 3 mm, 200 mesh uncoated copper grids and exposed on the grids for 15 min in a saturated aqueous solution of uranyl acetate (13) followed by 5 min in lead citrate (10). Sections were examined and photographed with a Zeiss EM-9A electron microscope.

RESULTS

Evidence from electron micrographs indicated that the palisade and mesophyll cells from green noninfected leaf tissue have normal cell walls, cytoplasm and vacuoles (Fig. 1). The cytoplasm is largely restricted to a thin parietal layer around the periphery of the cell, and bounded by the plasmalemma and the vacuolar membrane, the tonoplast. The dominant organelle in the cytoplasm is the chloroplast. These chloroplasts contain stroma, thylakoid units of the grana, starch grains and osmiophilic bodies or plastoglobuli. By contrast, in leaf cells from infected plants exhibiting a gradient of leaf discoloration from pale green to complete white, the lamellar structure of the chloroplasts progressively diminished with decreasing

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pigmentation (Fig. 2, 3, 4). Starch grains per chloroplast were reduced in number in pale green and yellow leaves and were totally absent in chloroplasts in cells from white leaves. The plastoglobuli increased two-fold in chloroplasts from discolored leaves over those in normal green leaves. The plastoglobuli aggregated in the chloroplasts in cells from white leaves making it impossible to determine the number present.

DISCUSSION

The degenerative changes observed in the chloroplasts of discolored alfalfa leaves are reminiscent of senescent changes reported in *Elodea* by Ikeda and Ueda (7), in wheat leaves by Shaw and Manocha (11), in kidney bean leaves by Barton (1), and in cucumber cotyledons by Butler (2). These workers reported that the first sign of any change was a localized swelling of the chloroplast thylakoids and a disappearance of free ribosomes. This was followed by an increased loss of thylakoids, disappearance of the stroma, and a marked accumulation of globules. The cells lost their tonoplast, ribosomes, and endoplasmic reticulum, but retained the degenerated plastids and mitochondria, and some spherosomes, all within an apparently intact plasmalemma. The increased amount of globules probably results from an accumulation of membrane breakdown products.

When leaves senesce, the loss of chlorophyll is accompanied by disappearance of a large proportion of the protein that was originally

