BROWNING OF CHRYSANTHEMUM LEAVES INFESTED WITH
*APHELENCHOIDES RITZEMABOSI*

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The rate of browning of infested chrysanthemum leaves increased with relative humidity and the number of eelworms. The eelworm is the primary cause of leaf discolouration although the brown sectors may contain a distinctive fungal population. Chlorogenic acid and isochlorogenic acid are the chief polyphenolic substrates for leaf browning. When cells are pierced by the eelworm stylet during feeding the polyphenols and the enzyme polyphenol oxidase probably meet resulting in oxidation and polymerisation to form the brown pigments.

The chemistry of leaf browning during the manufacture of tea and tobacco has been described by numerous authors. Plant pathogens may also cause discolouration of plant tissues and this subject has been studied by both mycologists and virologists. The nature of leaf discolouration caused by eelworms, however, has not been investigated. Hesling & Wallace (1960) have suggested that varietal susceptibility of chrysanthemums to *Aphelenchoides ritzemabosi* (Schwartz) may be associated with a hypersensitive browning reaction of the leaf. Before the problem of resistance or host-parasite relations can be studied, however, it is essential to know something of the factors which influence browning and the chemistry of the process.

**MATERIALS AND METHODS**

Chrysanthemum eelworms were obtained from dried infested chrysanthemum leaves. Leaves were infested by introducing a known number of eelworms on to the undersurface of the leaf by the method described previously (Wallace, 1960). Areas of browning on the leaf were measured by tracing on to paper and weighing the paper. The study of browning under sterile conditions was achieved by dipping leaves in a saturated calcium hypochlorite solution for 5 minutes followed by washing in sterile water. Eelworms were sterilised by immersion in a solution of 3.75 p.p.m. ethoxy-ethyl mercury chloride and 0.01% diocetyl sodium sulphosuccinate for 3 days (Crosse & Pitcher, 1952, 1953). The isolation and identification of fungi on the surface of infested leaves were made by Dr. F. T. Last using a technique already described by him (Last, 1955).

Polyphenols in the leaf were identified by paper chromatography. About 2 g of leaf were macerated in 80 per cent methyl alcohol in an Atomix 100 homogeniser for 1 minute. This was filtered and the filtrate concentrated in a rotary...
evaporator under vacuum to give a final volume of 5 ml. After centrifugation the leaf extract was stored at 0°C. Descending chromatograms were run on No. 1 Whatman paper 46 × 57 cm in Shandon tanks. About 0.01 ml of the leaf extract was spotted on the paper. Two-way chromatograms were run with the organic phase of n-Butanol: acetic acid: water (4 : 1 : 5) and 6 per cent acetic acid as the solvents. After drying, the papers were examined under U.V. with and without ammonia fumes. Different sprays were also used to identify the spots, and finally the \( R_F \) values were measured. After provisional identification by these criteria, the spots were eluted from the paper by immersion in 80 per cent methyl alcohol for 24 hours and re-run one way against authentic specimens in four different solvents: 6% acetic acid, n-Butanol: acetic acid: water (4 : 1 : 5), glacial acetic acid: conc. HCl: water (10 : 1 : 3) and water. The leaves were also hydrolysed in 2N HCl for 1 hour, the solution filtered, concentrated by rotary evaporation, centrifuged and finally spotted on to chromatography paper. Identification methods were similar to those for the unhydrolysed leaf extract. Spectrograms of the major spots on the papers were obtained using a Unicam U.V. spectrophotometer.

Isolation of polyphenol oxidase from leaves was made at 0°C. About 10 g of leaf was macerated in 70 ml of phosphate buffer (pH7). The leaf extract was centrifuged to remove plant debris and dialysed in distilled water for 24 hours. The enzyme solution was then removed from the dialysis bags, centrifuged and stored at 0°C.

RESULTS

**Influence of humidity and numbers of eelworms on rate of browning**

To measure quantitatively the influence of moisture on the rate of browning, leaves of variety Westfield Bronze were kept at three humidity levels and infested...