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Sink removal and leaf senescence in tobacco

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Abstract. The effect of sink removal (topping) on the senescence of selected leaevs of field-grown tobacco plants was investigated. Using the extent of loss of chlorophyll and soluble protein as criteria of senescence, sink removal resulted in significant delay in the rate of leaf senescence. The effect of sink removal on leaf senescence was not different between two tested cultivars. The loss of ribulose bisphosphate carboxylase (large subunit, 53 KD) of desinked plants was slower than controls. No new protein bands of desinked plants were detected. Sink removal resulted in a marked increase in leaf dry weight, specific leaf weight and phosphorus, indicating that in the absence of a reproductive sink, the leaf acts as an alternate sink.

Key words: Leaf senescence; *Nicotiana tabacum*; Sink removal; Topping.

Introduetion

The effect of sink (including seed, ear, pod, head and flower) removal has been extensively studied. It has been shown that sink removal accelerated leaf senescence of pepper (Hall and Brady, 1977) and barley (Mondahar and Garg, 1975) and retarded senescence of sunflower (Ho *et al.*, 1987; Purohit 1982), pigeon pea (Grover *et al.*, 1985) and *Brassica campestris* (Biswas and Mandal, 1987).

In maize there is conflicting evidence on the effects of ear removal or prevention of pollination on the onset of leaf senescence. Moss (1962) found that such treatments caused a delay of leaf senescence. Conversely, Allison and Weinmann (1970) and Christensen *et al.* (1981) reported a premature senescence. More recently, Crafts-Brandner *et al.* (1984) and Ceppi *et al.* (1987) found differential expression of leaf senescence in response to ear removal among maize hybrids or genotypes.

Flower or pod removal from soybean plants has been found to lead a slower rate of chlorophyll loss (Crafts-Brandner and Egli, 1987; Leopold *et al.*, 1959; Nooden, 1984). However, Wittenbach (1982) claimed that pod removal led to a more rapid functional senescence. The divergent senescence responses to sink removal mentioned above seem to stress the complexity of the senescence response.

Topping at onset of flowering is a standard practice in the production of tobacco. The effects of topping include improved quality, increased size and weight of leaves and a higher alkaloid content in leaves. The purpose of the present investigation was to study the effect of topping (sink removal) on leaf senescence

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of two tobacco cultivars, Taiwan Tobacco 8 (TT8) and Speight G-70, which were grown in the field.

Materials and Methods

Seeds of flue-cured cultivars, Taiwan Tobacco 8 (TT8) and Speight G-70, were germinated and seedlings were transplanted to the field. Soil type was sandy loam. Treatments were: (a) control; (b) sink removal (topping), in which all flowers were removed. Leaves were taken from the 7th-8th and 11th-12th node from the top for TT8 and Speight G-70, respectively. The first sampling of the leaves were conducted at the time of topping which was 64 DAP (days after planting). Leaf discs (1 cm diameter) were cut with a cork borer. Fresh leaf discs were used to determine chlorophyll, phosphorus and specific leaf weight and extract protein for SDS-PAGE. Leaf dry weight was measured by drying leaf to constant weight at 70°C.

Chlorophyll was extracted with boiling ethanol (80%) and expressed as A_{665} per 10 discs. Phosphorus was extracted by homogenizing leaf discs with distilled water and centrifuged. Aqueous TCA (trichloroacetic acid, 5%, w/v) was added to the supernatant and centrifuged again. Clear TCA-soluble fraction was used to determine phosphorus. Phosphorus was determined according to Yoshida *et al.* (1972).

For gel electrophoresis, leaf discs (6 g) were homogenized in Tris buffer (124 mM, pH. 6.8) with a Polytron homogenizer. After centrifugation of leaf extract, a 1-ml aliquot was taken from supernatant fractions and added to 1 ml 124 mM Tris (pH 6.8) containing 4.6% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol, and 0.01% (w/v) bromophenol blue. The proteins were completely dissociated by immersing the samples for 5 min in boiling water. Aliquots (10 μl) of the SDS dissociated extracts were applied to each well. The polypeptides were separated on a 5 to 10% polyacrylamide gradient slab gel overlaid with a 4.05% polyacrylamide stacking gel. Electrophoresis was carried out at a constant current of 30 mamp for 2.5-3 h. Gels were stained 40 min in a solution containing 0.1% (w/v) Coomassie Blue, 50% (v/v) methanol and 10% (v/v) glacial acetic acid and were destained

