THE EFFECT OF *PHYTOPHTHORA INFESTANS* (MONT.)DBy., INFECTION ON THE DISTRIBUTION PATTERN OF CARBON - 14 IN *SOLANUM TUBEROSUM* L.

by

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ABSTRACT

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THE EFFECT OF *PHYTOPHTHORA INFESTANS* (MONT.) DBY., INFECTION ON THE DISTRIBUTION PATTERN OF CARBON-14 IN *SOLANUM TUBEROSUM* L.

Autoradiograms of plants which had one of their leaves (presentation leaf) photosynthesizing in $C^{14}O_2$ show high concentration of radioactivity in the growing point and roots and low concentration in leaves below the presentation leaf. Infection on two of these lower leaves increases the concentration of radioactivity in them, especially around lesions, but has no marked and consistent effect on the concentration in other organs. Infection on the presentation leaf reduces photosynthesis and export of C^{14} to other parts. Heat girdling of petioles of healthy and infected leaves show that in both cases, C^{14} export takes place through phloem and its import through xylem. Radioactivity in detached leaves exposed to $C^{14}O_2$, either in light or in darkness, is uniformly distributed in healthy leaves, but accumulates around lesions in diseased ones. Starch has similar distribution. Pathological increase in transpiration and metabolic activity are probably responsible for the altered C^{14} distribution in diseased leaves.

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" In order to understand the parts we must first focus our attention upon the whole, because this whole is the field of study that is intelligible in itself ".

Arnold Toynbee: A Study of History (1946).

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I. GENERAL INTRODUCTION.

In diseased plants many physiological processes such as respiration, photosynthesis, growth and solute absorption can be pathologically altered. Studies on the pathology of these functions have been considerable. However the effect of infection on translocation and distribution of solutes has received relatively little attention, in spite of the possibility that alterations in translocation could be the cause - the result also - of a disturbance in many physiological processes.

Therefore to reach a better understanding of the nature of disease it seems important to carry out experiments to investigate the effects of infection on translocation and distribution of food substances in the host plant.

Late blight of potato (parasite - *Phytophthora infestans* (Mont.) DBy., race 0; host - *Solanum tuberosum* L. variety Irish Cobbler), was chosen for this study. The succulent stems, long internodes and easily manipulable leaves make potato plants favourable for use in translocation studies. In addition the vascular anatomy has been worked out in detail. *P. infestans* on the other hand, is easily cultured on synthetic media and the gene-for-gene relation between this parasite and the host is well known.

It is significant that whole plants have been used in these experiments, in view of the emphasis which has been placed hitherto, on detached organs - usually leaves or parts of leaves - in studying pathological alterations. Because of the interrelation between organs in the plant it is possible that they differ in their reaction to disease when separate from the plant. For example, it is possible that the movement of substances into and out of attached infected leaves is associated with a host reaction which in some respects differs profoundly from that which prevails in detached leaves.

In this study radioactive carbon (C^{14}), was used as a tracer with the hope that it would be possible to observe the effects of disease, if any, on the movement and distribution of photosynthates. C^{14} is a very convenient tool for this kind of work because it can be easily and naturally introduced into the plant by allowing a leaf to carry on photosynthesis with $C^{14}O_2$, and it can be detected at very low concentrations. Also, because of the small amounts of C^{14} used (8μc per experiment), only minimum handling precautions were necessary.

The research reported in this thesis was carried out in the Department of Plant Pathology, Macdonald College of McGill University from May 1960, to September 1961.

II. LITERATURE REVIEW

A. INTRODUCTION

The purpose of this review is to discuss briefly the importance, causal organism, symptoms and factors affecting development of late blight disease of potato; to review general aspects of the physiology of host-parasite relations which are significant to an understanding of the alterations in the physiology of potato as a result of infection by the late blight pathogen; and to review briefly current knowledge of translocation and distribution of solutes in plants. This should provide background information concerning the relation of disease to physiological processes including, translocation and distribution of solutes, and should contribute to an understanding of the information to be presented, from studies on the effect of late blight infection on the distribution of C^{14} in the potato plant.

B. DESCRIPTION OF THE DISEASE

1. IMPORTANCE OF LATE BLIGHT

The ravages of late blight on potato crops have been known since the beginning of the nineteenth century. Indeed, the occurrence of late blight epidemics in the nineteenth

century serves as an outstanding example of the influence of a disease on the course of social, political and scientific history (Keitt, 1959; Stakmann, 1959).

Late blight is world wide in distribution. In an extensive review on late blight epidemics throughout the world Cox and Large (1960), indicate that this disease occurs wherever the potato is grown. It causes damage to potato crops in all the provinces of Canada, but is most prevalent in the Maritime Provinces and Eastern Quebec (Cox and Large, 1960; Howatt, 1957).

2. THE CAUSAL ORGANISM

Phytophthora infestans (Mont.)DBy., is the organism which causes late blight of potato and was first fully described by DeBary in 1876 (cited in Bessey, 1950). Since that time extensive studies have been made on the biology of the organism (Crosier, 1934), penetration phenomena (Pristou and Gallegly, 1954), nutrition and physiology (Sakai, 1956); host-parasite relations (Ferris, 1955; Tomiyama, 1960; Müller, 1959), the nature of sexuality (Galindo and Gallegly, 1960), the genetics of pathogenicity (Black, 1952; Gallegly and Niederhauser, 1959), and epidemiology (Hirst and Stedman, 1960a; 1960b).

The life history of the fungus has been described in some detail (Cox and Large, 1960; Walker, 1957). Under

favourable conditions of temperature and moisture spores on leaves germinate and subsequently enter the leaf. The development of symptoms, which will be described presently, is followed by sporulation. Sporangia may be air-borne to other leaves where they germinate and cause further infection. Alternatively the sporangia may remain in the soil where they can infect potato tubers (Cox and Large, 1960; Hirst and Stedman, 1960b; Zan, 1956). The pathogen generally overwinters as living mycelium in potatoes infected during the previous season. Oospores are also believed to be important in survival (Yurova, 1960).

The Organism may be classifed arbitrarily as a type of facultative saprophyte (Walker, 1957). It becomes established on the host by means of excretory substances (Müller and Behr, 1949; Pristou and Gallegly, 1954), which weaken the host tissue in advance of the invading fungus. P. infestans, in some respects resembles an obligate parasite (Yarwood, 1956), as it may live for a while in association with the host cells before destroying them. There are a number of physiological races of the parasite (Black, 1952), some of which are virulent, others avirulent, on a given host variety.

In addition to the pathogenic characteristics of the parasite, however, it is generally recognized that the occurrence of infection, and the subsequent development of symptoms,

is determined by the nature of the host and the nature of the environment, which are in a complex relation with each other and with the parasite.

3. SYMPTOMS ON POTATO

a. Description of Symptoms.

The development of symptoms on the potato plant from the time of penetration to the time of sporulation has been studied and reported (Callbeck, 1950; Ferris, 1955; Lowings and Acha, 1959; Pristou and Gallegly, 1954; Walker, 1957). Leaf damage is most common and most spectacular in its effects. Therefore microscopic and macroscopic symptoms will be described here in some detail.

Microscopic studies indicate that initially biflagellate zoospores - in a film of water on a leaf - encyst, germinate and produce appressoria. On the under side of the appresorium an infection peg develops, which enters the leaf, usually through the epidermis and occasionally through the stomata (Pristou and Gallegly, 1954). In susceptible host tissue secondary mycelium develops from the primary mycelium - produced from the infection peg - and spreads freely through the mesophyll of the leaf, appearing as flecks in about 2 days and enlarging rapidly to large spreading lesions in 3 to 4 days (Ferris, 1955). In a resistant host, on the other hand, the

primary mycelium does not spread, and gets no further than the epidermal cell (Ferris, 1955; Pristou and Gallegly, 1954).

Macroscopic symptoms on the leaf are typical. They appear as brown lesions surrounded by a light green or yellowish zone which merges into the normal green of the uninfected part of the leaf (Callbeck, 1950; Walker, 1957). Sporulation occurs in this transition zone, usually on the underside of the leaf.

It is important to recognize that the symptoms of late blight outlined here, are only one aspect of the disease syndrome. This is evident from studies of the physiology of host-parasite relations, general aspects of which will be discussed in a later section.

b. Environmental Factors in Relation to Infection and Symptom Development.

Susceptibility to disease depends on the external environment and on the internal conditions of the host (Daly et al, 1961; Grümmer, 1955; Yarwood, 1956; 1959).

It is known that the spread of P. infestans and the occurrence of epiphytotics of late blight are dependent on a particular interplay of temperature and humidity (Crosier, 1934; Hirst and Stedman, 1960a).

The increased resistance to P. infestans associated with an increase in nitrogen nutrition (Lowings and Acha, 1959), is an indication of the importance of the internal environment of the plant. These workers also suggest that chemical changes in senescent leaves may influence the nutrition and growth of the parasite independently of any liberated during cell necrosis. Thus, Grainger's (1956) use of the term "disease potential" connotes, "the relative physiological aptitude of a host crop or plant to contact disease at different stages of its life history". He found that the percentage of carbohydrate in a crop plant was related to the disease potential. For example, periods of low disease potential in potato were related to a low percentage of carbohydrate in the whole plant and vice versa.

A knowledge of the factors influencing infection and symptom development, - predisposition, (Yarwood, 1959) - is of great value in studies of the physiology of susceptibility and the physiology of resistance.

C. THE PHYSIOLOGY OF HOST PARASITE RELATIONS.

1. INTRODUCTION

What we commonly think of as infection consists essentially of two stages. The first is the entrance of the parasite into the tissues of the host. The second is the

establishment of a parasitic relationship with the host (Wingard, 1953). In general changes in the host physiology that occur following infection are incited by the presence of the pathogen (Allen, 1953; Keitt and Boone, 1956; Müller and Behr, 1949; Sempio, 1950; 1959; Shaw and Samborski, 1956;1957). In this section the influence of infection on respiration, photosynthesis and water balance is discussed, in addition to some aspects of the physiology of host-parasite relations, because a disturbance of one of these processes would be expected to influence translocation and vice versa. This latter phenomenon will be discussed in a separate section.

2. ASPECTS OF THE PHYSIOLOGY OF HOST-PARASITE RELATIONS.

Physiological aspects of fungus diseases in potato (Müller, 1953; Tomiyama, 1960; Tomiyama et al., 1959), as well as in other plants (Allen, 1953; Brown, 1955; Farkas and Kiraly, 1958; Kiraly and Farkas, 1959; Walker and Stakmann, 1955), have been reviewed.

Although knowledge of the mechanism of host-parasite relations is still very meager, it is evident that obligate parasites, like Puccinia graminis Pers., and non-obligate parasites, like P. infestans are fundamentally similar. This view is supported by Brown (1955) who states that, "the agent inhibiting the growth of Puccinia graminis on an unsuitable

host may, possibly, be comparable to that which acts similarly on P. infestans in the cells of a resistant potato variety".

The establishment and spread of a parasite in a host is basically determined by two phenomena which are interrelated, and they are: nutritional phenomena (Garber, 1956; 1960; Keitt and Boone, 1956), and inhibitional phenomena (Müller, 1959; Müller and Behr, 1949; Sempio, 1950; Walker and Stakmann, 1955; Wingard, 1941).

An outstanding example of the role of nutritional phenomena in pathogenicity is reported by Keitt and Boone (1956), using Venturia inaequalis (Cke.)Wint., on apple. These workers showed that pathogenicity can be nullified or restored by nutritional controls and concluded that there is a direct relationship between the host as a growth medium and the specific nutritional requirements of the parasite.

Information of this kind has not been reported for P. infestans on potato plants. However, Lowings and Acha (1959), observed a relation between nitrogen nutrition and resistance of the potato leaves to attack by the pathogen, and Grainger (1956) noted a relation between the level of carbohydrate in the potato plant and susceptibility to late blight. Such preliminary observations suggest that nutritional phenomena are important in the development and spread of P. infestans on potato.

Inhibitional phenomena have been studied extensively, especially in studies relating to the nature of disease resistance (Stakmann and Walker, 1955; Wingrad, 1941). The mechanisms associated with inhibitional phenomena are mediated by physical and chemical antimicrobial agents (Garber, 1956; Walker and Stakmann, 1955). Chemical antimicrobial agents are operative after the entry of the parasite (Allen, 1953) and include, those occurring naturally within the host tissues (Kiraly and Farkas, 1959; Hayes, 1947; Walker and Stakmann, 1955), and those resulting from the response of the infected tissues to the presence of the pathogen. There is evidence that both of these types of chemical antimicrobial agents are present in potato plants (Johnson and Schaal, 1957; Kiraly and Farkas, 1959; Müller, 1959; Müller and Behr, 1949; Vallé, 1957), at concentrations which are, or become, inhibitory to an invading parasite.

Vallé (1957) indicates that chlorogenic acid is the substance in potato leaves which is responsible for resistance to P. infestans. Further, Johnson and Schaal (1952; 1957), showed that the chlorogenic acid content is higher in potato varieties resistant to scab, Streptomyces scabies (Thaxt.) Waksman and Henrici., than in susceptible varieties.

Although there is increasing evidence in the literature to show the important role of phenolic compounds

and their derivatives in host-parasite relations (Nienstaedt, 1953; Oku, 1960; Pridham, 1959; Rubin and Aksenova, 1957; Tomiyama et al., 1959), it is, nevertheless, important to recognize that the mere presence of a toxic compound does not warrant the conclusion that it plays a role in the resistance of the host to one or more of its parasites (Walker and Stakmann, 1955).

In addition to occurring under natural conditions, fungitoxic substances may be found in tissues in response to infection (Müller, 1956; 1959; Müller and Behr, 1949). For example Müller (1959), indicates that invasion of the inner epidermis of Phaseolus vulgaris pods with P. infestans and other pathogens results in the production of antibiotic factors or so called phytoalexins. He defines phytoalexins as, " .... antibiotics which are the result of an interaction of two different metabolic systems, the host and the parasite, which inhibit the growth of organisms pathogenic to plants".

Müller also draws attention to the fundamental difference between the phytoalexins and the classic antibodies of animals. Whereas in animals antibodies are highly specific and systemic, in plants the unspecified phytoalexins are, "restricted to the site of infection".

On the basis of the phytoalexin concept a mechanism of resistance of potato to P. infestans has been proposed (Müller and Behr, 1949). These workers suggest that infection

is accompanied by the formation or the activation of a principle, a phytoalexin, which is produced as a result of an interaction between the living host and the parasite. The substance exerts a toxic effect on the host cells which die and lose their ability to serve the parasite as a host.

Furthermore, it is claimed that there is a close relation between the degree of resistance and the rapidity with which necrosis is brought about (Müller and Behr, 1949). This is contrary to the view of Johnson and Schaal (1957), who claim that the degree of susceptibility or resistance of potato tubers depends on the amount of inhibitory substance produced or accumulated in response to infection rather than the rate. Also, Ferris (1955), claims that the rate of necrotic response of leaves of Solanum demissum derivatives to infection with P. infestans is not a dependable indication of susceptibility or resistance.

It is evident from the foregoing, that there is an interaction between the parasite and the host following infection. However, it is not known with certainty whether the initial response to infection originates in the cells of the host or those of the parasite (Müller, 1959). Nevertheless it is recognized that disease caused by either obligate parasites or non-obligate parasites is associated with an alteration in certain principal functions of the host including respiration, photosynthesis and water balance.

3. RESPIRATION

There is always a change in the respiratory rate of infected tissue (Allen, 1953). Alterations in respiration have been reported for rusted safflower (Daly and Sayre, 1957), rusted wheat (Shaw and Samborski, 1957), rusted bean (Daly *et al.*, 1961), mildewed wheat (Allen, 1942), and *P. infestans* infected potato (Tomiyama *et al.*, 1959). The respiratory changes observed are usually increases. For example Tomiyama *et al.* (1959), found that infection of potato tuber tissue with an incompatible race of *P. infestans* caused a definite increase in respiratory rate.

The mechanism of the respiratory increase following infection has not been determined with certainty (Hackett, 1959). Nor is the physiological significance of increased respiration in infected tissues known, as many agents can stimulate tissue respiration (Hackett, 1959). However a change in respiration following infection, is presumably only one manifestation of the complex interrelation between two metabolic systems: the host and the parasite. For example a change in the C^6/C^1 ratio of rust infected tissue (Daly *et al.*, 1961; Shaw and Samborski, 1957), suggests that infection can direct metabolism into abnormal pathways. Supporting evidence for an enhanced alternate pathway following infection of wheat by rust fungi is reported by Jain and Pelletier (1958), who showed that

radioactive sedoheptulose can be more readily detected in rusted tissue than in healthy tissue. Also, respiratory increases following infection are accompanied by increases in synthetic activity (Daly et al., 1961; Uritani and Akazawa, 1959). For example the formation of polyphenols is a common feature occurring concomitantly with respiration increase, and coumarins have been observed to accumulate in infected plants (Uritani and Akazawa, 1959).

From the foregoing the nature and rate of respiration can evidently be altered by infection and by other environmental changes. Increases in synthetic activity accompany pathological alterations in respiration. Such changes have been recorded in P. infestans infected potato and in other diseases.

4. PHOTOSYNTHESIS

It is known that infection with obligate parasites is accompanied by an alteration in photosynthesis (Sempio,1950; Allen, 1954). In practically all cases studied there is, ultimately, a decrease in the photosynthetic activity of the tissues affected. The effect of P. infestans infection on photosynthetic activity is not reported here, but it is known that the fundamental physiological processes affected by non-obligate parasites are analogous to those for obligate parasites (Kiraly and Farkas, 1959). Consequently, P. infestans infection would be expected to cause a general decrease in photosynthetic activity.

