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PASSAGE OF CARBON BLACK THROUGH SIEVE PLATES OF UNEXCISED HERACLEUM SPHONDYLIUM AFTER MICRO-INJECTION

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SUMMARY

A suspension of carbon black particles, 200–700 Å, was injected from a microcanula into relatively little damaged living sieve elements. The suspension was carried through the sieve plates by the translocation stream in a manner thought to be a type of mass flow.

1. INTRODUCTION

TAMMES & IE (1971) have recently reported that fine particles of carbon black could be sucked through sieve plates of *Yucca flaccida* Haw. Their work indicated that these inert particles, 200–700 Å diameter, could pass through sieve plate pores of short sections of inflorescence, excised from the main stalk at 2 °C. Gross slime plugging and callose deposition blocked the pores in some cases before fixation for the electron microscope was complete, but it was assumed that the blocking occurred after the carbon had passed.

We have evidence (ROBIDOUX, SANDBORN, FENSOM & CAMERON 1973) that some microfibrillar material which aggregates to form slime plugs upon damage, normally passes axially through both lumen and plate pores of the sieve elements of *Heracleum*. In this plant the pores are known to be about 0.4 μm in diameter and previous studies have shown that while microfibrillar material aggregates near the plates when the sieve elements are excised (LEE, ARNOLD & FENSOM 1971), it may at times be forced through the pores completely by a pressure wave. We therefore wished to inject carbon black into living, unexcised sieve elements, using the method of FENSOM & DAVIDSON (1970) to see whether particles of the same size as those used in *Yucca* would pass through relatively undamaged plates in living *Heracleum*.

2. MATERIALS AND METHODS

Plants of *Heracleum sphondylium* grown out of doors in plastic pots were transferred to the laboratory before using. The phloem strands were gently detached from the surrounding parenchymous and xylem tissue under a buffered 0.1 M sucrose solution (7×10^{-2} mM Na_2CO_3, 4.5×10^{-2} mM KH_2PO_4, pH 8.0 (MITTON et al., in prep)). India ink (Talens trade mark) similar to that used by TAMMES & IE (1971) was washed and dried three times and then diluted to 1/10 with buffer solution. The bathing solution contained 2% resorcinol blue

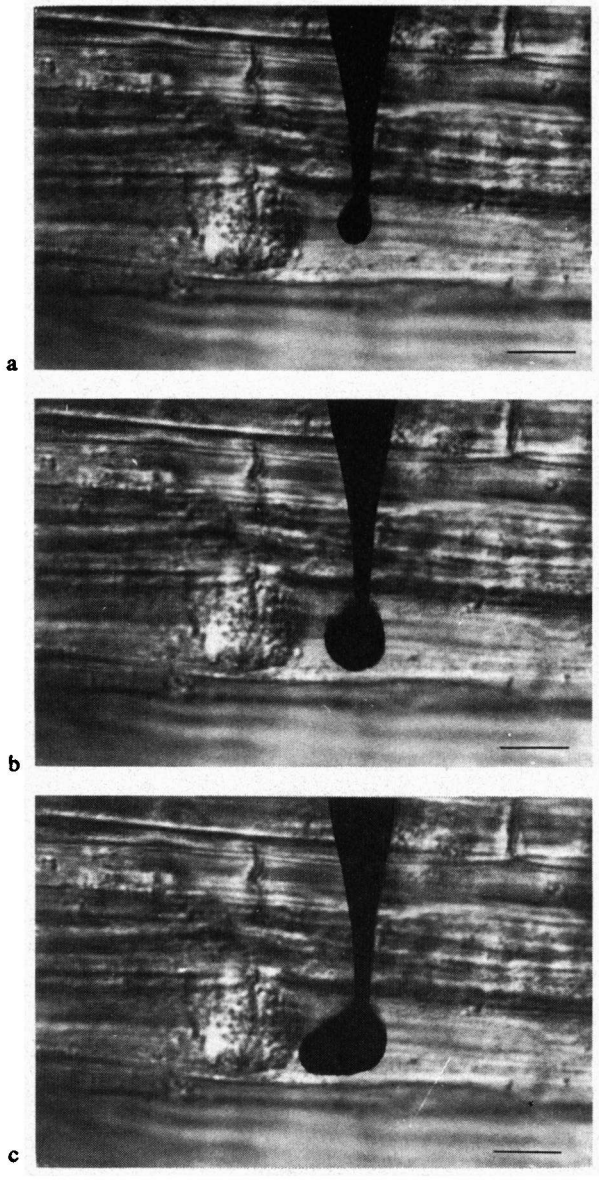

Plate 1 a), b) and c). Three flash photographs of a carbon black injection into a sieve tube at successive 5 second intervals. The injection was done apically to a sieve plate. Some slight background vibration of the tissue is detectable between flashes. Scale lines 10 μm; flash 1/2000 sec.

