TRACER TECHNIQUES IN PLANT PATHOLOGY*

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ABSTRACT

Isotope techniques have been valuable in understanding many fundamental aspects of plant disease, particularly those caused by obligate parasites like rusts and mildews. Autoradiography, microautoradiography and other tracer techniques have thrown considerable light on the mobilization of materials to the infection court, shifts in metabolic pathways, RNA and protein synthesis in the host-parasite complex as well as on the metabolic machinery of uredospores. The present article summarizes current knowledge on obligate parasitism gained through tracer techniques.

INTRODUCTION

UNDERSTANDING plant disease at the cellular and molecular level involves the use of many sophisticated biochemical techniques including isotopes as tracers. In plant pathology, tracer techniques have been successfully employed in the elucidation of metabolic pathways of the parasites, biosynthesis of toxins and other metabolites as well as in many areas of host-parasite interactions. Both stable and radioactive isotopes have been used, the former only to a limited extent, owing to the low sensitivity of their detection and high cost of instrumentation. Nevertheless, in studies on the fate of elements like N in biological systems, mass spectrometry has to be resorted to since the radioactive isotope of N has too little a half-life (10 minutes) to be of any practical value. Indeed, the ability of wheat stem rust uredospores to assimilate inorganic nitrogen into amino-acids and proteins could not have been feasible but for mass spectrographic analysis (McConnell and Underhill, 1966). It is hardly necessary to emphasize that mass spectrometry has to be fully exploited in investigations on the role of nitrogen

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in host susceptibility to many plant pathogens. Although tracer techniques have been employed in many facets of plant pathology, the present communication will be restricted to a consideration of the knowledge obtained through radioactive isotopes in the field of obligate parasitism, particularly in regard to rust diseases.

One striking effect of infection by obligate parasites is the mobilization of materials to the infection court. This was first demonstrated by autoradiographic techniques. Gottlieb and Garner (1946) showed that P^{32} accumulated around stem rust pustules on wheat. Later, several workers have confirmed this observation with suitable isotopic materials and reported that other elements like C, S, P and Ca also accumulate in the vicinity of the foci of infection (Yarwood and Jacobson, 1950; Shaw *et al.*, 1954; Yarwood and Jacobson, 1955; Shaw *et al.*, 1956). It has been further shown that the uptake of elements by tissues infected with obligate parasites could be several hundred times greater than that of the corresponding areas of healthy tissue and that accumulation is evident regardless of the natural occurrence of the compound. That host cells are responsible for at least part of this accumulation has been clearly shown in the case of bean leaves-infected with *Uromyces phaseoli* and barley infected with *Erysiphe graminis*. In the former accumulation could still be observed, though to a lesser extent, when the rust mycelium had been killed with heat in the infected tissue. In the latter, high radioactivity accumulated at the previous sites of infection although the mildew colonies had been removed before feeding the isotope (Shaw *et al.*, 1954). More recently, using a windowless gas-flow chromatogram scanner Johnson *et al.* (1966) presented evidence to the effect that wheat leaves bearing pustules of *Puccinia recondita* at the basal region mobilized P^{32} readily only when applied to the leaf tip. Application of the tracer to leaf parts proximal to the localized pustules resulted only in an extremely weak translocation. The same results were obtained when I^{131} was employed although the magnitude of accumulation was considerably less than that obtained with P^{32}.

The above-mentioned tracer studies led to the following conclusions: (1) accumulation is associated with an increase in the net uptake by the leaf and is not merely due to a redistribution of the same substance; (2) both an influx of solutes from the other parts of the leaf and also an enhanced synthetic activity of the host cells are involved in this process of accumulation; (3) DNP and treatments which interfere with aerobic respiration inhibit accumulation and the phenomenon is, therefore, dependant upon the aerobic metabolism of infected leaves especially of the tissues adjacent

to the site of infection; and (4) increased respiratory activity is associated with an increase in anabolic activities of the host.