The importance of a decline in photosynthesis on the balance of metabolic activities in the plant and the influence of such a decline on the susceptibility of the plant to disease, is recognized (Sempio, 1959).

5. WATER BALANCE

Most of the physiological disturbances associated with the disease syndrome in plants are traceable either directly or indirectly to a disturbed water balance (Subramanian and Saraswathi-Devi, 1959). These workers consider that water imbalance in diseased plants may be brought about by dysfunction of the conductive elements, the roots or the leaf and stomates.

Except in the case of wilt diseases the conductive elements are considered to be of little significance in determining water balance (Jensen et al., 1961; Lundegardh, 1946). Furthermore passive absorption i.e. absorption controlled and initiated by transpiration from the shoot, is of greater significance than active absorption (Slayter, 1960; van den Hornet, 1948). Consequently, if water supply to the roots is adequate, water balance in plants having the leaves as the only infected organs is not likely to be due to root dysfunction.

Enhanced transpiration is a feature commonly accompanying pathogenesis and is associated with leaf injury. The

development of spreading lesions on potato leaves (Ferris, 1955; Walker, 1957), as well as action in advance (Thatcher, 1942), is ample evidence of leaf injury associated with P. infestans infection. Moreover, it has been demonstrated that infected tissue or necrotic zones represent the leaf areas with the highest intensity of transpiration (Harvey, 1930; Sivadjian and Kern, 1958; Yarwood, 1947).

From the foregoing, there is reason to believe that a disturbance in the water balance can occur during the development of a disease on the leaves of a plant. However, it is not known whether disturbed water balance is the cause or the result of other alterations associated with pathogenesis (Subramanian and Saraswathi-Devi, 1959).

D. TRANSLOCATION AND DISTRIBUTION OF SOLUTES IN PLANTS

1. INTRODUCTION

Translocation and distribution of solutes in plants are interrelated but quite distinct events. According to Swanson (1959):

> "The term translocation of organic solutes, is reserved for transport processes which are more directly associated with specific transport tissues, mainly phloem, and are

characterized by a considerably higher rate (potential or capacity) as well as by movement over greater distances from organ to organ as opposed to cell to cell".

Plants, however, accumulate translocated solutes in specific areas. When these accumulations are viewed within a whole plant the term distribution or distribution pattern is applied (Langston, 1956).

The reader is referred to a number of extensive reviews and papers (Arisz, 1952; Bollard, 1960; Bukovac and Wittwer, 1957; Crafts, 1951;1961; Curtis, 1935; Kursanov, 1961; Mitchell et al., 1960; Swanson, 1959; Tukey et al.,1961; Williams, 1955; Zimmermann, 1960), for a comprehensive bibliography on translocation and distribution of solutes in plants.

In this section aspects of translocation and distribution of solutes will be discussed including; the distribution pattern of solutes, pathways of translocation and mechanisms of translocation. Finally, pathological alterations in translocation and distribution of solutes will be discussed at some length. The potato plant has not been used extensively in translocation studies (Crafts, 1933; 1961), so that information obtained from experiments with other plants will be presented.

2. ASPECTS OF TRANSLOCATION AND DISTRIBUTION OF SOLUTES IN PLANTS

a. The Distribution Pattern of Solutes.

The distribution pattern of organic as well as inorganic solutes exported from leaves has been studied extensively. Radioactive carbon (C^{14}) is commonly used as a tracer in translocation studies involving organic solutes (Aronoff, 1955; Jones et al., 1959; Kursanov, 1955; Nelson and Gorham, 1957a; 1957b; 1959a; 1959b; Shiroya et al., 1961), and radioactive phosphorous (P^{32}), is commonly used in studies involving inorganic solutes (Biddulph, 1941; Biddulph and Biddulph, 1959; Biddulph and Markle, 1944; Colwell, 1942).

In general the initial movement of C^{14}-labelled translocates is predominantly downward to the roots and to a lesser extent upward to the shoot. For example, Jones et al., (1959), studied the translocation of C^{14} in tobacco plants by allowing single leaves (presentation leaves) to assimilate $C^{14}O_2$ for 2 to 3 hours. They found that 20 to 30 percent of the radioactivity was irreversibly incorporated into the presentation leaf, and 40 to 50 percent of the total radioactivity moved out, mainly as sucrose, after 7 or 8 hours. Of the material exported 3 percent reached the upper leaves and stem apex, and the remainder was in the stem and roots. There was no radioactivity in leaves below the presentation

leaf, but the distribution in the leaves above the presentation leaf followed a well defined pattern determined by the vascular interconnections. Similar studies by Shiroya et al.,(1961), with tobacco indicate that C^{14} is translocated to leaves both below and above the presentation leaf. Like Jones et al. (1959), they found that the distribution of C^{14} is influenced by the anatomical connections among leaves and their stage of development. Also, C^{14} was translocated to flowers or developing seeds, suggesting movement of solutes to the most active sites metabolically; - sinks.

The influence of metabolic sinks on the distribution pattern is emphasized by Aronoff (1955), who concluded from translocation studies in bean that, "under normal circumstances the flow of photosynthate is greatest to the growing regions .. ... No physiological condition approaches this as a causative agent for the direction and magnitude of translocation". A similar sort of inference was made by Nelson and Gorham (1959b), who ascribed the initial distribution pattern of C^{14} in soybean plants to the influence of the roots which they concluded exerts a strong "demand" which favours translocation in a downward direction more than in an upward direction in the stem. Further, Colwell (1942), noted that P^{32} movement in squash is correlated with food movement in the plant and is predominantly towards the nearest sinks for organic solutes.

The distribution pattern of solutes in a plant is not static but dynamic. For example Kursanov (1955), showed that C^{14} may move from the leaves to the roots of sugar beet, and later to the growing sprouts and fruits. Alternatively C^{14} may migrate directly into growing sprouts, or young fruits or leaves, without entering the roots.

The distribution pattern of solutes may vary with the plant species, solute mobility or the stage of development of the plant (Biddulph, 1959; Swanson, 1959). However, a similar distribution pattern determined by the source to sink relation (Crafts, 1961), of phosphorous and other solutes as well as of carbohydrates was shown for cotton (Biddulph and Markle, 1944); for bean (Biddulph and Biddulph, 1959), and for tobacco (Shiroya et al., 1961).

b. Pathways of Transport of Solutes

In general the circulation and distribution of food substances and other solutes throughout the plant is considered to be via the vascular system: phloem (Swanson, 1959; Zimmermann, 1960); xylem (Biddulph, 1959; Bollard, 1960).

Solute movement in the phloem is predominantly downward (Biddulph, 1956; Biddulph et al., 1958; Biddulph and Cory, 1957; 1960; Biddulph and Markle, 1944; Chen, 1951; Nelson et al., 1959; Rabideau and Burr, 1945). However, Biddulph et al.,(1958),

using microautoradiography to determine the histological pathway of movement of C^{14} and P^{32} in the stem of bean, found that solutes move upward and downward in the phloem, with some lateral movement into the xylem especially at higher levels in the stem. More recently, Biddulph and Cory (1960), found that upward and downward movement of solutes can take place in the same phloem bundle.

Solute movement in the xylem is predominantly upward (Biddulph, 1959; Stout and Hoagland, 1939). It has been conclusively shown with radioactive tracers that salts absorbed by the roots are carried towards the leaves under the influence of the transpiration stream. However Biddulph (1957), cites a number of papers in which it has been shown that dyes and salts are capable of rapid downward movement in the xylem. More recently Nelson et al. (1958), have shown rapid downward movement of C^{14} in the xylem. This claim is disputed by Crafts (1961), who suggests that diffusion of $C^{14}O_2$ via the intercellular space system cannot be discounted when considering rapid translocation.

It is evident from the foregoing that the phloem is the main pathway for downward movement of solutes, but there is some upward movement, and the xylem is the main pathway for upward movement, with some downward movement.

c. Mechanisms of Translocation

Attempts to explain the mechanism of transport in the xylem and the phloem are frustrated by the great variation in rates of transport (Nelson and Gorham, 1957a; 1957b; Nelson et al., 1958), in direction of movement (Biddulph and Cory, 1960) and in distribution patterns (Langston, 1956).

For xylem transport, the generally held concept is that solutes move "en masse" in the xylem transpiration stream (Biddulph, 1959; Stout and Hoagland, 1939). Recently, however, Biddulph et al., (1961), using THO and Ca^{45} have shown that neither water nor calcium ascends exclusively in the vessels, but that the stem as a whole functions as a pathway for ascent. They concluded that a translocation mechanism involving an exchange process explains the observed unequal rates of Ca^{45} and THO, and their different distribution characteristics in different plant parts, more successfully than a mechanism based on mass flow.

The mechanism of translocation in the phloem is still not fully understood. A number of theories have been proposed to explain phloem translocation (Swanson, 1959). Two theories are currently popular: the mass flow hypothesis first proposed by Münch in 1932 and strongly advocated by Crafts (1951; 1961), and the protoplasmic streaming hypothesis, advocated by Kursanov (1961).

Recently, Biddulph and Cory (1960) demonstrated two distinctively different patterns of bidirectional movement in the phloem of bean plants which they suggest are explainable by different mechanisms: one primarily dependent upon physical forces such as pressure flow; the other more closely allied with vital forces such as protoplasmic streaming.

From the foregoing it is evident that no single mechanism has been proposed which will explain *in toto*, phloem transport, or xylem transport. Consequently it will be difficult to explain clearly the nature of pathological alterations in translocation and subsequent distribution of solutes in plants.

3. PATHOLOGICAL ALTERATIONS IN TRANSLOCATION AND DISTRIBUTION OF SOLUTES.

a. Pathological Alterations in Translocation and Distribution of Solutes in Whole Plants.

Whole plants have been used to investigate pathological alterations in organ to organ transport of tagged solutes (Baldacci *et al.*, 1958; Gottlieb and Garner, 1946; Hampson, 1960; Yarwood and Jacobson, 1955).

Gottlieb and Garner (1946) used wheat seedlings to study the distribution of radiophosphorus (P^{32}) in normal and rusted leaves. The seedlings were grown throughout the experiment in Hoagland's complete nutrient solution containing

radioactive phosphorus. When the plants were about 10 cm. high, one side of the distal half of the first true leaf of a number of seedlings was inoculated with the rust fungus. All other leaves were clipped off when they appeared. These workers made Geiger readings of leaf tissue digests and autoradiograms of distribution in leaves at different stages following inoculation, but they did not study the distribution of the radioisotope in the plant as a whole. However, they found that although the total amount of phosphorus absorbed by the plant was not significantly influenced by the disease there was greater accumulation in the rusted parts of infected leaves, which concentrated in areas invaded by the parasite. Thus, they concluded that rust infection stimulates the accumulation of P^{32} in the diseased areas of leaves.

Yarwood and Jacobson (1955), studied the distribution via translocation, of P^{32} or C^{14} in primary leaves of bean (Phaseolus vulgaris L.), 5 to 8 days following inoculation with rust (Uromyces phaseoli Arthur). They supplied P^{32}-labelled H_3PO_4 or C^{14}-labelled sucrose to plants, with or without roots, by immersing a healthy or half-rusted primary leaf in a solution of the labelled solute for 63 to 91 hours. The opposite half-rusted primary leaf was either exposed to air or immersed in water. After treatment the healthy areas and diseased areas of the leaves were assayed for radioactivity using a Geiger

counter. They found that C^{14} as well as P^{32} was translocated to the opposite primary leaf whether it was immersed in water or not. However, much less total radioactivity moved to the opposite primary leaf when it was immersed in water than when it was not immersed. Furthermore, there was, in every case, a higher concentration of C^{14} and P^{32} in the rusted half of the leaf than in the non-rusted half.

Yarwood and Jacobson also noted that the degree of selective accumulation of C^{14} and P^{32} in half-rusted bean leaves was much greater following translocation than following direct application of these radiochemicals to rusted detached leaves. From a point of view of pathological alterations in translocation, however, the significance of this observation cannot be adequately appraised because the experiments with whole plants and detached leaves involved different intensities of rust infection, different intervals from inoculation to start of tests, and different durations of tests.

Hampson (1960), also studied the effect of infection on the distribution of C^{14}-labelled photosynthates in bean plants. One primary leaf of each of a number of plants was allowed to photosynthesize with $C^{14}O_2$ (in an exposure apparatus containing 100μc C^{14}), for 0.5 hour at different times following inoculation of the opposite primary leaf. The plants were

allowed a period of 0.5 hour in light to permit migration of the photosynthates, then heat killed. From autoradiograms he noted that radioactivity accumulated in infected leaves, where it concentrated in leaf veins and around rust lesions. In addition, he noted abnormal accumulation in non-infected organs. Accumulation in infected leaves was evident even before symptoms appeared and became less marked at later stages of infection. He concluded from his observations that infected primary leaves as well as other non-infected organs can act as metabolic sinks, accumulating translocates in competition with meristematic tissues. It is noteworthy that for the duration of the exposure period, the infected leaves were exposed to conditions favouring rapid transpiration. Hampson did not indicate whether accumulation of radioactivity in infected leaves occurred in the absence of transpiration. This would have been a valuable observation in view of the work of Yarwood and Jacobson (1955), who found that there is a reduction in translocation of tagged substances to infected leaves in the absence of transpiration.

Baldacci et al. (1958), found that in tissues damaged and then inoculated with fungi the accumulation of P^{32} via translocation was greater than in comparable tissues of healthy plants. This accumulation was evident in bean leaves even before the development of external symptoms.

From the foregoing it is evident that investigators using whole plants to study pathological alterations in translocation and distribution have given little or no attention to the effects on the plant as a whole, and still less attention to the phenomenon of translocation. Instead, attention has been directed mainly to the localized effects of disease on the distribution within leaves.

b. Pathological Alterations in Transport and Distribution of Solutes in Detached Leaves.

Detached leaves are commonly used (Shaw, 1961; Shaw et al., 1954; Shaw and Samborski, 1956; Wang, 1960; Yarwood and Jacobson, 1950; 1952; 1955), to study pathological alterations in transport and distribution of tagged solutes. Less commonly, leaves attached to plants (Shaw et al., 1954; Wang, 1961), are used for such studies, but no consideration is given to the possible influence of the remainder of the plant on the final result. However, with the use of the above-mentioned experimental material, some very interesting observations have been made concerning the effects of infection on the distribution of solutes.

Yarwood and Jacobson (1955), studied the distribution of radiochemicals in detached leaves infected with a variety of pathogens. The leaves were exposed directly to

S^{35} vapour or floated on $H_3P^{32}O_4$ or C^{14}-sucrose. They assayed the leaves for radioactivity using a Geiger counter, or by means of autoradiograms, and found that there was a greater concentration of radiochemical in the diseased areas of a leaf, than in comparable healthy areas (selective accumulation), in the majority of the host pathogen combinations studied but not in all. In some cases diseased leaf showed less accumulation than comparable healthy tissue and in other cases there was no clear difference. However, in a similar experiment performed earlier (Yarwood and Jacobson, 1952), they found that in most cases H_2S (toxic), H_3PO_4 and C^{14}-sucrose (nutritive), and $Cs^{137}Cl$ (inert) were selectively accumulated. They concluded that there was no obvious relation between the nutritive or toxic value of chemicals and their selective accumulation.

Shaw et al. (1954), studied the uptake of radioactive carbon (C^{14}), and phosphorus (P^{32}) by wounded leaves, and leaves infected with facultative and obligate parasites (wheat and sunflower rust and mildew of wheat and barley; 3 to 4 days following inoculation). C^{14} - sugars or P^{32}-phosphate were introduced either by localized injection of attached leaves or by standing the cut leaf bases in 0.25 per cent solutions for from 20 minutes to 24 hours. C^{14} was also fed to leaves as $C^{14}O_2$. Autoradiograms indicated that following

feeding with labelled sugars or phosphate the concentration of radioactivity in uninfected tissue wounded by pricking or removing the epidermis was sometimes the same or less than in undamaged areas and was similar to that found with facultative parasites. On the other hand there was a rapid and marked accumulation of radioactivity at infection sites of obligate parasites which was most pronounced on susceptible hosts. Also exposure to $C^{14}O_2$ in light indicated that the parasite depresses photosynthesis in the uninvaded host tissue and the subsequent influx of fixed C^{14}. On the basis of such observations these workers concluded that, for obligate parasites at least, the rate of movement of substrates plays an important part in determining susceptibility to disease.

In a later study Shaw and Samborski (1956), dipped the bases of excised parasitized leaves in radioactive solutions for from 20 minutes to 24 hours and found that in less than 2 hours from the start of an experiment several groups of compounds including sugars, sugar derivatives, acids, phenols (toxic), and ions, accumulated at infection sites of rust and powdery mildew. In contrast to their earlier work (Shaw et al., 1954), they concluded that accumulation at infection sites bears little or no relation to the effect of the accumulated substance.

Also, in contrast to Shaw et al., (1954), Shaw and Samborski (1956), found that the products of light and dark fixation of carbon dioxide were transported into and accumulated at infection sites of rust and powdery mildew. Furthermore, they found that in short-term experiments photosynthesis (or $C^{14}O_2$ fixation in light) was depressed at some non-erumpent 5- to 6-day old wheat rust infection sites and stimulated at other infection sites of a similar type.

Wang (1961), on the other hand, exposed rust infected leaves on bean plants to $C^{14}O_2$ in light for 0.5 to 3 hours and assayed the leaves for radioactivity after 3.5 hours in ordinary air in light. He found a consistent increase in radioactivity at infection sites from 3 to 11 days following inoculation, and concluded that this was due to de novo synthesis from carbon dioxide at the infection sites and not due to enhanced translocation of photosynthates from non-infected areas.

