SHORT COMMUNICATION

Autoradiography of Haustoria of *Erysiphe pisi*

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(Received 17 July 1979)

Leaves of *Pisum sativum* infected with *Erysiphe pisi* were exposed to $^{14}\text{CO}_2$ for 2 h, and then haustorial complexes were isolated. The distribution of ^{14}C in the isolated fraction was studied by light microscopic autoradiography. Solvent extraction prior to autoradiography indicated that most of the ^{14}C resided in compounds insoluble in ethanol, chloroform and methanol. The haustorial complexes contained 82 to 84% of the ^{14}C in the fraction. Considerable variation, not correlated with haustorium age, was found in the labelling of individual haustorial complexes.

INTRODUCTION

In spite of the widely held view that fungal haustoria are instrumental in taking nutrients from their hosts, there is little complete evidence of this function. Much of the evidence is indirect. When host tissues were supplied with radioisotopes of sulphur (Slesinski & Ellingboe, 1971) or phosphorus (Martin & Ellingboe, 1978), isotopes did not enter other fungal cells until the developing haustoria approached maturity. Even more indirect evidence is the correlation of hyphal extension rates with either haustorium development (Hirata, 1971) or the incidence of mechanical damage inflicted on haustoria or infected cells (Bushnell *et al.*, 1967; Sullivan *et al.*, 1974). Few experimenters have detected isotopes in fungal haustoria. Some of these (Manocha, 1975; Mendgen, 1977) have supplied the hosts with an arbitrarily chosen amino acid, and only two groups (Takahashi *et al.*, 1977; Manners & Gay, 1978*a, b*) have used $^{14}\text{CO}_2$. The last authors showed that the photosynthates were both translocated into haustoria and subsequently metabolized.

The experiments by Manners & Gay (1978*a, b*) depended on isolation of haustorial complexes from leaves infected with a powdery mildew (*Erysiphe pisi*). The haustorial complex comprises the haustorium with its cytoplasm, the extrahaustorial membrane (invaginated host plasmalemma) around it and the materials and structures between them (Gil & Gay, 1977; Manners & Gay, 1977). However, the results of Manners & Gay (1978*a, b*) are averages for populations and the possibility of differences in the translocatory activity of individual haustoria has not been explored. Further, the fractions isolated included contaminants of host origin and, although several precautions were taken, the extent of this interference is not known. Even uninfected plants were not entirely satisfactory as controls because infection reduces the rate of photosynthesis (Ayres, 1976; Manners, 1979) and increases the proportion of photosynthates incorporated into ethanol-insoluble host components (Manners, 1979) which constitute the main contaminants.

In order to elucidate the activity of individual haustoria and to clarify the distribution of assimilates within an haustorial complex fraction, autoradiographs were prepared from the fraction obtained from pea leaves infected with *Erysiphe pisi* and exposed to $^{14}\text{CO}_2$ in the light. These were examined by light microscopy.

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METHODS

Plants and inoculum. *Pisum sativum* cv. Onward was cultivated and infected with *Erysiphe pisi* de Candolle ex St Amans as before (Manners & Gay, 1978a).

$^{14}\text{CO}_2$ exposure and fractionation. Plants infected 7 d previously were trimmed to provide 30 g leaves with the mycelium attached and prepared for $^{14}\text{CO}_2$ exposure as described by Manners & Gay (1978a). They were exposed to 5 mCi $^{14}\text{CO}_2$ (60 mCi mmol $^{-1}$, 2.2 GBq mmol $^{-1}$) for 2 h in the light, then taken from the chamber and the mycelia were removed. Haustorial complexes were isolated as before (Manners & Gay, 1978a) except that, to increase the yield, the leaves were comminuted in the Omni-mixer (Sorvall) for two additional periods of 1 min at 16000 rev. min $^{-1}$. The final preparation was divided into two approximately equal fractions. The first was washed twice with double-distilled water by centrifuging (3000 g) at 4 °C and finally suspended in double-distilled water. The frequency of haustorial complexes was estimated by counting in a haemocytometer and adjusted to 10 5 ml $^{-1}$. The second fraction was treated to remove low molecular weight compounds and lipids. It was washed successively with a series of ethanol concentrations (once in each of 10, 20, 30, 45 and 60% and twice in 70%, v/v), then centrifuged (3000 g) in chloroform/methanol (2:1, v/v), suspended in ethanol (70%) and the concentration was adjusted to 10 5 ml $^{-1}$.