As mentioned earlier, C^{14}, P^{32}, and S^{35} labelled compounds have been useful for the autoradiographic demonstration of the movement of materials to the site of infections. However, these tracers emit relatively high energy beta particles which penetrate deeply into photographic emulsions and are thus not suitable for the study of such problems as whether the tracers move preferentially in the parasites or the host tissue. Tritium is the tracer of choice for such studies at the cellular level because of the shallow penetration of its low energy beta particles into photographic emulsions and is thus well-suited for the production of microautoradiographs with a resolution at the micron range. Feeding Tritiated-glycine to rusted bean leaves Staples and Ledbetter (1958) concluded that an increase in glycine incorporation took place primarily in the fungus than in the host. Similar results were obtained by Sydow and Durbin (1962) in their microautoradiographic studies of the distribution of C^{14} labelled metabolites in stem rusted wheat leaves. They showed that the parasite accumulated appreciably more C^{14} than the host tissue at infection sites. On the other hand, when tritium labelled thymidine was incorporated into nuclei of bean leaves-infected with *U. phaseoli*, no label was found within the fungus and the number of labelled nuclei gradually decreased in leaf areas more distant to the uredium (Staples and Ledbetter, 1960). In line with this observation, Nielsen and Rohringer (1963) also did not find any incorporation of tritiated cytidine into the leaf rust fungus in the infected tissue. They, however, observed that the host cells in infected leaf areas contained considerably less label in their nuclei and cytoplasm than cells farther from the site of infection. These results were difficult to reconcile with the observation that both incorporation of P^{32} into RNA and the total level of RNA increased in rust-infected susceptible leaves (Rohringer and Heitefuss, 1961; Heitefuss and Fuchs, 1961; 1962).

In an attempt to resolve these findings, Bhattacharya and Shaw (1967) fed wheat leaves inoculated with the stem rust fungus with tritiated leucine, cytidine, uridine or thymidine. Their results showed that mesophyll cells in infected zones incorporated more leucine into protein and more cytidine and uridine into RNA than did cells in adjacent uninfected tissue. Leucine, cytidine and uridine were also heavily incorporated into the fungal mycelium and developing uredospores. There was no detectable incorporation of tritiated thymidine into either the fungus or the host cells. The greater incorporation of tritiated uridine, cytidine and leucine into rust-infected

nuclei was taken as evidence for increased RNA and protein synthesis. Accumulation thus represents not merely an influx of solutes from other parts of the leaf but also an enhanced synthetic activity which can be attributed to host cells. There is evidence that the increased respiratory activity is associated with an increase in anabolic activities of the host tissues.

Infected plant tissues show not only increased respiratory rate but also a shift in the metabolic pathways. Clearest evidence for this was provided by Shaw and Samborski (1957) and by Daly *et al.* (1957) with the help of specifically labelled sugars.

In comparing the relative contribution of EMP and PP pathways to respiratory CO_2 production, glucose specifically labelled in positions 1 and 6 is particularly useful (Bloom and Stetten, 1953). The rationale behind this approach is that in the EMP pathway carbons 1 and 6 of glucose appear as the methyl carbon of pyruvate which on further oxidation in the TCA cycle is evolved as CO_2. Thus CO_2 is produced equally and indistinguishably from C_1 and C_6 of glucose. On the contrary, in the PP pathway CO_2 arises only from C_1 of glucose. Thus, if the EMP pathway alone contributes to CO_2 production, the ratio of $C^{14}O_2$ from glucose-6-C^{14} and glucose-1-C^{14} respectively (*i.e.*, the C_6/C_1 ratio) would be 1·0. If only the PP pathway is operating, the ratio would be zero. A combination of the two pathways would yield a ratio less than 1·0 and would indicate approximately the relative proportion of CO_2 arising out of the EMP pathway. There are, however, serious limitations to this approach. Data are valid only for short-term experiments since interconnection between the EMP and PP pathways results in randomization of the radiocarbon and the pentose phosphate formed may eventually be degraded completely. Drainage of certain intermediates from the TCA cycle into cellular constituents may preclude the appearance of certain carbons as CO_2 which could lead to an underestimation of the EMP sequence (Beevers, 1961). In spite of the limitations, the C_6/C_1 ratio is an index of the relative contributions of EMP and PP pathways to the release of C_1 of glucose as CO_2. Thus a low C_6/C_1 ratio characteristic of rust-infected tissue (Shaw and Samborski, 1957; Daly *et al.*, 1957; 1961) as well as that of the parasite itself (Shu *et al.*, 1956; Shaw, 1961) indicates a shift in the respiratory pathway of the host in favour of a pathway (PP) that apparently plays an important role in the parasite.