It is evident from the foregoing that leaves from a variety of hosts, infected with a variety of pathogens, at different stages following inoculation and under different environmental conditions, usually - but not always - accumulate a greater concentration of tagged solutes in infected areas than in comparable healthy areas. The exact

significance of this event in the physiology of host-parasite relations is not known with certainty; there are conflicting views concerning the factors or phenomena responsible for such accumulation.

c. Phenomena Associated with Accumulation of Substances at Infection Sites.

The term infection site refers to the diseased area corresponding to the fungus and the host tissue closely associated with it (Shaw, 1961). As indicated earlier the greater concentration of externally applied chemicals in diseased than in comparable normal tissues is known as selective accumulation. The ratio of the amounts of chemical accumulated in these areas is known as the accumulation ratio.

Attempts have been made to explain selective accumulation. There is much evidence to show that it is metabolically dependent (Gottlieb and Garner, 1946; Shaw, 1961; Shaw and Samborski, 1956; Wang, 1960; 1961; Yarwood and Jacobson, 1955). However, there is evidence that, in part at least, transpiration plays a role in selective accumulation (Baldacci et al., 1958; Harvey, 1930; Shaw and Samborski, 1956; Yarwood and Jacobson, 1955).

In attempting to explain selective accumulation in terms of metabolic phenomena it is important to consider

the relative roles of the host tissue and the mycelium of the invading pathogen.

Accumulation of radioactivity was found to be predominantly in the host cells, beneath the mycelium in mildewed bean leaves (Yarwood and Jacobson, 1955), and in mildewed and rusted wheat leaves (Shaw et al., 1954; Shaw and Samborski, 1956), 4 to 6 days following inoculation. These latter workers also found radioactivity in mildew conidia and mycelium isolated from these leaves, and in rust uredospores and teliospores. However, Baldacci et al., (1958), noted that P^{32} concentrated mainly in the diseased tissues of rusted bean leaves during the early stages of infection, but mostly in the mycelium and spores during the latter stages of infection.

There is usually an increase in respiration and dry weight in the host tissue at infection sites. For example Shaw and Samborski, (1956), noted that the oxygen consumption and dry weight of wheat rust infected leaf discs increased relative to healthy tissue and was accompanied by accumulation, at infection sites, of radioactive glucose or its products. Furthermore, they claimed (Shaw and Samborski, 1956), that accumulation roughly paralleled the course of respiration and was inhibited by respiratory inhibitors and by anaerobiosis. They concluded from their studies that accumulation at infection sites is metabolically dependent.

Yarwood (1953), noted significant increases in temperature of rust infected bean leaves even after the mycelium was killed and concluded that this increase was principally due to host respiration. Later Yarwood and Jacobson (1955), suggested that the accumulation of P^{32} and C^{14} at infection sites, via translocation, and in the absence of transpiration could be metabolically dependent. This, they claim, could also explain the zones of accumulation of S^{35} about rust pustules.

Selective accumulation is, however, not necessarily metabolically dependent. For example, there was no apparent relation between selective accumulation and the toxic or nutritive value of the substance accumulated (Shaw and Samborski, 1956; Yarwood and Jacobson, 1952; 1955). Furthermore, in leaves of Pyrus malus infected with Podosphaera leucotricha, and Quercus agrifolia infected with Sphaerotheca lanestris - obligate parasites which presumably stimulate host respiration at infection sites - Yarwood and Jacobson (1955), found less S^{35} and P^{32} in diseased tissue than in comparable healthy tissue.

Transpiration, at least in part, is considered to account for selective accumulation. The accumulation of dyes (Harvey, 1930) around: pustules of P. graminis infected wheat leaves; mildew infections and insect injuries on squash leaves; and Septoria spots on celery, is considered to demonstrate

the effect of certain local infections by fungi on transpiration.

The observation (Yarwood and Jacobson, 1955) that rusted bean leaves exposed to air accumulated more C^{14} and P^{32}, via translocation, than rusted leaves immersed in water suggests an effect of transpiration.

Baldacci et al., (1958), noted with bean leaves that the accumulation of P^{32} at lesions produced with carborundum - which resembled the hypersensitive spots formed at the onset of disease - was correlated with transpiration.

Indeed, it has been demonstrated that fungus infected leaf tissues, including those of bean, lose water more rapidly than comparable healthy tissues (Yarwood, 1947), and this is considered to be due to increased permeability of the host cells. Permeability increase has been demonstrated in association with tissue invasion by several parasites on their susceptible hosts (Thatcher, 1939; 1942). Moreover, an increase in the intensity of respiration at infection sites is also considered to generate an increase in permeability (Sempio, 1959). This suggests that, in part at least, transpiration may be metabolically dependent. If this is the case then, indirectly, selective accumulation due to transpiration is metabolically dependent.

In view of the foregoing, one should exercise the utmost caution in attempting to account for the cause of

accumulation of substances at infection sites as it may be due to the activities of the host tissue or the parasite, or both; depending upon the nature of the host-parasite relationship, the stage of infection, and the environmental conditions. At any rate it appears that accumulation in the diseased tissues of the host is, directly or indirectly, metabolically dependent.

E. CONCLUSIONS

It is concluded from this review that, in general, infection of a plant with an obligate or non-obligate parasite is accompanied by a disturbance of such physiological processes as respiration, photosynthesis, water balance, and so on. The nature and extent of such physiological disturbances in the potato plant infected with P. infestans is not known with certainty because of the limited available information.

Further, it has been demonstrated with radioactive tracers that the distribution of solutes in attached and detached leaves is pathologically altered. But, pathological alterations in translocation - as defined - are not known because the rates and pathways of solute transport have never been studied in conjunction with disease. Moreover, the mechanisms of translocation are still not known with certainty.

The available data, however, do not provide any information concerning the translocation or distribution of radioactive tracers in healthy or P. infestans infected potato plants. A study of the effect of P. infestans infection on the distribution of C^{14}- labelled solutes in the potato plant should help to determine whether a non-obligate parasite acts similarly, in this regard, to an obligate parasite. In addition, such information should contribute to knowledge concerning the nature of disease.

III. MATERIALS AND METHODS

A. INTRODUCTION

Materials and methods of general application are described in this section under the following headings:

- Culture of potato plants
- Plant material used (host)
- Inoculation with P. infestans (pathogen)
- Administration of $C^{14}O_2$
- Killing of plants
- Autoradiography

Materials and methods used in one or a few experiments will be described with the results of the experiments concerned.

B. CULTURE OF POTATO PLANTS

Potato plants of the Irish Cobbler variety were used. This variety was chosen because it is susceptible to all races of P. infestans, and consequently was expected to show relatively great and easily detected pathological alterations in 'translocation' and distribution of solutes.

Seed pieces were prepared from whole tubers, which had previously been washed and dried. Wedge-shaped pieces,

about 4 cm. long, and containing only one bud were excised from tubers, by means of a small kitchen knife. About 4 seed pieces were obtained from each tuber. The seed pieces were kept for 12 to 24 hours, in a tray and covered with a moist paper towel to allow time for the suberization of the cut surfaces then, 25 to 30 seed pieces were planted 3 cm. deep, in Vermiculite (a brand of exploded mica), contained in plastic trays. The Vermiculite was watered thoroughly and covered with an opaque cover, so that emerging shoots would be in darkness. The moisture level was maintained by occasional light watering with tap water. The planted trays were kept at 23°C. in a plant growth chamber.

About 7 to 10 days after planting, i.e. when the developing sprouts emerged, the Vermiculite was kept moist with nutrient solution instead of tap water. At that stage the sprouts were devoid of chlorophyll.

When the sprouts had attained a height of about one to 3 cm., they were exposed to light by substuting the opaque cover with a transparent one made of polyethylene sheet. The germination trays are illustrated in figure 1. Under the prevailing conditions there was rapid growth of the young plants.

About 10 to 12 days later the shoot and roots of the plants were about 12 and 20 cm. long, respectively. At this stage the plants were transferred to nutrient solution in 8-liter crocks. The nutrient solution used for culturing the potato plants was the same composition as that used by Hougland (1950), and included the following salts:

Calcium nitrate -	$Ca(NO_3)_2$	9.00 m e /liter
Ammonium phosphate -	$NH_4H_2PO_4$	4.00 " "
Potassium chloride -	KCl	2.55 " "
Magnesium sulfate -	$MgSO_4$	4.60 " "
Boric acid -	H_3BO_3	0.026 " "
Manganous sulfate -	$MnSO_4$	4.60 " "
Copper sulfate -	$CuSO_4$	0.0016 " "
Zinc sulfate -	$ZnSO_4$	0.0014 " "
Ferric citrate -	$FeC_6H_5O_7$	0.1612 " "

In each crock were placed 4 plants with comparable root and shoot development. These plants were previously washed in water in a large container until free of Vermiculite. Shoots other than the main shoot were removed.

Each plant was held upright by means of a cork stopper with a hole in the center and split through the middle, and the part of the stem passing through the cork stopper was protected by cotton batting. The plants were held in the nutrient solution by means of crock covers placed on the crocks

Figure 1.

Two-week old potato plants in Vermiculite in a plastic tray with a cover of polyethylene sheet (right), and without a cover (left).

and having 4 large holes into which the cork stoppers fitted. This is illustrated in figure 2. Lots of 16 or 32 plants were grown at a time.

The daily regime of illumination during growth of the plants consisted of 14 hours light and 10 hours dark. Fluorescent lamps - warm white - together with incandescent lamps were used (Dunn and Went, 1959; Leister et al., 1960). The light intensity at the level of the top of the crocks was 1500 foot candles. There was a temperature range of 16°C. to 24°C. during growth, being above 21°C. during most of the light period, and below this temperature during most of the dark period. There was a range in the relative humidity of 50 to 60 per cent. The nutrient solution was aerated daily for a period of 3 hours and was replenished with tap water or culture solution as required. Under these conditions the plants grew rapidly and had succulent stems with long internodes and relatively small leaves. After about 4 weeks in nutrient solution the plants were used in the experimental work.

C. PLANT MATERIAL USED

Whole plants about 7 weeks old and detached leaves from plants of the same age were used in the experiments. Whole plants were grown in the manner described previously. Two leaves of each plant, to be used in a given experiment as

Figure 2.

Three-week old potato plants in culture solution. Crocks of glazed porcelain; a cover of "Masonite"; potato plants held upright in cork stoppers, and tubes for aeration of culture solution, are illustrated herein.

a diseased plant, were inoculated as described in the following section. Other plants which were left uninoculated were used as controls.

In experiments with detached leaves the infected leaves were inoculated 3 or 4 days before removal from the whole plants. The healthy leaves to be used as controls were taken from healthy plants.

D. METHOD OF INOCULATION

1. INOCULUM

Cultures of *P. infestans*, race 0, were obtained from W.A.Hodgson, Plant Pathologist, Canada Department of Agriculture Research Station, Fredericton, N.B. Subcultures were grown on lima bean agar and stored under oil in a refrigerator at about 8°C. Cultures for making inoculum were prepared from these subcultures by transferring mycelium to slants on lima bean agar in test tubes. The cultures were incubated in the same chamber as the plants at a temperature of about 20°C,(±4°C.). Inoculum was prepared from these cultures after a 2- to 3-week period of incubation. The amount and type of growth at this stage is shown in figure 3.

To prepare the inoculum sterile distilled water (about 10 ml.), was poured into a test tube containing a

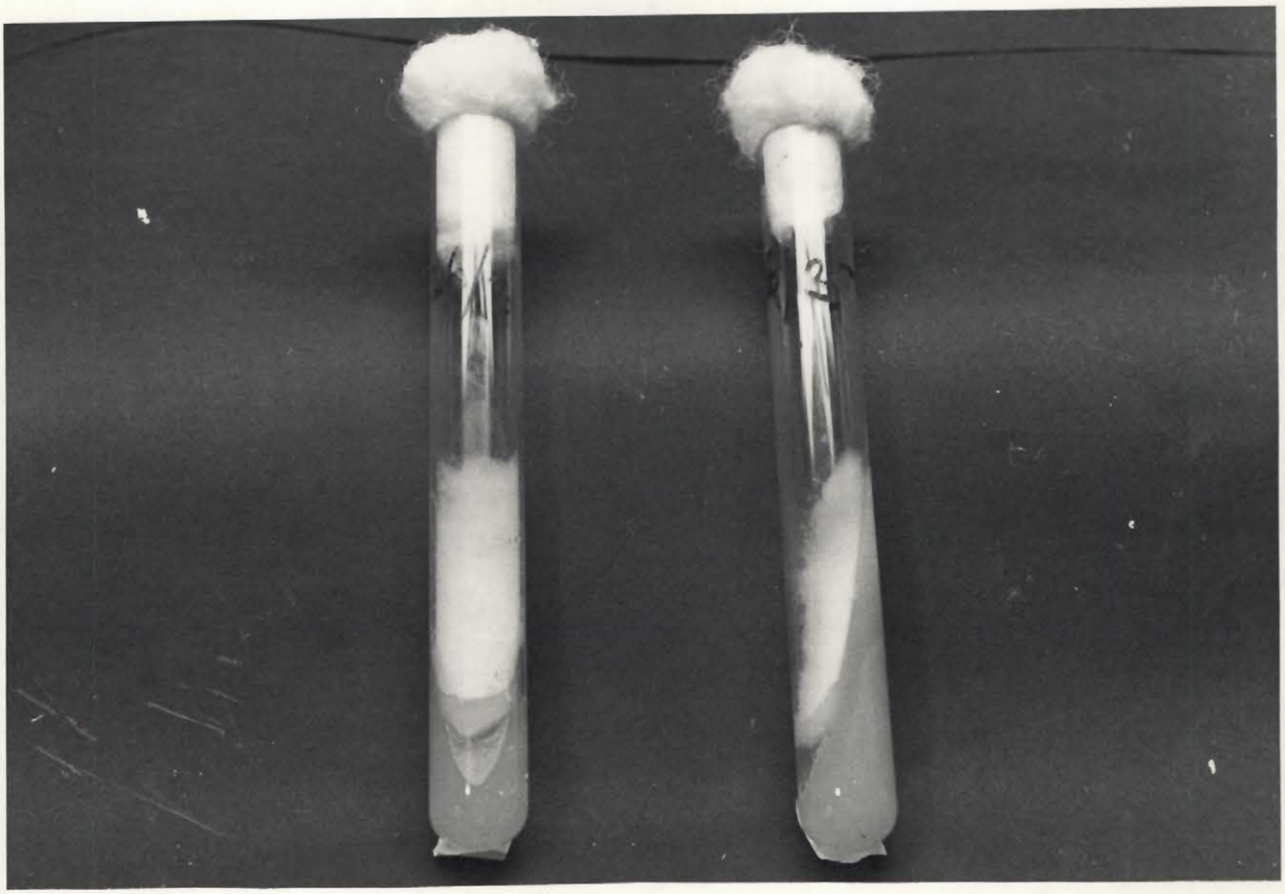


Figure 3.

Two-week old cultures of P.infestans grown on slants of lima bean agar in test tubes. Cultures such as these were used to prepare inoculum.

culture of the fungus, and a suspension of spores and mycelium was produced by scraping the fungus from the surface of the culture medium. This suspension was poured out into a clean test tube and stored in an incubator at 12°C. for 3 to 4 hours. Then a loopful of inoculum was examined under the microscope to determine the presence of zoospores and sporangia.

2. INOCULATION

Leaves were inoculated when the potato plants were about 7 to 8 weeks. Leaf inoculation was done at a time of day which preceded, almost immediately, a dark period. Each leaf to be inoculated was sprayed with water, using an atomizer, until thoroughly wetted. The excess water was wiped off and the suspension of inoculum was spread on the leaf by means of a dropper. Each inoculated leaf was covered with a small polyethylene bag for 18 to 24 hours to maintain a high relative humidity around the leaf during the incubation period. Under these conditions the first symptoms were usually evident in about 36 to 48 hours following inoculation.

Inoculation of individual leaves in the manner described above was quite tedious; it took about one hour to inoculate 12 leaves. However, under the conditions the inoculations resulted in 100 per cent infection.