Autoradiography. The stripping film technique described by Rogers (1973) was employed. Haustorial complex fractions were smeared on to microscope slides and covered with stripping film (AR-10, Kodak). After drying, slides were incubated in the dark for 5 d and then developed and fixed.

The following control slides were employed (Rogers, 1973): (i) unlabelled haustorial complexes treated as described above, to detect positive chemography, and (ii) labelled complexes exposed to light prior to development and fixation. The latter detected negative chemography and variations in the sensitivity of the emulsion. All slides were observed by dark-field microscopy with an anoptal (Reichert) phase-contrast objective lens.

RESULTS AND DISCUSSION

 ^{14}C in haustorial complexes

Haustrorial complexes were not distinguishable on dry emulsion-coated slides, but when water and a cover glass were added they were easily recognized by their characteristic shape with the extrahaustorial matrix swollen around the central haustorial body (Figs 1, 3). The neck-bands and septum in the neck region (Gil & Gay, 1977) were also visible (Figs 1, 3). Dark-field microscopy permitted simultaneous observation of silver grains and haustorial complexes (Fig. 3), although the silver grains were usually viewed in a different focal plane (Figs 1, 2). Grains for haustoria and extrahaustorial matrices could not be distinguished as separate classes.

Silver grains were located directly over most haustorial complexes but no difference in total counts ($P = < 0.1$) was observed for the complexes mounted directly and those where low molecular weight solutes had been removed by pretreatment with ethanol and chloroform/methanol (Table 1). However, silver grains were not confined to the area immediately over each haustorial complex and were also found in a surrounding zone about 10 μm wide. Solvent treatment significantly decreased ($P = < 0.02$) the number of grains in the peripheral zones suggesting that some activity resulted from soluble compounds diffusing out of the complexes. The emulsion also increases in area by a factor of 1.38 when hydrated (Rogers, 1973) but such an increase would only result in a small increase in radius. Thus, although mounting in water for observation caused displacement of some grains directly over the complexes, it could not account for the whole width of the zone further suggesting that diffusion of compounds had occurred.

Controls for positive and negative chemography showed that these phenomena did not influence the labelling observed. Haustorial complexes isolated from unlabelled plants and not treated with the solvents had a mean number of 2.8 ± 2.1 silver grains per complex. This is only 6% of the value for the labelled fraction. Areas lacking silver grains were not detected on slides exposed to the light prior to development and fixation.

A great deal of variation was observed in the number of silver grains associated with individual haustorial complexes. Although the number averaged about 40, some complexes were unlabelled. This variation is further shown by the large standard deviations of the

Light microscope autoradiographs of an haustorial complex fraction isolated from *Pisum sativum* infected with *Erysiphe pisi* and exposed to $^{14}\text{CO}_2$ for 2 h. Specimens were mounted in water and examined by dark-field illumination and an anoptral objective lens. The age classes recognized are referred to in the text. Bar markers represent $20\text{ }\mu\text{m}$.

Fig. 1. A mature haustorial complex (H1, age class 3) with well developed extrahaustorial matrix (MA) and haustorial body (B). Two other complexes (H2 and H3) are present and neckbands (N) are evident on one (H3).

Fig. 2. The same area as in Fig. 1 but at the focal plane of the emulsion. Many silver grains are visible over and around two of the complexes (H1 and H3), but H2 is less labelled.

Fig. 3. The young haustorial complex (H2, age class 1) shows a clear neck region (N) and numerous silver grains over it and the surrounding area. Two other haustorial complexes (H1 and H3) of intermediate age are almost unlabelled. The extrahaustorial membrane (M), matrix and body (B) are visible in H1. A few grains are associated with the highly refractile crystalline contaminants (C) and starch grains (S).

Fig. 4. A group of contaminant particles (starch grains and xylem cell walls). The absence of silver grains indicates that they had not incorporated photosynthate.