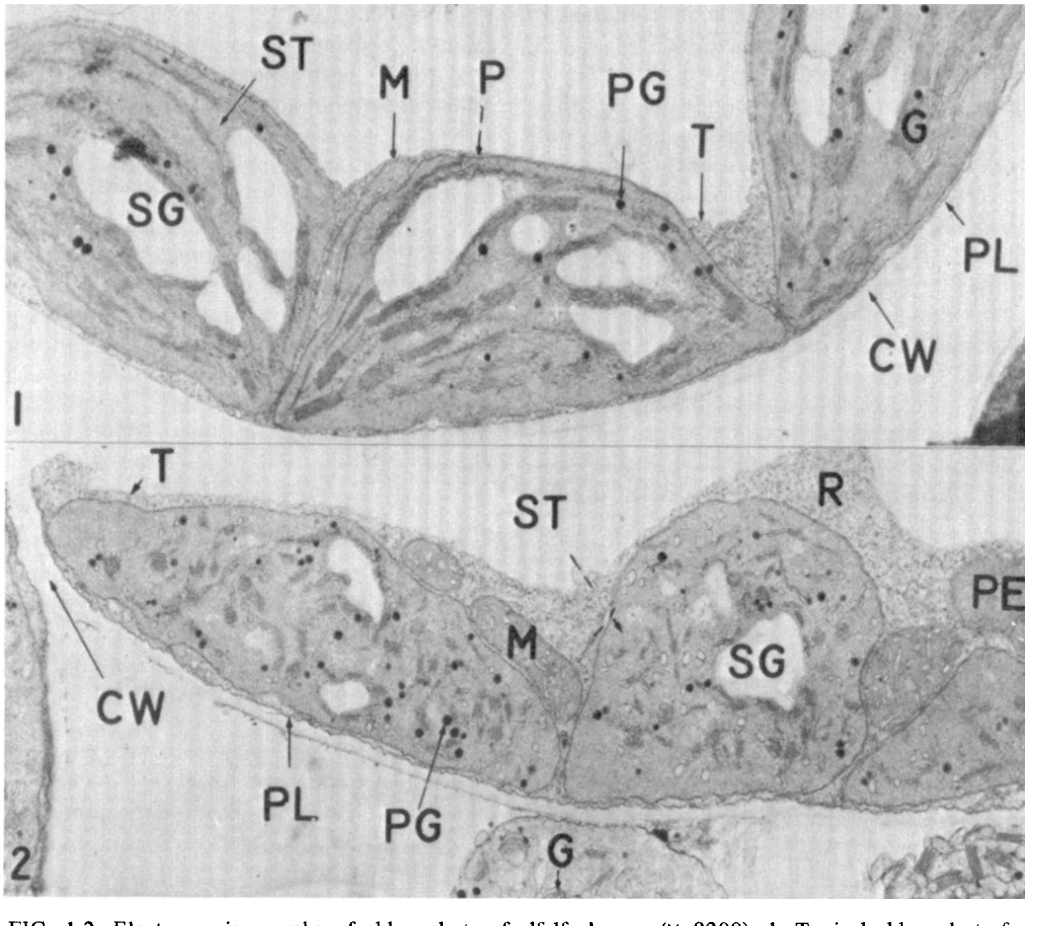

FIG. 1-2. Electron micrographs of chloroplasts of alfalfa leaves. (× 9200). 1. Typical chloroplasts from noninfected normal green alfalfa leaves. 2. Abnormal chloroplasts from pale green leaves of a plant infected with *Ditylenchus dipsaci*. CW = Cell Wall; G = Granum (Grana); M = Mitochondrion (ia); P = Plastid; PE = Peroxisome; PG = Plastoglobuli; PL = Plasmalemma; R = Ribosomes; SG = Starch Grains; ST = Stroma; T = Tonoplast.

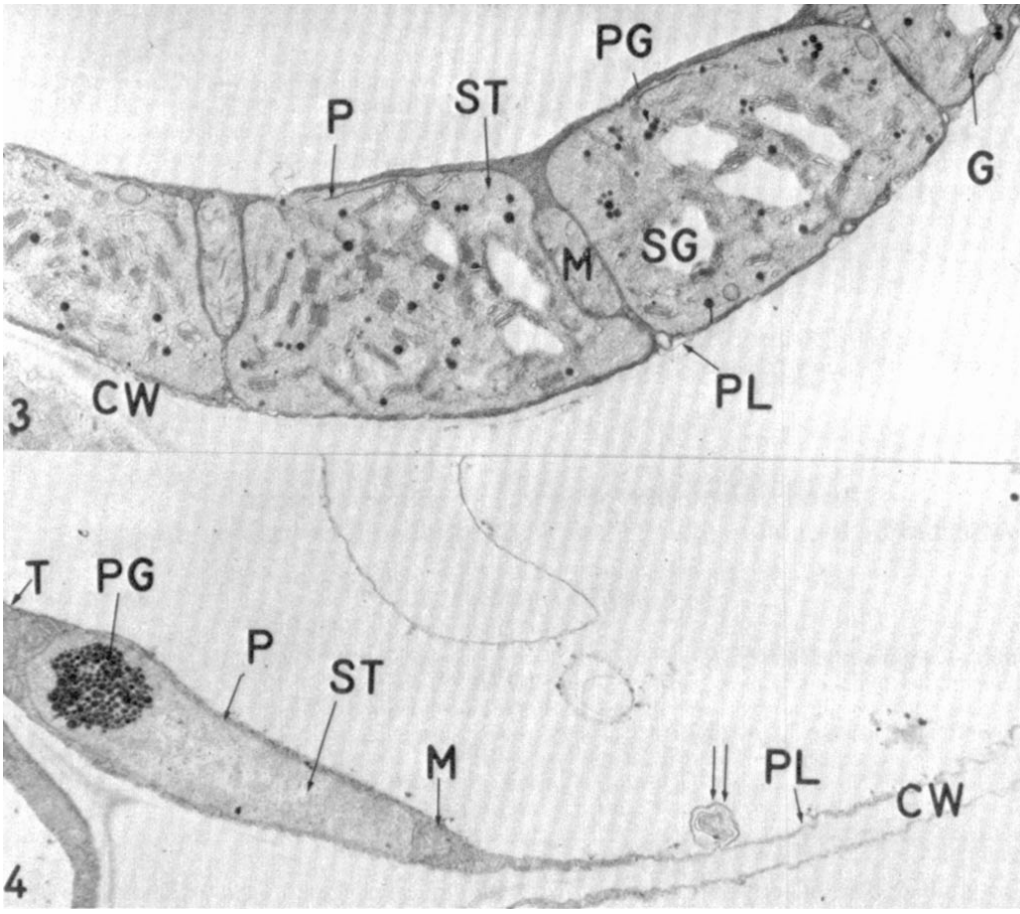

FIG. 3-4. Electron micrographs of chloroplasts of alfalfa leaves (× 9200). **3.** Abnormal chloroplasts from yellow leaves of a plant infected with *Ditylenchus dipsaci*. **4.** Abnormal chloroplasts from nematode-infected alfalfa leaf completely devoid of green pigmentation. For symbol legend see caption of Fig. 1-2 on page facing.

present (1, 2, 9, 11, 14). Since most of the protein in green leaves is located in the chloroplasts, a leaf cannot suffer much protein loss without harm to its chloroplasts. Loss of chlorophyll and protein has been correlated with changes in chloroplast ultrastructure (2, 11). *D. dipsaci* can cause changes in cells great distances from their actual site (5). The nematodes may be extracting protein from the leaves, which in turn, leads to the chloroplast and other organelle breakdown.

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