E. ADMINISTRATION OF $C^{14}O_2$

1. SOURCE OF $C^{14}O_2$

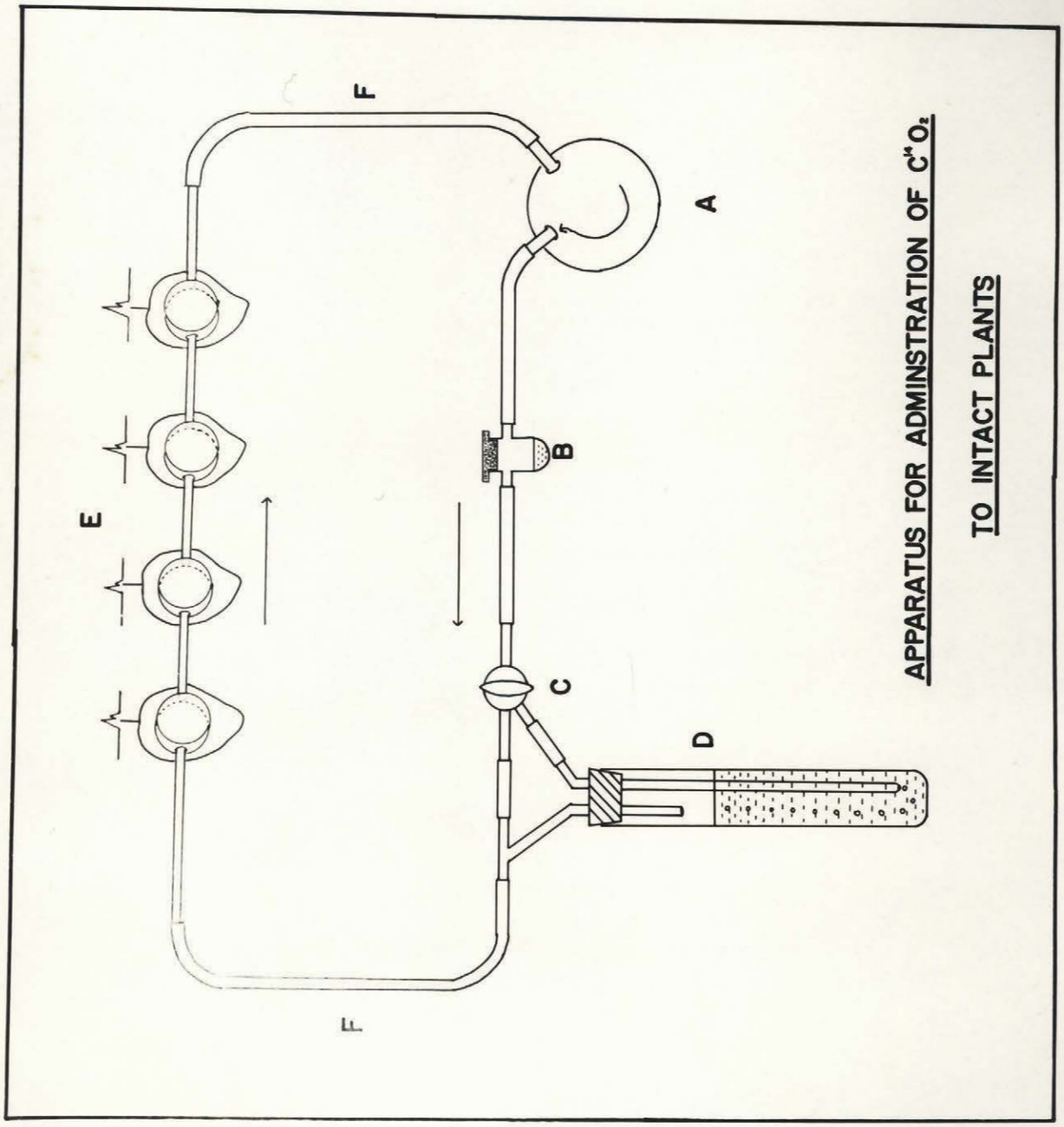
Sodium carbonate ($Na_2C^{14}O_3$, specific activity 778 μc/ml.), dissolved in sodium hydroxide and obtained from Atomic Energy of Canada Limited in June 1957, was used as the source of $C^{14}O_2$. The $C^{14}O_2$ was generated by adding an excess of 20 per cent sulphuric acid to 0.01 ml of the sodium carbonate solution diluted in a small volume of water. The amount of radioactivity (about 8 μc) liberated in the apparatus used for administering $C^{14}O_2$ to plants (see below), gave a reading of over 30,000 cpm on the "Tracerlab Laboratory Monitor", Model SU3B, using a Tracerlab geiger tube (TGC-2/$_1$B84), window weight 1.9 mg/cm^2.

2. APPARATUS FOR ADMINISTRATION OF $C^{14}O_2$ TO PLANTS.

The basic equipment for administration of $C^{14}O_2$ to whole plants is represented diagrammatically in figure 4. It includes: (A), a circulation pump, (Roll-Flex, Cole-Palmer Instrument and Equipment Co.), used to circulate gas within the system; (B), a reaction chamber in which was placed $Na_2C^{14}O_3$. The open end of the chamber was sealed with a serum bottle cap through which was introduced, by means of a hypodermic needle the acid which caused the liberation of $C^{14}O_2$ into the system.

Figure 4.

Diagram of apparatus for administration of $C^{14}O_2$ to whole plants in light. (A), circulation pump; (B), reaction chamber for generating $C^{14}O_2$; (C), stopcock for diverting the flow of gas through, (D), test tube with sodium hydroxide for absorption of excess $C^{14}O_2$ remaining after $C^{14}O_2$ administration; (E), cups for exposing small areas of leaves to $C^{14}O_2$; (F), polyethylene tubing. Arrow indicates direction of flow.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APPARATUS FOR ADMINSTRATION OF C¹⁴O₂
TO INTACT PLANTS

(C) is a stopcock used to divert the circulation of gas; (D), a test tube containing 10 per cent sodium hydroxide and through which the gas flow was diverted at the end of the exposure period to absorb the excess $C^{14}O_2$; (E), exposure cups connected in series and used for exposing small areas of leaves to $C^{14}O_2$; (F), polyethylene tubing for interconnection.

The plants to be exposed to $C^{14}O_2$ were placed in a fume cupboard. To the upper surface of the terminal leaflet of a selected leaf of each plant was attached an exposure cup, made of perspex and measuring 2 cm. in diameter and 0.5 cm. in depth. Each cup was sealed to the surface of a leaflet by means of silicone grease which had been applied to the rim of the cup. The cup was appressed to the surface of the leaflet by means of a wire clip, with a paper pad placed on the under surface of the leaflet. The leaflets attached to the cups were so arranged as to receive a comparable amount of the available light. The light source consisted of a bank of 10 fluorescent tubes (5 day light; 5 warm white), set up in a fume cupboard about 2 ft. above the top of the plants.

When it was time for administration of $C^{14}O_2$ to the plants a volume of sodium carbonate solution containing about 8 μc of C^{14} was poured into the reaction chamber which was then sealed with a serum bottle stopper. The circulation pump

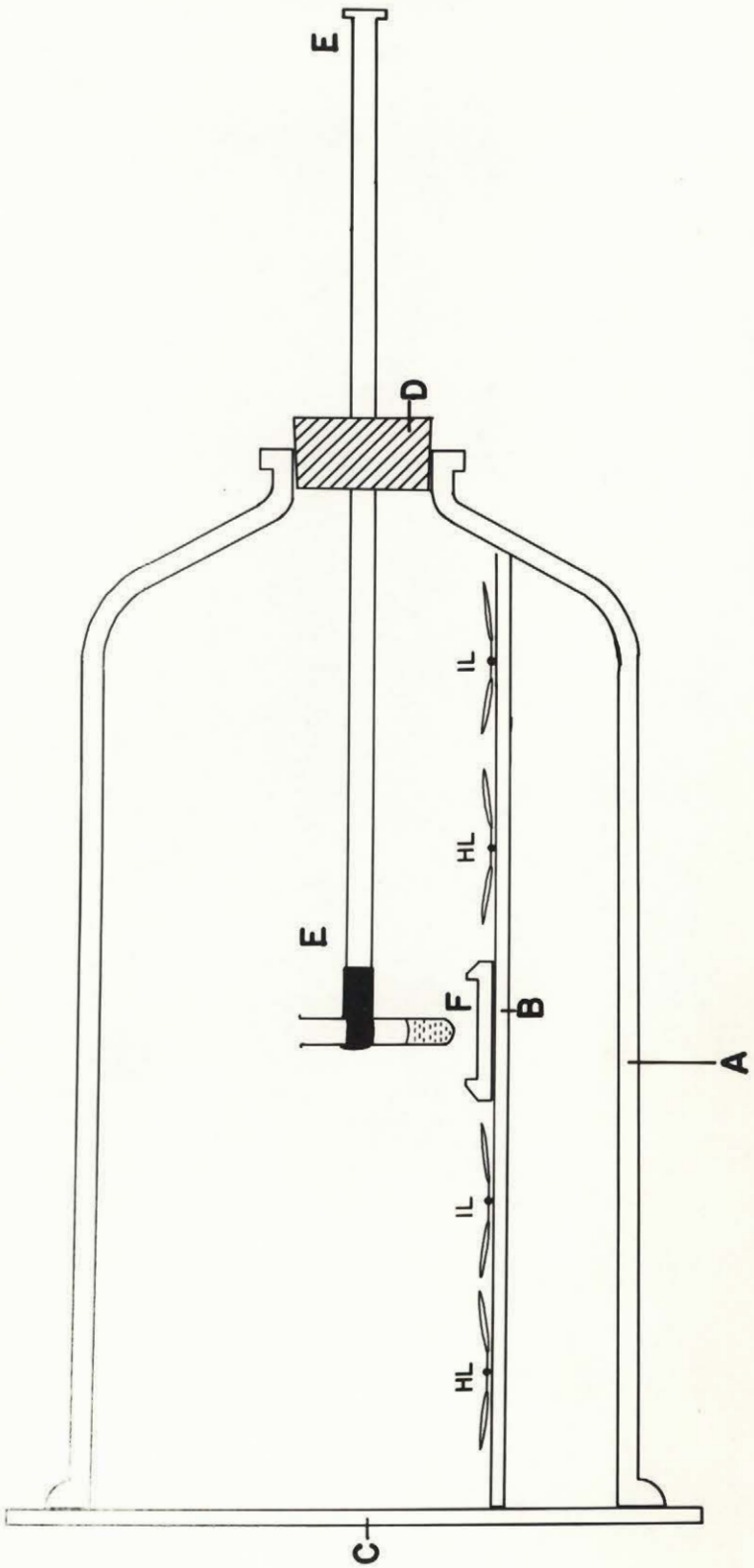
was started and the $C^{14}O_2$ was liberated into the system by injecting an excess of 20 per cent sulfuric acid into the generator using a hypodermic syringe. The leaflets were allowed to carry on photosynthesis with $C^{14}O_2$ for 40 to 45 minutes. At the end of this period the lights were turned off and the $C^{14}O_2$ was removed from the system by directing the gas flow through a 10 per cent solution of sodium hydroxide contained in a test tube (see figure 4.). The leaflets were then detached from the exposure cups and the plants were removed to a dark cupboard where they were kept for a period of varying duration to allow time for migration of the photosynthate to take place. The plants were then heat-killed as described in the following section.

The equipment for exposing detached leaves to $C^{14}O_2$ is illustrated in figure 5. It includes: (A) 10 liter bell jar; (B), glass plate; (C) glass cover; (D), rubber stopper; (E), glass rod with a small test tube, containing sulfuric acid solution, attached by means of rubber tubing; (F), syracuse dish containing $Na_2C^{14}O_3$.

The detached leaves to be exposed to $C^{14}O_2$ were cut away from the plant a few minutes before the commencement of the experiment. The cut end of each petiole was immediately wrapped in wet cotton batting. The leaves were then put on to

Figure 5.

Diagram of apparatus for administration of $C^{14}O_2$ to detached leaves. (A), bell jar; (B), glass plate supporting infected (IL), and healthy (HL), leaves; (C), glass cover; (D), rubber stopper; (E), glass rod passing through rubber stopper with test tube containing sulphuric acid; (F), syracuse dish containing sodium carbonate ($Na_2C^{14}O_3$) solution. The $C^{14}O_2$ was liberated by pouring the acid into the dish containing the $Na_2C^{14}O_3$ solution.



APPARATUS FOR ADMINISTRATION OF $C^{14}O_2$

TO DETACHED LEAVES

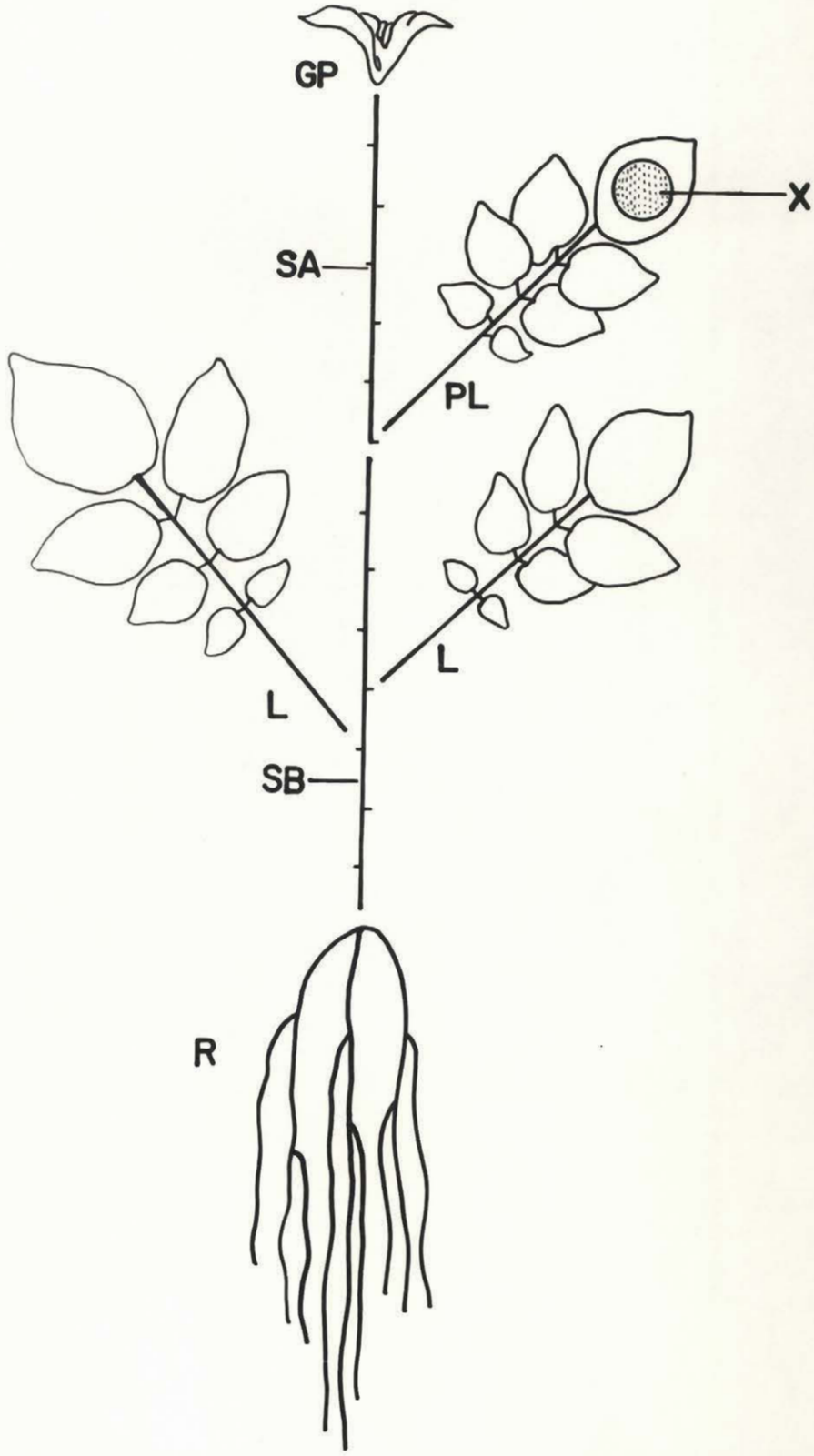
the glass plate together with a syracuse dish containing $Na_2C^{14}O_3$ solution and the whole setup was inserted in a bell jar in a horizontal position. The wide end of the bell jar was closed with a sheet of glass (C), and the contacting surfaces were sealed with a mixture of melted beeswax and petroleum. The $C^{14}O_2$ was liberated into the bell jar by pouring the acid from the test tube into the dish containing the $Na_2C^{14}O_3$ solution. This was done in the light or in the dark depending on the conditions of the experiment. After periods of exposure varying with the experiment the leaves were removed from the jar and immediately heat-killed.

F. KILLING OF PLANTS BY HEAT.

In experiments involving the use of whole plants, each plant was cut into a number of segments immediately preceding heat killing (see figure 6.). These include : (PL), presentation leaf, i.e. the leaf which had one of its leaflets exposed to $C^{14}O_2$; (SB), the stem below the presentation leaf; (SA), the stem above the presentation leaf; (R), the roots; (GP), the growing point, and 2 leaves below the presentation leaf (L). These were the infected leaves on diseased plants. Results obtained in preliminary experiments in which all parts of the plant were heat killed indicated that autoradiograms of only these segments were necessary to establish

Figure 6.

Diagram of plant parts which were autoradiographed. (PL), presentation leaf with the area of the terminal leaflet (X), exposed to $C^{14}O_2$ in light; (SB), stem segment below the presentation leaf; (SA), stem segment above the presentation leaf; (R), roots; (GP), growing point, and two leaves below the presentation leaf (L). (See text).



PLANT PARTS USED FOR AUTORADIOGRAPHY

the effects of the treatments used on distribution.

For heat-killing of the different segments the equipment illustrated in figure 7 was used. It includes: an iron plate 30 cm. long, 14 cm. wide and 0.5 cm. thick, set on an electrical hot plate with thermostat (Tempco); a cloth iron connected to a transformer, and pairs of blotting paper sheets. The plant segment to be heat-killed was put between a pair of blotting paper sheets cut to an appropriate size. The number and description of each segment and the plant to which it belonged was written on the blotting paper. The material so prepared was heat-killed by gently pressing between the hot plate,and the iron as illustrated in figure 8. The temperature of the iron, and of the hot plate, was adjusted to obtain rapid drying of the plant material without scorching. Pressing caused a small amount of the plant juice to escape, especially out of the stem and out of the petioles. This juice was quickly absorbed by the blotting paper and did not contaminate other parts. Prolonged heating and drying was avoided as it caused the plant material to become very brittle and to adhere to the blotting paper. The heat-killing method described here was also used in the experiments involving detached leaves. The heat-killed components were stacked away to be used later in autoradiography.

Figure 7.

Equipment used for heat-killing plant parts. An iron plate set on a hot plate, a cloth iron connected to a transformer to control temperature, and blotting paper, are illustrated herein.

Figure 8.