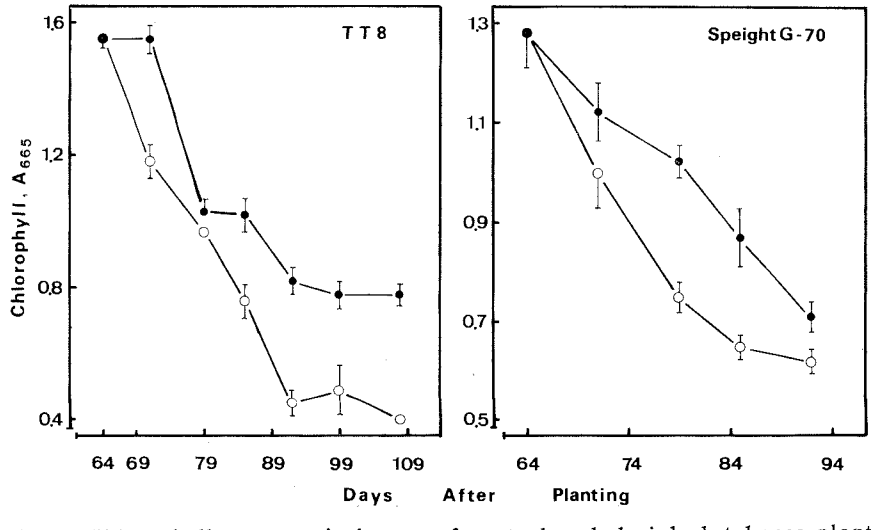

Fig. 1. Chlorophyll contents in leaves of control and desinked tobacco plants. Control plants are indicated by open symbols (○) and desinked plants are indicated by closed symbols (●).

in 10% (v/v) methanol and 10% (v/v) glacial acetic acid. Photographs were taken after the gels were dried.

Results

Sink removal clearly delayed loss of leaf chlorophyll in two tobacco cultivars (Fig. 1). SDS-PAGE was used to visualize differences in leaf soluble proteins caused by sink removal treatment (Fig. 2). For all cultivars, staining intensity of all bands decreased progressively with each successive sampling date. The loss in the staining intensity of protein bands was greater for the control plants than the plants

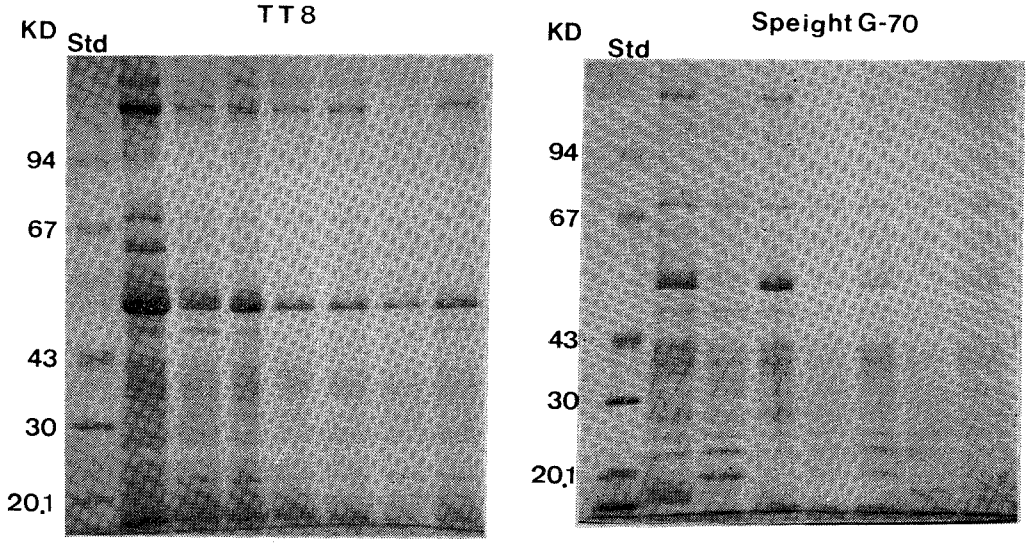

Fig. 2. Polypeptide profiles from SDS-polyacrylamide gel electrophoresis. For TT8, lanes from left to right were: standard polypeptide; 64 DAP; control, 78 DAP; desinked, 78 DAP; control, 92 DAP; desinked, 92 DAP; control, 106 DAP; desinked, 106 DAP. For Speight G-70, lanes from left to right were: standard polypeptides; 64 DAP; control, 71 DAP; desinked, 71 DAP; control, 85 DAP; desinked 85 DAP; control, 99 DAP; desinked, 99 DAP.