Besides what has been mentioned earlier, tracers, particularly C^{14} have been widely employed in studying the metabolism of uredospores.

Specifically labelled fatty acids (C_2—C_9) have been useful in understanding whether the TCA and glyoxylate cycles are functional in uredospores (Reisener *et al.*, 1961; 1963 *a*, *b*; 1964; Staples, 1962; Suryanarayanan and McConnell, 1964; 1965). Analysis of the extensive labelling data indicate that fatty acids undergo β-oxidation in uredospores and enter the TCA and/or glyoxylate cycles as acetyl units. The distribution of label in the glutamic acid skeleton gives a reasonable indication of the route by which acetyl units have been channelled into the two cycles. Granting that the TCA cycle alone is functioning, glutamate derived from carboxyl labelled acetate or any other fatty acid with a corresponding carbon in it would contain the label only in its terminal carbons and the ratio of activity between carbons 5 and 1 would be 2 : 1. On the contrary, the exclusive functioning of the glyoxylate cycle would be reflected in the same specific activities of the carboxyl groups of glutamic acid, *i.e.*, a ratio of 1 : 1 between C_5 and C_1 (Suryanarayanan and McConnell, 1965). In practice, however, one notices intermediate values between 1 and 2 for rust uredospores suggesting that both cycles are operative and that when pelargonate is fed to the uredospores the glyoxylate cycle seems to be more pronounced than the TCA cycle. With methyl labelled acetate or other fatty acids with a corresponding carbon, only the 3 internal carbons of glutamic acid would get labelled by way of the TCA cycle. In uredospores one finds, however, some amount of labelling also in carbons 5 and 1 of glutamic acid indicating CO_2 fixation to a certain extent which has indeed been shown to occur by Staples and Weinstein (1959). Studies with specifically labelled propionate and metabolic inhibitors suggest that propionate metabolism in rust uredospores departs considerably from hitherto described pathways (Suryanarayanan and McConnell, 1967).

Tracers have again been helpful in understanding why germinating uredospores do not continue to grow. Using leucine-U-C^{14} and methionine-S^{35}, Staples *et al.* (1961, 1962) showed that there is no net synthesis of protein in germinating uredospores although protein turnover could be observed. Staples *et al.* (1966) also used C^{14}-leucine in studying protein synthesis by uredospores *in vitro*. It is needless to emphasize that cell-free systems have to be increasingly employed to understand the fundamental aspects of *de novo* synthesis of proteins and isoenzymes which appear after infection not only in rust diseases (Staples and Stahmann, 1963; 1964; Andreev and Shaw, 1965) but also in other infections (Akazawa *et al.*, 1957; Heitefuss *et al.*, 1960; Uritani and Stahmann, 1961 *a*, *b*; Deway *et al.*, 1967; Akazawa and Ramakrishnan, 1967; Uritani *et al.*,

1967; Stahmann, 1967). Although it is presently known that qualitative and quantitative changes in RNA and protein are characteristic of parasitised tissues, information is lacking at the messenger, transfer and ribosomal RNA level in host-parasite associations. Hopefully, these cell biological apects of host-parasite relations could be solved by diligent use of tracer techniques and cell-free systems.

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