Heat killing. Plant segment between blotting paper sheets is gently pressed between hot surfaces of plate and iron.

G. AUTORADIOGRAPHY

1. PREPARATION OF SPECIMENS

The heat-killed segments from a given plant were removed from between the blotting paper sheets and mounted on kraft paper (see figure 9). The paper mounts were cut to the same dimensions - 43.2 cm. long by 35.6 cm. wide - as the X-ray films used for autoradiography. Usually the components of one plant were set up on one paper mount. In some cases, however, the components of 2 plants were set up on one mount. The components were arranged in a set order on the mounts. Detached leaves grouped according to the experiment were also set up on paper mounts.

All mounts were labelled according to the date of the experiment, plant number, treatment and other details, so as to permit identification after autoradiography.

2. METHOD OF AUTORADIOGRAPHING

The mounts with the plant components were set up with Kodak no-screen X-ray film in a press for autoradiographing, and in a manner similar to that described by Yamaguchi and Crafts (1958), and Hampson (1960).

The press for autoradiographing consisted of foam rubber

Figure 9.

Heat-killed segments of a plant mounted on kraft paper. Included are: presentation leaf with most of the terminal leaflet removed; stem segment below the presentation leaf; stem segment above the presentation leaf; roots; growing point, and two leaves with three-day old infection lesions.

sheets, 43 cm. long, 36 cm. wide and 1.3 cm. thick; aluminum sheets, used as shields, 43 cm. long, and 35 cm. wide; 2 plywood covers 56 cm. long, 46 cm. wide and 1.3 cm. thick; 4 threaded iron bolts, 18 cm. long and 1 cm. in diameter, with nuts.

The mounts and X-ray films were set up in the dark in the dark room. This was done by placing one of the plywood covers on a workbench in the darkroom and building up a stack in the following sequence: aluminum shield - film - mount - rubber - mount - film - aluminum shield - film - mount etc. The other plywood cover was placed on top of the stack. The threaded bolts were passed through corresponding holes in top and bottom plywood covers and the stack was compressed, to ensure close contact between the film and the plant material, by screwing down the nuts against the top plywood cover to finger tightness. The press was then put into a light proof box and kept in the cold room at 6°C. to 8°C. until the time of development.

The plant segments used in experiments with whole plants were exposed to X-ray film for 4 weeks, and the leaves used in experiments with detached leaves were exposed for 12 hours.

Kodak X-ray developer (D-19b) and Kodak acid fixer

were used to develop the X-ray film. Groups of 4, 8, or 12 films were developed simultaneously by first immersing the films in tap water at room temperature then in developer for 3 minutes, at a temperature of about 20°C. then, fixer for about 20 minutes. After fixing the films were put together with the mounts from which they were produced. Photographs of the autoradiograms produced in the manner described above are presented as results.

IV. EXPERIMENTAL RESULTS

SECTION I: STUDIES WITH WHOLE PLANTS.

A. PRELIMINARY EXPERIMENT ON C^{14} DISTRIBUTION IN PLANTS WHICH HAD ONE LEAFLET EXPOSED TO $C^{14}O_2$ IN LIGHT.

The distribution pattern of C^{14} following assimilation of $C^{14}O_2$ by a single leaf (presentation leaf), of tobacco has been reported (Jones et al., 1959). These workers observed that radioactivity exported from the presentation leaf moved to the stem, roots, stem apex, and young upper leaves. Further, they noted a lack of activity in the leaves below the presentation leaf. However, it was not known whether or not a similar distribution would occur in potato plants if similar experimental conditions were used. Therefore, a preliminary experiment was performed to determine the distribution of C^{14} in whole potato plants under conditions resembling those of Jone et al., (1959), and using the gross autoradiographic technique described previously under the section "Materials and Methods".

In this experiment 2 potato plants, about 9 weeks old, were used. A leaf at a comparable position on the stem of each plant was used as a presentation leaf. The terminal leaflet of the presentation leaf of each plant was attached

to the apparatus for administration of $C^{14}O_2$, and the plants were allowed to carry on photosynthesis for 45 minutes. One plant was left in air in the dark for 3 hours and the other for 5 hours in order to permit migration of the photosynthate throughout the plant. At the end of each migration period each plant was cut into segments including the presentation leaf, the stem above the presentation leaf, the roots, the growing point and the leaves (see figure 6 and figure 9).

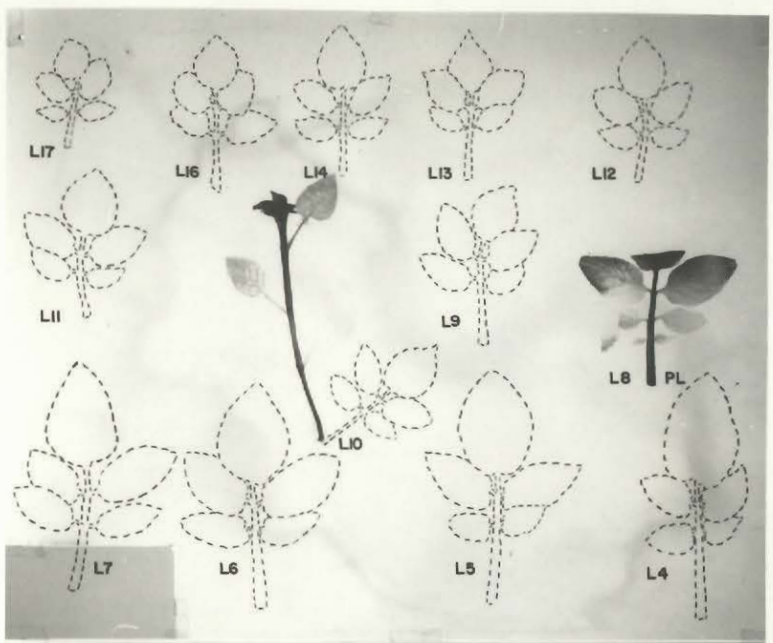
The segments were immediately heat-killed and autoradiographed as described earlier. X-ray films were left in contact with the plant parts before they were developed.

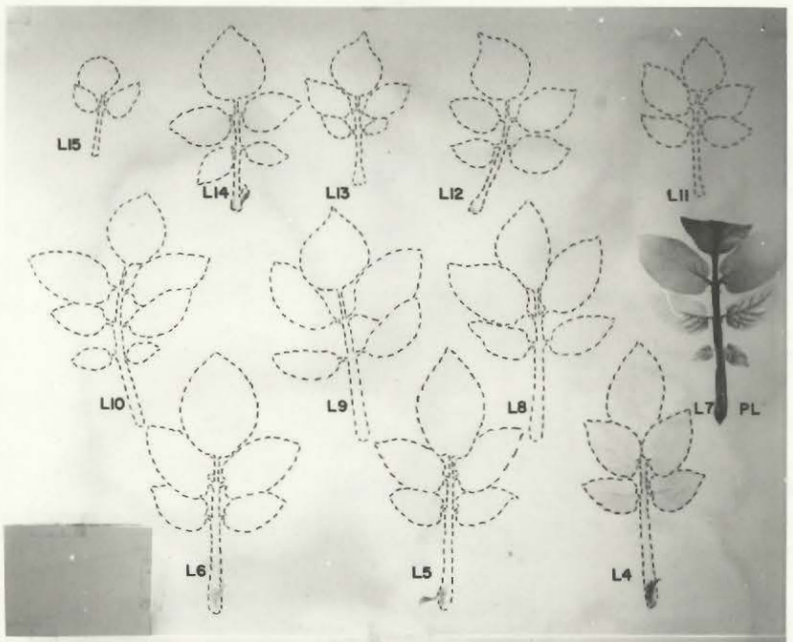
Autoradiograms of the plants used in the experiment are presented in figure 10. Before discussing the distribution of C^{14} in the plants, it should be mentioned that the optical density of the autoradiographic image was found to be related to counts per minute (cpm), as determined by scanning with a geiger tube connected to a ratemeter - scaler. Thus, a darker image on the autoradiogram corresponds to higher radioactivity and vice versa.

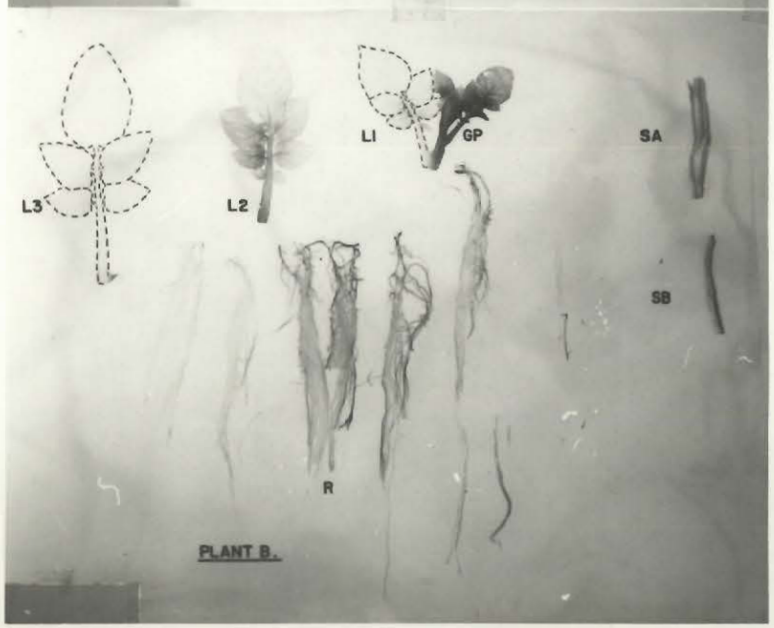
It is evident from the autoradiogram that the distribution of activity in a potato plant after 3 hours in the dark (plant A), is similar to the distribution after 5 hours in the dark (plant B). In both of the plants, there is relatively

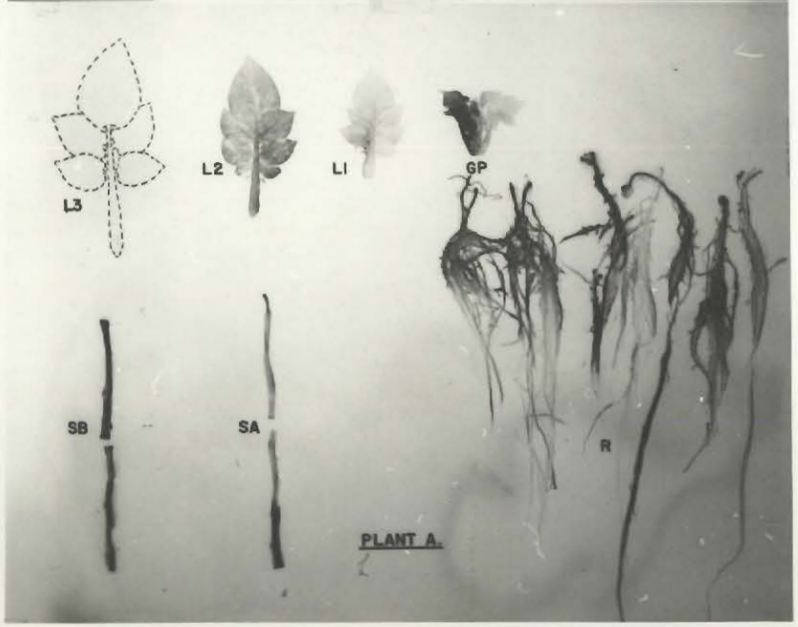
Figure 10.

Autoradiograms of segments of healthy potato plants showing the distribution of C^{14}. The terminal leaflet of the presentation leaf (PL), of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration period was 3 hours for plant A, and 5 hours for plant B. (PL) the presentation leaf with most of the terminal leaflet removed; (SB), stem segment below the presentation leaf; (SA); stem segment above the presentation leaf; (R), the roots; (GP), the growing point, and leaves (L1, L2, L3, etc.). The darker areas correspond to locations of greater radioactivity. The broken lines outline those segments that have produced little or no image.


L17
L16
L14
L13
L12
L11
L9
L8
PL
L10
L7
L6
L5
L4


L15
L14
L13
L12
L11
L10
L9
L8
L7
PL
L6
L5
L4


L3
L2
L1
GP
SA
SB
R
PLANT B.


L3
L2
L1
GP
SB
SA
R
PLANT A.

high radioactivity in the petiole and somewhat less in the leaflets of the presentation leaf (PL). There is more radioactivity in the stem below (SB), the presentation leaf than in the stem above it (SA), and there is accumulation of radioactivity in the roots (R) and the growing point (GP). The 2 leaves (L1, L2), of plant A and one leaf (L2) of plant B contain some radioactivity, but there is little or no detectable activity in the other leaves. However the shoot in the axil of leaf 10 (L10) in plant B contains radioactivity. In the growing point of plant A and in the roots of plant B there is greater activity on one side of the presentation leaf than on the opposite side.

In subsequent experiments, autoradiograms were not prepared for any of the leaves which, on the basis of these results, were not expected to accumulate C^{14}, unless these leaves were infected. Therefore, only the following plant segments were autoradiographed: the presentation leaf (PL), the stem below the presentation leaf (SB), the stem above the presentation leaf (SA), the roots (R), the growing point (GP), and the 2 leaves immediately below the presentation leaf (see figure 6 and figure 9).

B. THE DISTRIBUTION OF C^{14} IN HEALTHY AND IN DISEASED PLANTS WITH TWO INFECTED LEAVES.

Results of the preceding experiments indicate that leaves older than the presentation leaf do not accumulate C^{14} to a level that is detectable with the methods used. It is known, however, that infected leaves of other plants accumulate translocates (Hampson, 1960; Shaw and Samborski, 1956; Yarwood and Jacobson, 1955). But it is not known, if late blight infection can cause accumulation in those leaves which do not normally accumulate translocate, nor is it known if plants at various stages of disease and under conditions causing different rates of transpiration, differed in this respect. Therefore, experiments were performed to elucidate these points.

1. THE DISTRIBUTION OF C^{14} IN PLANTS EXPOSED TO $C^{14}O_2$ AT DIFFERENT STAGES OF DISEASE.

Four experiments, each with diseased plants at slightly different stages, were performed. A number of plants were inoculated as described in Materials and Methods, and the remainder were left uninoculated and served as controls. In one experiment 2 diseased and 2 healthy plants were exposed to $C^{14}O_2$, 3 and 5 days following inoculation. In the other experiments pairs of healthy and diseased plants were exposed to $C^{14}O_2$ 3, 4 and 5 days following inoculation. The plants

were 8- to 9-weeks old. In each case the terminal leaflet of the presentation leaf was exposed to $C^{14}O_2$-enriched air, in light, for 45 minutes, and then left in ordinary air, in the dark, for 4 to 5 hours. At the end of this period, the plants were cut into segments which were immediately heat-killed and subsequently autoradiographed. The autoradiograms produced by the segments of each plant were given a number corresponding to the rank they had when they were placed in order of decreasing optical density which was estimated visually.

The ranks and the sum of the ranks of the different segments of healthy and diseased plants, when these segments were placed in order of decreasing optical density, are presented in Appendix Ia and Ib. The ranks of the sum of the ranks of the autoradiograms of these segments are presented in table I.

In almost all cases, the presentation leaf had the highest concentration of C^{14}, and the stem below the presentation leaf, the root and the growing point had an intermediate concentration, with a low concentration in the stem above the presentation leaf. The leaves below the presentation leaf had the lowest concentration of C^{14} of all the plant segments.

Table I.

Ranks of the sum of the ranks of the autoradiograms obtained from different segments of healthy and diseased potato plants, when segments of each plant are placed in order of decreasing optical density. Results are given for different times after 2 of the leaves below the presentation leaf had been inoculated with P. infestans. (see appendix Ib)

	Ranks of (A)					
	3 Days*		4 Days*		5 Days*	
Plant Segment	H	D	H	D	H	D
Presentation leaf (PL)⊙	1	1	1	1	1	1
Stem above PL	5	5	5	5	5	5
Stem below PL	3	4	3	3	2	3
Growing point	4	3	4	4	4	4
Roots	2	2	2	2	3	2
Leaf 1 #	6.5	6	6.5	6	6	6
Leaf 2 #	6.5	7	6.5	7	7	7

* Number of days following inoculation with P. infestans.

\# Leaves below presentation leaf; infected ones on diseased plants.

⊙ Ranks based on optical density of leaf petiole.

See figure 9 for illustration of the different segments.