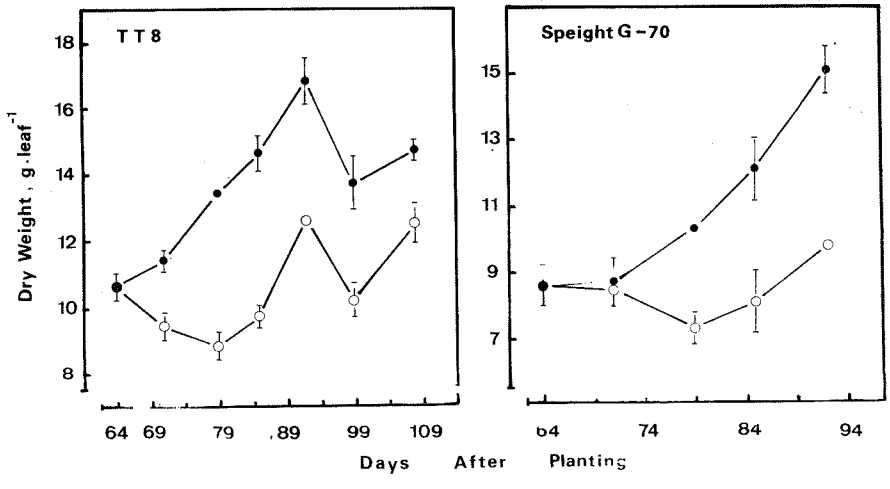

Fig. 3. Leaf dry weight of control and desinked tobacco plants. Control plants are indicated by open symbols (○) and desinked plants are indicated by closed symbols (●).

with sink removal treatment, indicating that sink removal delayed loss of soluble protein. No new or different protein bands were detectable on the gels following sink removal regardless of cultivar.

Leaf dry weight was greater for sink removal plants than control plants in the two tested cultivars (Fig. 3). For cultivar TT8, leaf dry weight following sink removal increased up to DAP 92 and then declined. For desinked Speight G-70 plants, leaf dry weight increased throughout the sampling period. Regardless cultivar, specific leaf weight of desinked plants was always greater than that of control plants (Fig. 4). Sink removal also caused an increase in leaf phosphorus content in the two tested cultivars (Fig. 5). The increase of leaf dry weight (presumably carb-

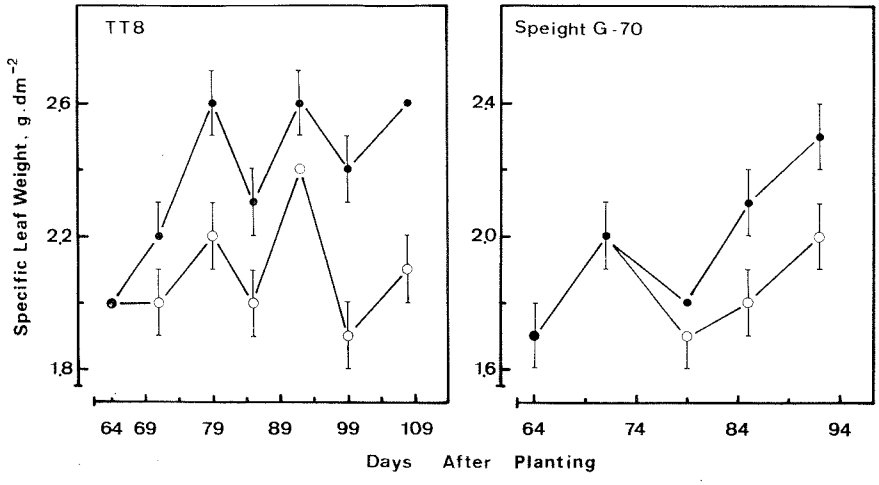

Fig. 4. Specific leaf weight of control and desinked tobacco plants. Control plants are indicated by open symbols (○) and desinked plants are indicated by closed symbols (●).