Table 1. *Distribution of radioactivity in an haustorial complex fraction isolated from infected leaves after exposure to $^{14}\text{CO}_2$*

The distribution of radioactivity in haustorial complexes and contaminant particles in the fraction was shown by autoradiograms prepared by the stripping film technique. Silver grains were counted directly over the subjects and within the 10 μm zone surrounding them. Results are expressed as mean numbers of silver grains per haustorial complex or particle and each value is derived from at least 100 haustorial complexes or particles. Standard deviations are given for the 95% confidence limits.

	Fraction washed with 70% ethanol and chloroform/methanol (2:1)		Untreated fraction	
	Haustorial complexes	Contaminants	Haustorial complexes	Contaminants
Directly over subject	22.4 $\pm$ 15.9	2.8 $\pm$ 4.5	21.7 $\pm$ 22.9	3.2 $\pm$ 4.3
Over 10 μm peripheral zone	15.0 $\pm$ 13.5	1.7 $\pm$ 2.7	22.1 $\pm$ 15.5	2.7 $\pm$ 3.5
Total	37.2 $\pm$ 28.1	4.5 $\pm$ 6.1	43.8 $\pm$ 27.6	5.9 $\pm$ 6.7

mean counts for the complexes. In order to test whether the variation was correlated with haustorial age the complexes were grouped into three classes indexed by the degree of lobe and matrix development (Figs 1, 3). The mean numbers of counts per complex for these classes were as follows: (1) young haustoria, 41.9 $\pm$ 26.2; (2) intermediate haustoria, 47.1 $\pm$ 27.6; (3) mature haustoria, 43.7 $\pm$ 26.1. The differences between these means were not significant indicating that haustoria of all ages (about 1 to 6 d) assimilated photosynthate at similar rates. This finding agrees with the results of Hirata (1971) who estimated the activity of a single haustorium of *Erysiphe graminis* by measuring hyphal extension on the leaf surface. He concluded that it remained active for at least 4 d.

Some haustorial complexes in each age group were unlabelled (Fig. 3). This may have been due to a low photosynthetic potential of the particular leaves from which they were isolated but it seems more likely that these haustoria may have been unable to take up and incorporate photosynthate. This is particularly relevant in view of the work on *E. graminis* by Slesinski & Ellingboe (1971) and Martin & Ellingboe (1978) and that of Manocha (1975) and Mendgen (1977) with haustoria of *Puccinia graminis* and *Uromyces phaseoli*, respectively. These workers found low rates of incorporation of label in resistant host cultivars and although a susceptible pea cultivar was used in the present work there may have been some individual resistant cells.

^{14}C in contaminants

Starch grains and cell wall debris were easily recognized but very few silver grains occurred over and around these and other unidentified contaminant particles (Table 1; Figs 3, 4). The ratio of the total numbers of silver grains counted for haustorial complexes and contaminant particles was 7.4:1.0 for untreated fractions and 8.9:1.0 for fractions washed with ethanol and chloroform/methanol. Since the ratio of numbers of particles to haustorial complexes from 50 random fields of view was 1.67:1.0, the label in the fractions was calculated to be 82% and 84% associated with haustorial complexes in untreated and solvent-washed fractions, respectively. It is most probable that the autoradiographic technique used here, particularly after solvent extraction, only detected high molecular weight ^{14}C -labelled compounds. Previous analysis of ^{14}C assimilates in the haustorial complex fraction also showed that most (60 to 70%) of the ^{14}C was in an ethanol-insoluble form (Manners & Gay, 1978a; Manners, 1979) and results here indicate that this was mainly incorporated into the haustorial complexes.

In conclusion, these experiments have shown that in a susceptible host the activity of individual haustoria of *E. pisi* differs widely. The basis of this variation is unexplained and the average haustorium maintains a steady level of transport of host assimilates during a period of at least 6 d. It has also been shown that when haustorial complex fractions are

obtained from host plants previously exposed to CO₂ for short periods, the haustorial complexes contain over 80% of the photosynthate in the fraction. Thus the experimental system can be used to determine the biochemical composition of haustoria and their associated interfacial structures.

J. M. Manners acknowledges the support of a Science Research Council CASE student-ship awarded in conjunction with Imperial Chemical Industries (Plant Protection Ltd., Jealott's Hill, Research Station). We are grateful to Professor E. Barnard for use of his autoradiographic darkroom.

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