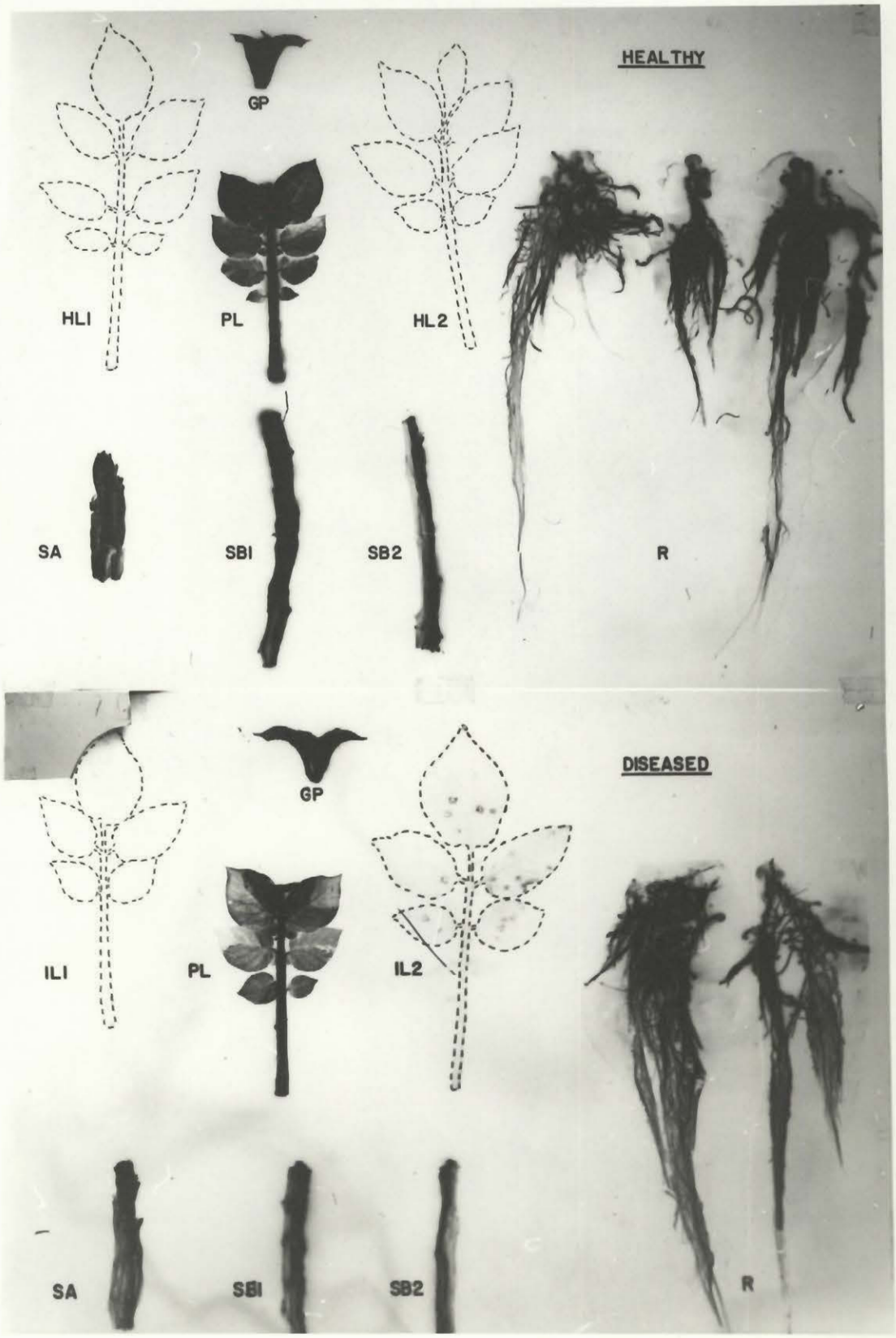
Autoradiograms of segments of a healthy and of a diseased plant are presented in figure 11. In both the healthy and the diseased plants there is high radioactivity in the presentation leaf (PL), the stem below the presentation leaf (SB), the roots (R), and the growing point (GP), with less activity in the stem above the presentation leaf (SA). In the stem segments below the presentation leaf (SB1; SB2), there is higher radioactivity on one side than on the other. There is no detectable radioactivity in the healthy leaves (H1; H2), but there is radioactivity in one infected leaf (IL2), where radioactivity is greatest in a zone around the lesions.

Three, 4 or 5 days following inoculation of 2 leaves with P. infestans, the distribution of C^{14} in the segments of the potato plants studied is not apparently altered to any great extent. However, at all the stages studied infection causes an abnormally high concentration of C^{14} in infected leaves, where it accumulates in a zone around lesion sites. In the few cases where comparable healthy leaves accumulate C^{14} in detectable concentrations the distribution is uniform.

It is concluded that infection causes an increase in the concentration and an alteration in the distribution of C^{14} in an infected leaf but does not have a detectable influence on the concentration and the distribution in the stem, root and growing point of the potato plant.

Figure 11.

Autoradiograms of segments of a healthy and a diseased potato plant, 3 days following inoculation of 2 of the leaves below the presentation leaf with P. infestans. The terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration period was 4 hours. (PL), presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA), stem above the presentation leaf; (R), roots; (GP), growing point; (IL1, IL2), infected leaves; (HL1, HL2), comparable healthy leaves. The darker areas correspond to locations of higher radioactivity. The broken lines outline those segments that have produced little or no image.


HEALTHY
GP
HL1
PL
HL2
SA
SB1
SB2
R
DISEASED
GP
IL1
PL
IL2
SA
SB1
SB2
R

2. THE DISTRIBUTION OF C^{14} IN HEALTHY AND DISEASED PLANTS WITH TWO LEAVES UNDER DIFFERENT CONDITIONS FOR TRANSPIRATION.

The results of the preceding experiment have shown that C^{14} translocates accumulate in infected leaves. It is known that such leaves also transpire at a faster rate than healthy ones (Sivadjian and Kern, 1958; Yarwood, 1947). This increase in transpiration can possibly be the cause of the accumulation of C^{14} compounds in infected leaves (Shaw and Samborski, 1956). Therefore, an experiment was performed in which the transpiration rate of some of the infected leaves on the plants used was reduced by enclosing them in polyethylene bags and the effect of this treatment on C^{14} accumulation determined.

Two healthy and 2 diseased plants were used. Each diseased plant had 2 infected leaves below the presentation leaf. Three days following inoculation, the terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. During this period both infected leaves on one of the diseased plants were covered with a polyethylene bag. The infected leaves of the other diseased plant were not covered. After administration of the $C^{14}O_2$, the plants were kept in the dark for a 4 to 5 hour

migration period in air. They were then segmented and autoradiographed as described previously.

Autoradiograms prepared from these plants are presented in figure 12. There is accumulation of radioactivity around lesion sites of infected leaves which had no polyethylene cover for the duration of the experiment (figure 12A). On the other hand, there is no detectable radioactivity in the infected leaves with a polyethylene cover during this period; nor in comparable healthy leaves (figure 12B).

It is concluded that reducing the transpiration rate of infected leaves prevents accumulation of C^{14} -labelled translocates in them.

C. THE DISTRIBUTION OF C^{14} IN HEALTHY AND IN DISEASED PLANTS WITH INFECTION ON THEIR PRESENTATION LEAVES.

Preceding experiments have shown that infection of leaves other than the presentation leaf does not to any great extent affect the distribution of translocates in the plant except for accumulation in the infected leaves. The question that arises is: would the effect on distribution be different if the infection had been on the presentation leaf? In an attempt to find an answer to this question, experiments were performed in which; (a) the exposed leaflets were infected,

Figure 12.

Autoradiograms of healthy potato plants and diseased plants 3 days following inoculation of 2 of the leaves below the presentation leaf with P. infestans. The terminal leaflet of the presentation leaf was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration period was 4 to 5 hours. (PL), presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA), stem above the presentation leaf; (R), roots; (GP), growing point; (IL1; IL2), infected leaves; and (HL1, HL2), comparable healthy leaves. The darker areas correspond to locations of higher radioactivity. (A); a healthy plant and a diseased plant without a polyethylene bag on the infected leaves (IL1, IL2), during the exposure and migration periods; (B) ibid., but with infected leaves (IL1, IL2), enclosed in polyethylene bags.


A
B
HEALTHY
HL1
HL2
GP
PL
R
SA
SB1
SB2
DISEASED
IL1
IL2
A
B

and (b), other leaflets of the presentation leaf and not the exposed leaflets were infected.

1. THE DISTRIBUTION OF C^{14} WITH INFECTION ON THE LEAFLETS EXPOSED TO $C^{14}O_2$.

Two healthy and 2 diseased plants with infection only on the terminal leaflet of the presentation leaf were used when the plants were 8 weeks old. Three days after inoculation the terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. On one of the healthy plants, a disc of black paper about the size of the necrotic area of lesions on diseased plants was placed on the surface of the presentation leaflet, in order to shade part of the area enclosed by the exposure cups, and thus prevent photosynthesis in this area. No paper disc was placed on the corresponding leaflet of the other healthy plant. The plants were left after exposure to $C^{14}O_2$ in ordinary air in the dark for 3 to 4 hours to allow time for photosynthate translocation. At the end of this period plants were segmented, heat-killed and autoradiographed in the usual manner.

Autoradiograms of the healthy plant, which had no shading paper on the terminal leaflet, and of a diseased plant, are presented in figure 13A. There is less radioactivity in

all segments of the diseased plant than in the healthy plant. However, the C^{14} distribution among segments is apparently the same for both of the plants. In figure 13B are presented the autoradiograms of the healthy plant, which had a shading paper on the presentation leaf, as well as those of a diseased plant. There is less radioactivity in all of the segments of the diseased plant than in those of the healthy one; except the growing point which had more.

Autoradiograms of the leaf discs which were under the exposure cups are presented in figure 14. There is uniformly distributed radioactivity in the leaf disc (A), of the healthy control plant. In the leaf disc (B), of the healthy plant that was partly covered by a black shading paper, radioactivity is detected chiefly in the unshaded area. Radioactivity is not uniformly distributed in the discs (C;D), from infected leaflets, it is accumulated in a zone around the lesions. Also, there appears to be more radioactivity in healthy discs than in infected ones. Comparable results were obtained when this experiment was repeated on 2 other occassions.

It is concluded that infection of the terminal leaflet of the presentation leaf causes a considerable decrease in the concentration of radioactivity found in the plant but does not affect, to any great extent, its distribution among the different plant parts.

Figure 13.

Autoradiograms of segments of healthy and diseased plants 3 days after inoculation of the terminal leaflet of the presentation leaf. The terminal leaflet of each presentation leaf was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration period was 4 hours. (PL), presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA), stem above the presentation leaf; (R), roots; (GP), growing point. The darker areas correspond to locations of higher radioactivity. (A), autoradiogram of a healthy plant (right), and of a diseased plant (left); (B), autoradiogram of a healthy plant (right), which had the terminal leaflet partly shaded from light during exposure to $C^{14}O_2$, and of a diseased plant (left).

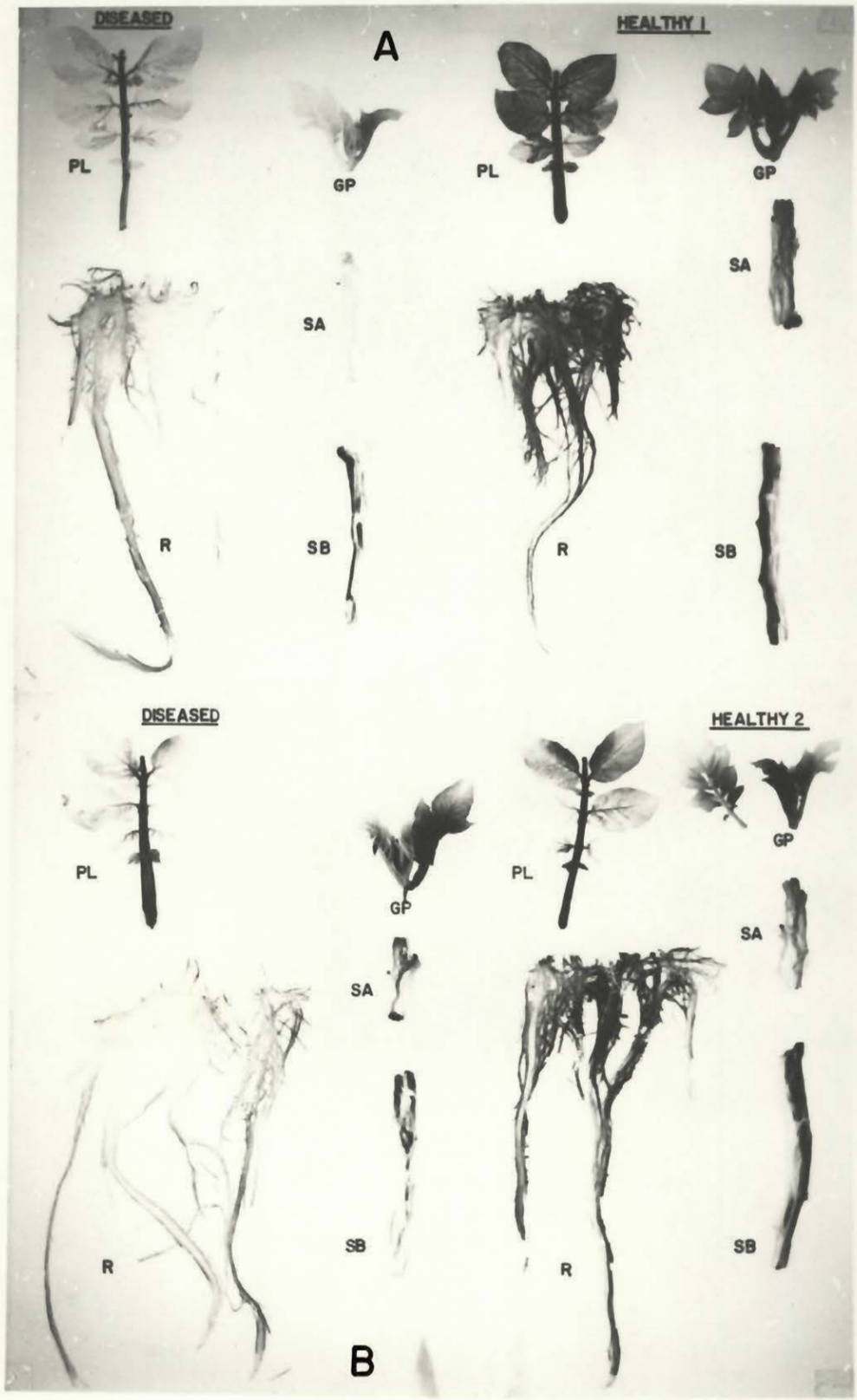

DISEASED
A
HEALTHY 1
PL
GP
PL
GP
SA
SA
R
SB
R
SB
DISEASED
HEALTHY 2
PL
GP
PL
GP
SA
SA
R
SB
R
SB
B

Figure 14.

Autoradiograms of healthy and infected discs of tissues removed from the leaflet area which was under a cup containing $C^{14}O_2$ (see figure 13.). This tissue was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration period was 4 hours. (A) disc from healthy plant; (B), disc shaded in the center during exposure to compensate for necrotic spots on infected discs; (C), and (D), discs with lesions 3 days after inoculation. The darker areas correspond to locations of higher radioactivity.

2. THE DISTRIBUTION OF C^{14} IN PLANTS WITH INFECTION ON LEAFLETS OTHER THAN THE TERMINAL LEAFLET OF THE PRESENTATION LEAF.

Two healthy and 2 diseased plants, with infection only on 2 lateral leaflets of the presentation leaf, were used when they were 9 weeks old. Three days after inoculation the terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The plants were then left in ordinary air to allow migration of the photosynthate. Two plants were segmented and heat-killed after 4 hours and the other 2 after 10 hours.

Autoradiograms of plants which were allowed a 10-hour migration period are presented in figure 15. The concentration of radioactivity in the stem below the presentation leaf (SB), and the roots (R), is less in the diseased than in the healthy plant but comparable in the stem above the presentation leaf (SA), and the growing point (GP). Similar results were obtained with plants, left in ordinary air for 4 hours, the autoradiograms of which are not presented here.

On the basis of these results and those of the preceding section, it is concluded that the migration and distribution of C^{14} is retarded by infection of either the terminal leaflet or the lateral leaflets of the presentation leaf.

Figure 15.

Autoradiograms of segments of a healthy and a diseased plant 3 days after inoculation of 2 of the leaflets (marked X) of the presentation leaf. The uninfected terminal leaflet was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration period was 10½ hours. (PL) presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA) stem above the presentation leaf; (R), roots; (GP), growing point. The darker areas correspond to locations of higher radioactivity.


HEALTHY
PL
GP
SA
SBI
SB2
R
DISEASED
X
X
PL
GP
R
SA
SBI
SB2

D. THE DISTRIBUTION OF C^{14} IN HEALTHY AND IN DISEASED PLANTS WITH HEAT-GIRDLED PETIOLES.

Photosynthates and other solutes are known to be exported out of leaves chiefly through living tissues; mainly the phloem (Biddulph et al., 1958), and imported into leaves through non-living tissue; mainly the xylem (Stout and Hougland, 1939). It was not known if late blight infection, which preceding experiments have shown to affect distribution, can alter also the path of translocation. Therefore, experiments were performed with the hope of obtaining useful information on this problem.

1. THE DISTRIBUTION OF C^{14} IN UNINFECTED AND INFECTED PLANTS HAVING THEIR PRESENTATION LEAVES WITH HEAT-GIRDLED PETIOLES.

Two healthy and 2 diseased plants with infections on 2 of the leaves below the presentation leaf were used 3½ days after inoculation and when the plants were 8 weeks old. A portion of the petiole of the presentation leaf of a healthy plant and a diseased plant was killed by scalding in the following manner: a strip of cotton batting 1 cm. wide was put around the petiole, midway between the stem and the proximal pair of lateral leaflets, and boiling water was poured onto this region in a narrow jet for one minute.

According to Colwell (1942), such treatment kills all living cells in the scalded region.