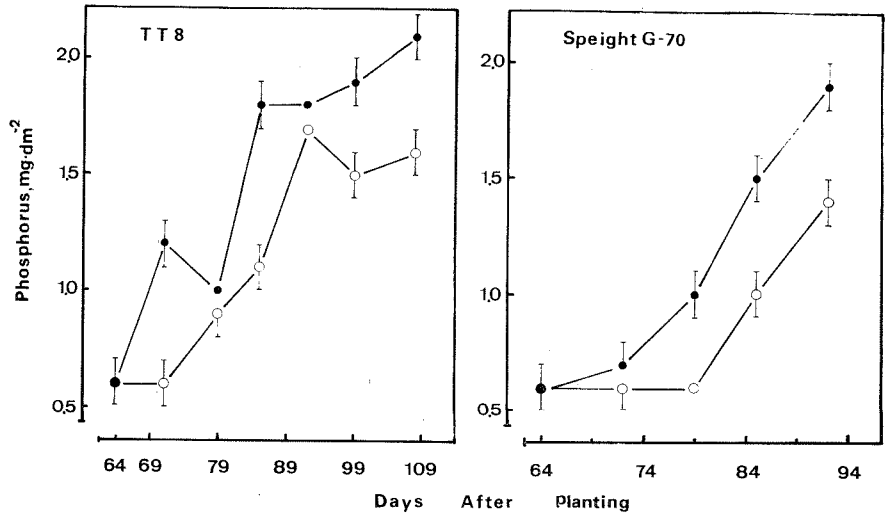

Fig. 5. Phosphorus contents in leaves of control and desinked tobacco plants. Conrol plants are indicated by open symbols (○) and desinked plants are indicated by closed symbols (●).

ohydrates) and phosphorus content by sink removal are possibly attributed to the decrease of export of carbohydrates and phosphorus in leaves, respectively.

Discussion

Using the loss of chlorophyll (Fig. 1) and soluble protein (Fig. 2) as criteria of senescence, sink removal resulted in significant delay in the rate of leaf senescence in field-grown plants. This finding is consistent with the results obtained in sink removal studies with soybean, sunflower and other plant species (Biswas and Mandal, 1987; Crafts-Brandner and Egli, 1987; Grover *et al.*, 1985; Ho *et al.*, 1987; Nooden, 1984; Purohit, 1982) The effect of sink removal on leaf senescence was not different between two tested tobacco cultivars which is in agreement with the results obtained in sink removal studies using different soybean cultivars (Crafts-Brandner and Egli, 1987).

Wittenbach (1982) reported that ribulose bisphosphate carboxylase (Rubisco) level was lower in depodded than in control, podded soybean plants. However, Crafts-Brandner and Egli (1987) showed that the effect of sink removal in Rubisco level was different among soybean cultivars. For soybean cultivar Harper, deflowering led to a more rapid decline in Rubisco relative to control. In contrast to Harper, deflowered McCall and Maple Amber plants lost Rubisco much more slowly than controls. In our study, results of two cultivars all showed that the loss of Rubisco (large subunit, 53 KD) of desinked plants was slower than controls (Fig. 2). Leaf photosynthesis has been observed to be partially inhibited by sink removal (Mondal *et al.*, 1978; Wittenbach, 1982). The decline in photosynthesis by sink removal was claimed to be, at least in part, associated with the loss of Rubisco (Wittenbach, 1982). It remains to be elucidated whether desinked tobacco plants have higher photosynthesis than controls.

The work of SDS-PAGE was designed to identify different (newly synthesized) proteins in desinked tobacco leaves. No new protein bands of desinked plants were detected. Thus, our work is in disagreement with the work with soybean, in which sink removal resulted in the formation of new protein bands (Crafts-Brandner and Egli, 1987; Wittenbach, 1982; 1983). However, our work is in agreement with similar work with maize and sunflower (Crafts-Brandner *et al.*, 1984; Ho *et al.*, 1987).

Our work with tobacco plants clearly showed that sink removal resulted in a marked increase in dry weight (presumably mostly carbohydrates), specific leaf weight and phosphorus. A possible interpretation is that in the absence of a reproductive sink, the leaf acts as an alternate sink for photosynthate. In other words, sink removal alters the partitioning of metabolites within tobacco plants. These findings are consistent with the results obtained in similar studies using sunflower plants (Ho *et al.*, 1987).

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積儲切除對於草葉片老化之影響

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本研究之主要目的爲探討積儲切除（摘芯）對菸草葉片老化之影響，如以葉綠素與可溶性蛋白質含量之降低做爲老化指標，則積儲切除可明顯的延緩葉片之老化，兩種菸草品種均表現積儲切除延緩葉片老化之效應，積儲切除之植株其Rubsico大次級單位（53 KD）含量之喪失較對照植株爲慢，積儲切除不會產生新的蛋白質，但明顯的會使葉乾重，比葉重與磷含量增加，積儲切除可能使得葉片由始源轉變爲積儲。