(aqueous). In this way sieve plates with appreciable callose could easily be-detected by their blue colour and avoided.

Injection was done with a glass micropipette drawn to a 3–6 μm tip (*plates 1–3*), using a Leitz micromanipulator. The injection process was observed under a Zeiss photomicroscope using a 40 × water immersion objective (NA 0.75) for insertion and a 100 × oil immersion planapochromat lense (NA 1.30) for photographs. An electronic flash was used to take photographs of the injected tissue. (see LEE et al. 1971). Electron microscopy of the carbon black has confirmed that the mean diameter of unaggregated particles was around 500 Å.

3. RESULTS

Many micro-injections were attempted, usually the carbon black moved basally too quickly to be photographed but did pass sieve plates unless the plates were clearly blocked in advance by an aggregation of slime. We were successful in photographing a few runs, one of which is shown on *plates 1* and *2*.

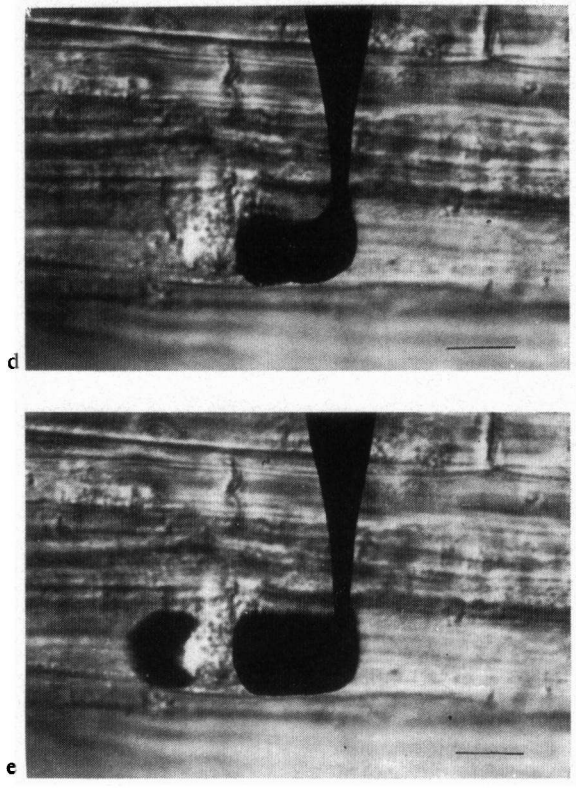

Plate 2 d) and e). Two further flash photographs in the same sequence as Plate 1 but with the carbon black touching and passing through the sieve plate. Scale lines 10 μm.

Plate 3. A micro injection photograph showing the carbon black passing a sieve plate. The plate is at right angles to the page, but carbon black may be seen in several pores. Note marker particles in this case, apparently intact, also that the injection passes through one side of the plate only. Scale line 10 μm.

These show successive stages of the microinjection taken 5 seconds apart. They show that the injection fluid has in this case been carried basally from the point of injection as a mass of liquid. The mass travelling at about 1 cm hr^{-1} reached half of a plate and passed through this half without apparent hindrance. The visual flow profile was of the mass-flow type.

A subsequent injection near a sieve plate on its side has been photographed (*plate 3*). It shows carbon black actually in the pores, which seem to be relatively callose free. Also it shows that the plate has not acted as a barrier, but rather seems to have allowed the particles to move through it readily and to rejoin on the downstream side.

4. DISCUSSION

We have confirmed in living *Heracleum* phloem the findings of Tammes and Ie that particles averaging 500 Å can pass through sieve plate pores. Since a type of mass flow was visible, we conclude that some translocation was actually occurring in our sieve tubes at the time of injection, though it was much less than normal for mass flow in this plant.

It is clear that the "marker particles" (LEE et al. 1971) were not in evidence or in their usual saltatory motion in the series of *plates 1–2*. Therefore it is possible that the usual network of microfibrillar material (ROBIDOUX et al. 1973) was

damaged or absent in these cells and that these sieve plate pores were open. But an alternative possibility is that some microfibrillar material traversed the pores with interfibrillar spaces at least 500 Å wide (FENSOM 1972). Marker particles were present and their movement was apparently normal in the cells shown in *plate 3*, therefore we think that in some cases the micro-injection was made into relatively little damaged cells and that the carbon black flowed by a type of mass flow across functioning sieve plates.

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