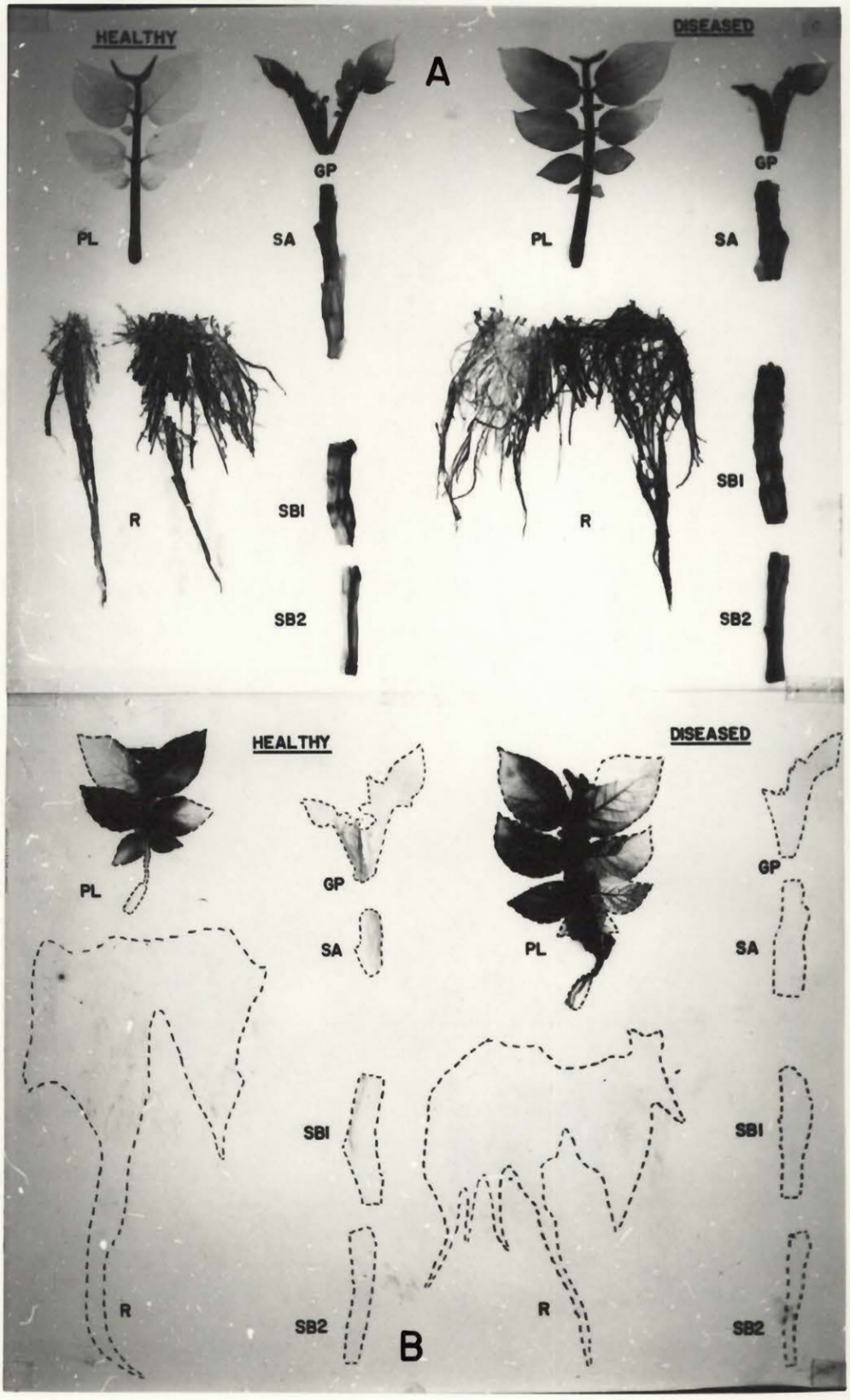
One hour following scalding of the petioles, the terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The plants were then left in ordinary air in the dark for 4 to 6 hours, then heat-killed and autoradiographed.

Autoradiograms of a healthy and a diseased plant without killed petioles are presented in figure 16A. In both of the plants there is a comparable concentration of radioactivity in the stem segments (SA;SB), the roots (R), and the growing point (GP). Figure 16B, illustrates the autoradiograms of an uninfected and an infected plant with heat-girdled petioles. In the presentation leaf of both plants there is a high concentration of radioactivity above the killed section of petiole. There is no detectable activity in the other segments of the diseased plant but there is a trace of radioactivity in the stem segment above the presentation leaf (SA), and the growing point of the healthy plant.

It is concluded from this experiment that killing a portion of the petiole prevents photosynthate from moving out of the leaf, of both the infected and uninfected plants, and thus prevents accumulation in the infected leaves.

Figure 16.

Autoradiograms of segments of: (A) a healthy and a diseased plant each with an intact presentation leaf petiole; (B) a healthy and a diseased plant each with a presentation leaf having a heat girdled petiole. The terminal leaflet of each presentation leaf was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes, 3 days after inoculation of 2 leaves on each diseased plant. The migration period was 4 to 6 hours. (PL), presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA), stem above the presentation leaf; (R), roots; (GP), growing point. The infected leaves and the corresponding healthy leaves were autoradiographed separately, (see figure 18). The darker areas correspond to locations of higher radioactivity.


HEALTHY
DISEASED
A
PL
GP
SA
R
SBI
SB2
PL
GP
SA
R
SBI
SB2
HEALTHY
DISEASED
B
PL
GP
SA
R
SBI
SB2
PL
GP
SA
R
SBI
SB2

2. THE DISTRIBUTION OF C^{14} IN UNINFECTED AND INFECTED PLANTS WITH HEAT-GIRDLED PETIOLES ON LEAVES BELOW THE PRESENTATION LEAF.

The results of the preceding experiment indicate that scalding of the petiole of the presentation leaf prevents movement of C^{14} to other plant segments. In a number of other experiments it was shown that infection causes accumulation in infected leaves. It is not known, however, whether or not scalding of petioles of infected leaves affects this accumulation and also the general distribution of C^{14} migrating out of the presentation leaf. An experiment was performed to investigate this problem.

Two healthy and 2 diseased plants, with infection on 2 of the leaves below the presentation leaf, were used. A portion of the petiole of each infected leaf on one of the diseased plants, and of comparable leaves on one of the healthy plants, was scalded in the manner described previously. Petioles of leaves on the other healthy and diseased plants were not scalded.

Three and a half days following inoculation of the diseased plants, and one hour following scalding of the petioles, the terminal leaflet of the presentation leaf of each was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The plants

were left in ordinary air in the dark for 4 to 6 hours then heat-killed and autoradiographed.

Figure 17A illustrates the autoradiograms of segments of an uninfected plant and an infected plant; both with scalded petioles, and figure 17B illustrates those of healthy and diseased plants both with unscalded petioles. It is evident that there is a similar distribution and concentration of radioactivity in the different segments of each plant examined.

Figure 18 illustrates the autoradiograms of infected and uninfected leaves with and without scalded petioles. No radioactivity is detected in uninfected leaves with heat-girdled petioles (C) or without heat-girdled petioles (A). On the other hand, infected leaves with heat-girdled petioles (D), and without heat-girdled petioles (B) both contain radioactivity, which accumulates in a zone around the lesion sites.

It is concluded that scalding the petioles of leaves on uninfected and infected plants does not affect the distribution of C^{14} in those plant; not even the characteristic accumulation in infected leaves.

Figure 17.

Autoradiograms of segments of: (A), a healthy plant with 2 of the leaves below the presentation leaf having heat-girdled petioles, and a diseased plant with the infected leaves having heat-girdled petioles; (B), comparable healthy and diseased plants with intact petioles (same as figure 16A). The terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes, $3\frac{1}{2}$ days following inoculation of 2 leaves below the presentation leaf. The migration period was 4 to 6 hours. (PL), presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA), stem above the presentation leaf; (R), roots; (GP), growing point. The infected leaves, and the comparable healthy leaves, were autoradiographed separately (see figure 18). The darker areas correspond to locations of higher radioactivity.

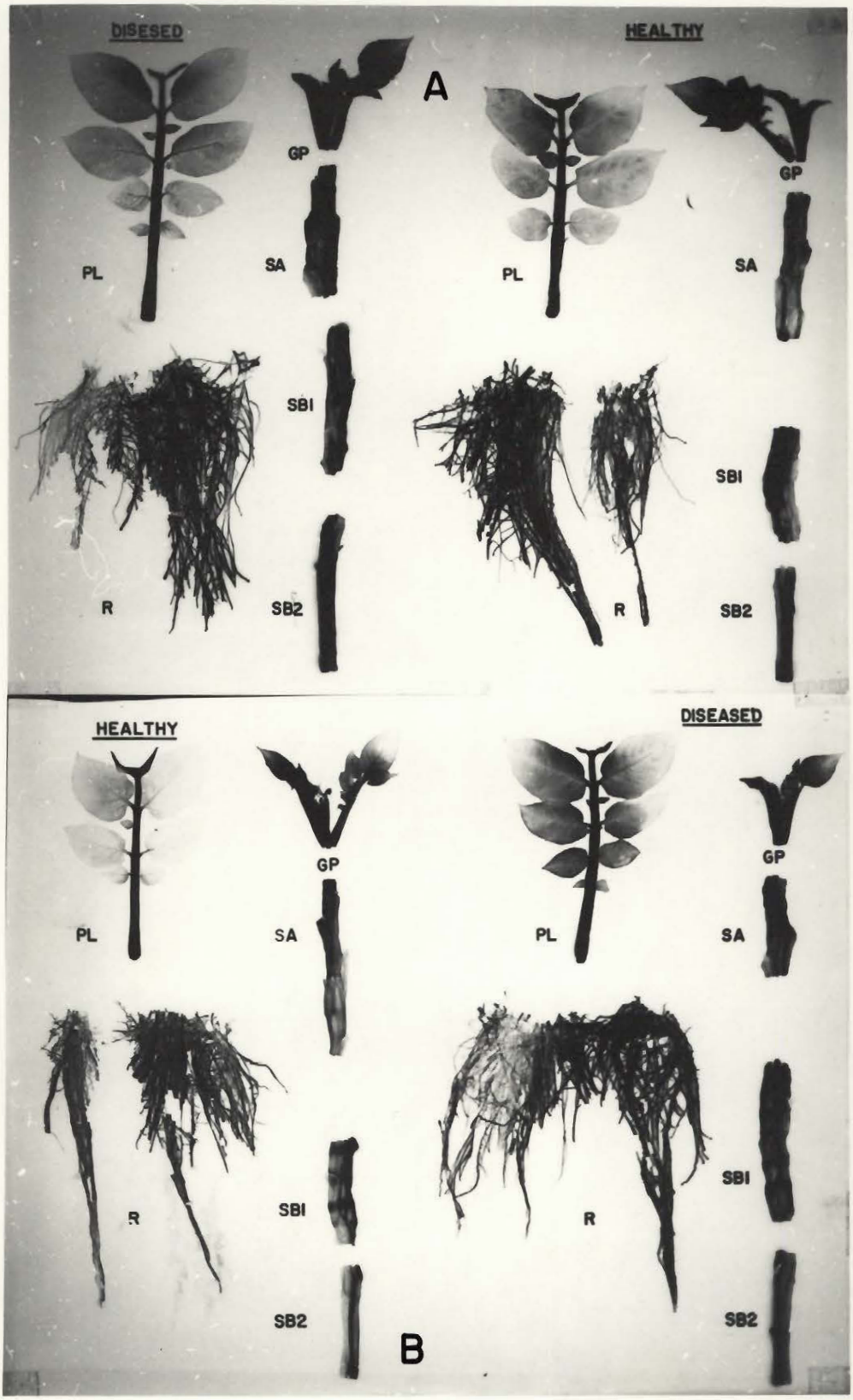
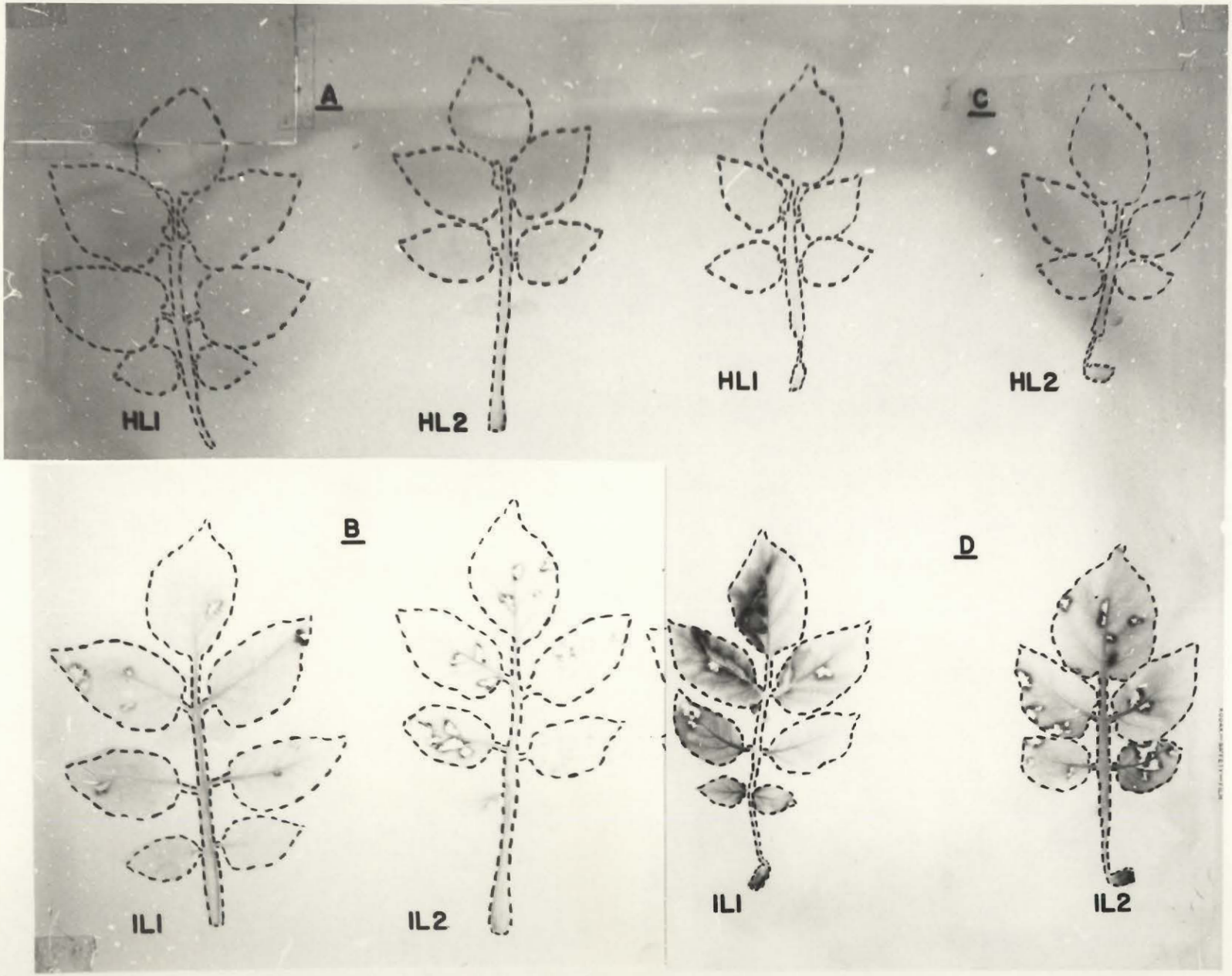

DISESED
HEALTHY
A
GP
PL
SA
SBI
R
SB2
HEALTHY
DISEASED
B

Figure 18.

Autoradiograms of healthy and infected leaves detached from nodes below the presentation leaves of healthy and diseased plants, autoradiograms of which are shown in figure 16, and figure 17. (A), healthy leaves (HL1, HL2), with intact petioles; (B), infected leaves (IL1, IL2), with intact petioles; (C), healthy leaves (HL1, HL2), with heat-girdled petioles; and (D) infected leaves (IL1, IL2), with heat-girdled petioles. The darker areas correspond to locations of higher radioactivity.


A
HL1
HL2
C
HL1
HL2
B
IL1
IL2
D
IL1
IL2

E. THE DISTRIBUTION OF C^{14} IN HEALTHY AND IN DISEASED PLANTS AFTER DIFFERENT MIGRATION PERIODS IN THE DARK.

The distribution patterns represented so far were obtained with plants which had migration periods of 2 or more hours. If a series of shorter migration periods were used, one could possibly find the chronologic order in which the different plant parts became labelled. From these results it would be possible to infer the pathway of the translocates. If in such an experiment healthy and diseased plants were used one would perhaps be able to observe the effect, if any, of infection on the chronological order of labelling. Therefore, an experiment was performed, taking into consideration these factors.

1. THE DISTRIBUTION OF C^{14} IN HEALTHY PLANTS AFTER DIFFERENT MIGRATION PERIODS IN THE DARK.

The terminal leaflet of the presentation leaf of each of 4 healthy plants was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. At the end of this period one plant was cut up into segments and heat-killed immediately. Similarly, one of the other 3 plants was heat-killed after each of the following periods in ordinary air in the dark: 0.5 hour; 1 hour and 2 hours. The heat-killed plant segments were subsequently autoradiographed.

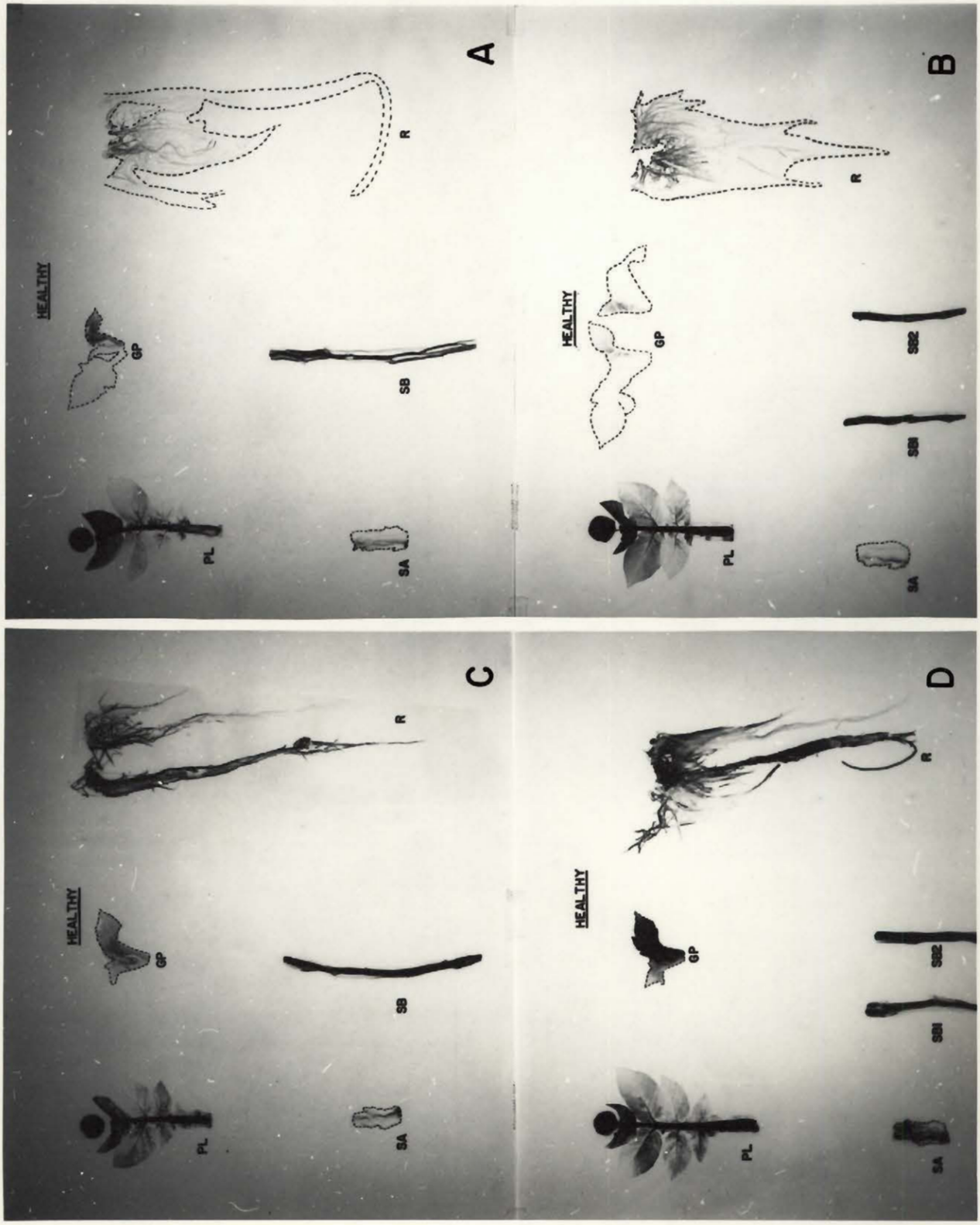
Figure 19 represents the autoradiograms of the plants used. With no migration period (figure 19A), there is radioactivity in the petiole of the presentation leaf, and in stem below the presentation leaf (SB), with traces in the other segments. After 0.5 hour in the dark (figure 19B), there is increased radioactivity in the stem below the presentation leaf (SB), and the root (R), with no apparent increase in the other segments. After 1 hour in the dark (figure 19C), there is considerably greater radioactivity than before in the roots and the growing point, and a slight increase in the stem above the presentation leaf. After 2 hours (figure 19D), there is high radioactivity in all segments, and differences in optical density of autoradiograms of the different segments become difficult to evaluate.

The results of other experiments of similar design, but probably not under as well controlled conditions, indicate that the migration pattern described above may be difficult to reproduce.

It does not appear possible to arrive at some reasonably sure conclusions on the basis of the results.

Figure 19.

Autoradiograms of segments of healthy potato plants after different migration periods following photosynthesis, with $C^{14}O_2$ for 45 minutes, by the terminal leaflet of the presentation leaf. The duration, in hours, of each migration period in ordinary air in the dark is: (A) 0; (B), 0.5; (C), 1; (D) 2. (PL), Presentation leaf; (SB), stem below the presentation leaf; (SA), stem above the presentation leaf; (R), roots; (GP) growing point. The darker areas correspond to locations of higher radioactivity.


A
HEALTHY
GP
PL
SA
SB
R
B
HEALTHY
GP
PL
SA
SB1
SB2
R
C
HEALTHY
GP
PL
SA
SB
R
D
HEALTHY
GP
PL
SA
SB1
SB2
R

2. THE DISTRIBUTION OF C^{14} IN DISEASED PLANTS AFTER DIFFERENT MIGRATION PERIODS IN THE DARK.

Results of the preceding experiment, indicated that the initial direction of migration of C^{14} in healthy plants is towards the root. It is not known, however, what would be the effect, if any, of disease on this pattern. Consequently, experiments were performed using diseased plants.

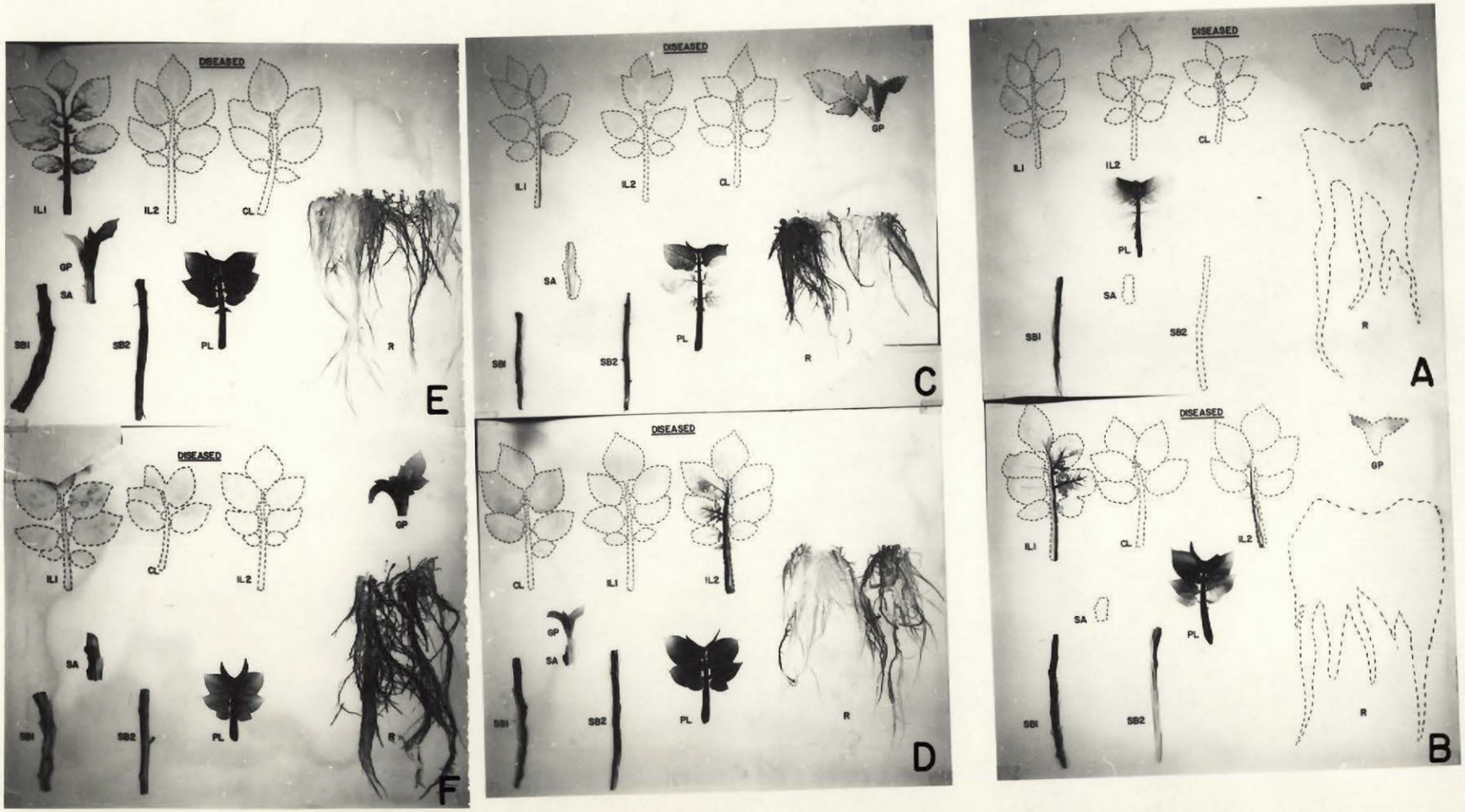
Six diseased plants with infection on 2 of the leaves below the presentation leaf were used. Three days following inoculation the terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration periods used were: 0, 0.5, 1, 1.5, 2, and 4 hours in the dark.

In addition to the presentation leaf, the stem, the roots, the growing point, and 3 leaves below the presentation leaf of each plant were heat-killed, these were; the infected leaves and one uninfected leaf.

Figure 20 illustrates the autoradiograms of the plants used. With no period in the dark (figure 20A), there is radioactivity in the presentation leaf (PL), and in a stem segment below the presentation leaf (SB1), but no detectable radioactivity in the other segments. After 0.5 hour in

Figure 20.

Autoradiograms of segments of diseased potato plants after different migration periods following photosynthesis, with $C^{14}O_2$ for 45 minutes, by the terminal leaflet of the presentation leaf. The duration, in hours, of each migration period in ordinary air in the dark is: (A), 0; (B), 0.5; (C), 1; (D), 1.5; (E), 2; (F), 4. (PL) presentation leaf with most of the terminal leaflet removed; (SB) stem below the presentation leaf; (SA) stem above the presentation leaf; (R) roots; (GP), growing point; (IL1, IL2), infected leaves; (CL) uninfected leaf used as a check. The darker areas correspond to locations of higher activity.


DISEASED
IL1
IL2
CL
GP
SA
SB1
SB2
PL
R
A
B
C
D
E
F

the dark (figure 20B), there is radioactivity in most of the stem below the presentation leaf (SB), the growing point (GP), and the infected leaves (IL1; IL2), but none in the roots or the check leaf. After 1 hour, 1.5 hours, 2 hours, and 4 hours in the dark (figure 20C; 20D; 20E; 20F) there is radioactivity in stem, root and growing point of each plant. However, after 1 hour (figure 20C) there is only a trace of radioactivity in an infected leaf (IL1). After 1.5 hours (figure 20D), there is radioactivity in an infected leaf (IL2), and a trace in the uninfected leaf (CL). After 2 hours in the dark (figure 20E), there is radioactivity in an infected leaf (IL1), with a trace in the other infected leaf (IL2), and the uninfected leaf (CL). Finally, after 4 hours in the dark (figure 20F), there is radioactivity in an infected leaf (IL1), which accumulates around the infection lesions, and a trace in the other infected leaf (IL2), and in the check leaf.

On the basis of these results and those of the preceding section it is concluded that disease does not affect the migration pattern of C^{14} to stem, root and growing point. However, infection is associated with enhanced movement of radioactivity to leaves within one hour following export from a presentation leaf in the dark, and accumulation around lesion sites follows some time later.

F. THE DISTRIBUTION OF C^{14} IN PLANTS WITH THE PETIOLE STUMPS ABOVE THE PRESENTATION LEAF TREATED WITH IAA.

In some of the experiments reported so far there was enhanced movement of photosynthates to infected leaves. If an increase in the concentration of indoleacetic acid reported for certain infected tissues (Shaw and Hawkins,1958; Daly and Inman, 1958), is chiefly responsible for the enhanced movement of photosynthates to infected leaves, then it should be possible to reproduce in some way the effect of disease on distribution. Consequently the effect of IAA on the distribution of photosynthates was studied.

Four plants were used in this experiment. One day before the experiment the leaves above the presentation leaf of each of 2 plants, were debladed;leaving the petiole stumps. Lanolin paste containing 1 per cent indoleacetic acid (IAA), was smeared on to the bare tips of the petiole stumps of one plant and lanolin paste without indoleacetic acid on the other. The remaining plants were used as untreated controls.

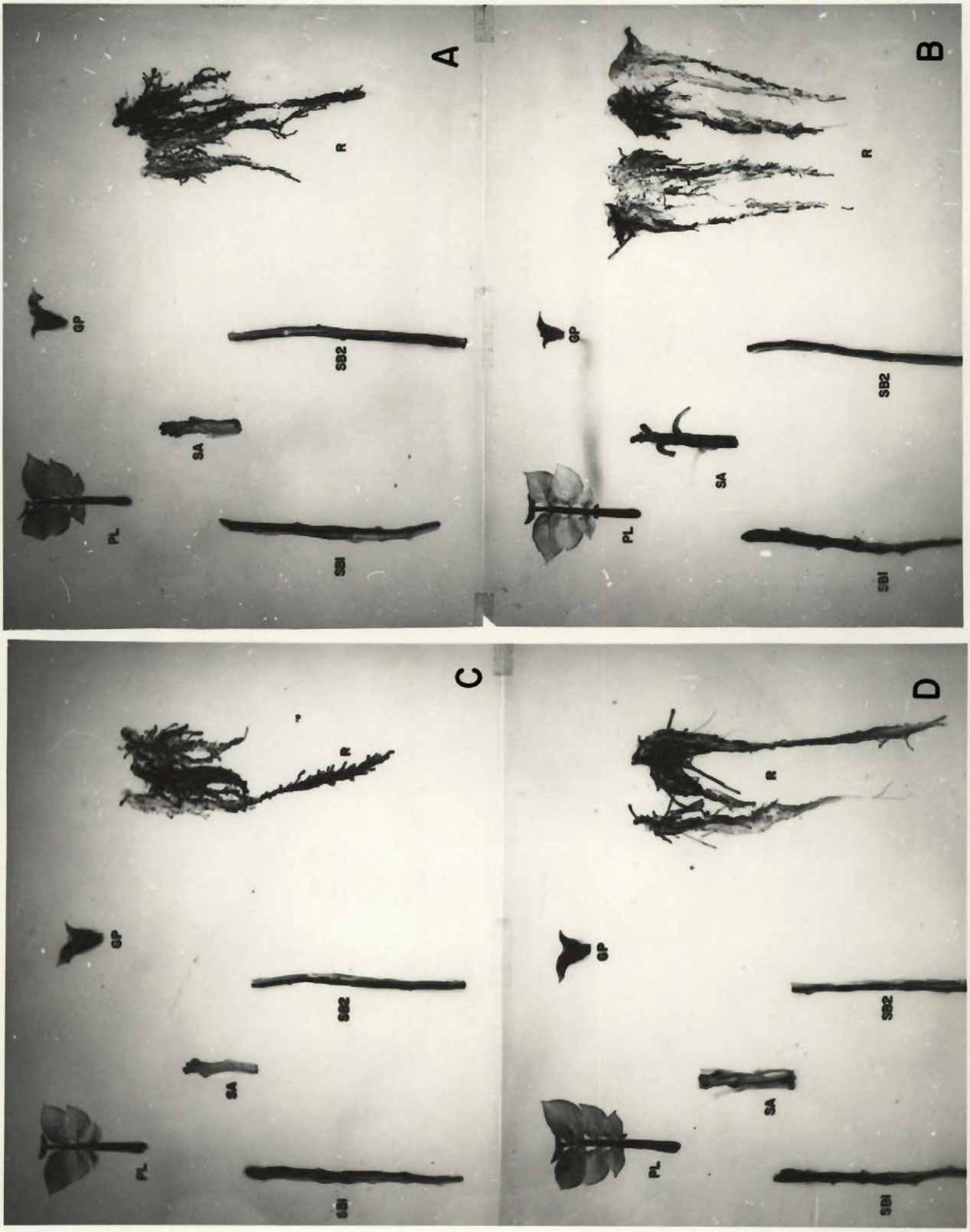
The terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes then the plants were left in ordinary air in the dark for 5 to 6 hours. At the end of this period the plants were segmented, heat-killed, and autoradiographed.

Figure 21 illustrates the autoradiograms of the plants used in the experiment described above. The distribution and concentration of radioactivity, in the stem below the presentation leaf (SB), the roots (R), and the growing point (GP), in the IAA treated plant (figure 21B), is similar to that in the lanolin treated plant (figure 21D), and to the control plants (figure 21A and 21C). However, the stem above the presentation leaf of the IAA treated plant has a higher concentration of radioactivity than the corresponding segments of either the control plants or the lanolin treated plant. Furthermore, there is a high concentration of radioactivity in the IAA treated petiole stumps with no detectable radioactivity in the axillary buds. On the other hand the lanolin treated petiole stumps contain only a trace of radioactivity but have a high concentration of radioactivity in the axillary buds. Similar results were obtained in 2 other experiments of this kind, carried out at different times.

It is concluded, that indoleacetic acid stimulates accumulation of C^{14} in debladed petioles and prevents accumulation in buds in the axils of those petioles.

Figure 21.

Autoradiograms of segments of: plants without debladed petioles, (A), and (C); a plant with debladed petioles above the presentation leaf treated with 1 per cent IAA in lanolin, one day before the plant was exposed to $C^{14}O_2$, (B); a plant with the debladed petioles above the presentation leaf treated with lanolin (D). The terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The duration of the migration period in ordinary air in the dark was 5 to 6 hours. (PL) presentation leaf with most of the terminal leaflet removed; (SB) stem below the presentation leaf; (SA), stem above the presentation leaf; (R) roots, (GP) growing point. The darker areas correspond to locations of higher radioactivity.


A
B
C
D
R
GP
SB2
SA
PL
SB1

G. THE DISTRIBUTION OF C^{14} IN PLANTS ALLOWED TO PHOTOSYNTHESIZE WITH $C^{14}O_2$ AFTER A PERIOD IN LIGHT OR DARKNESS.

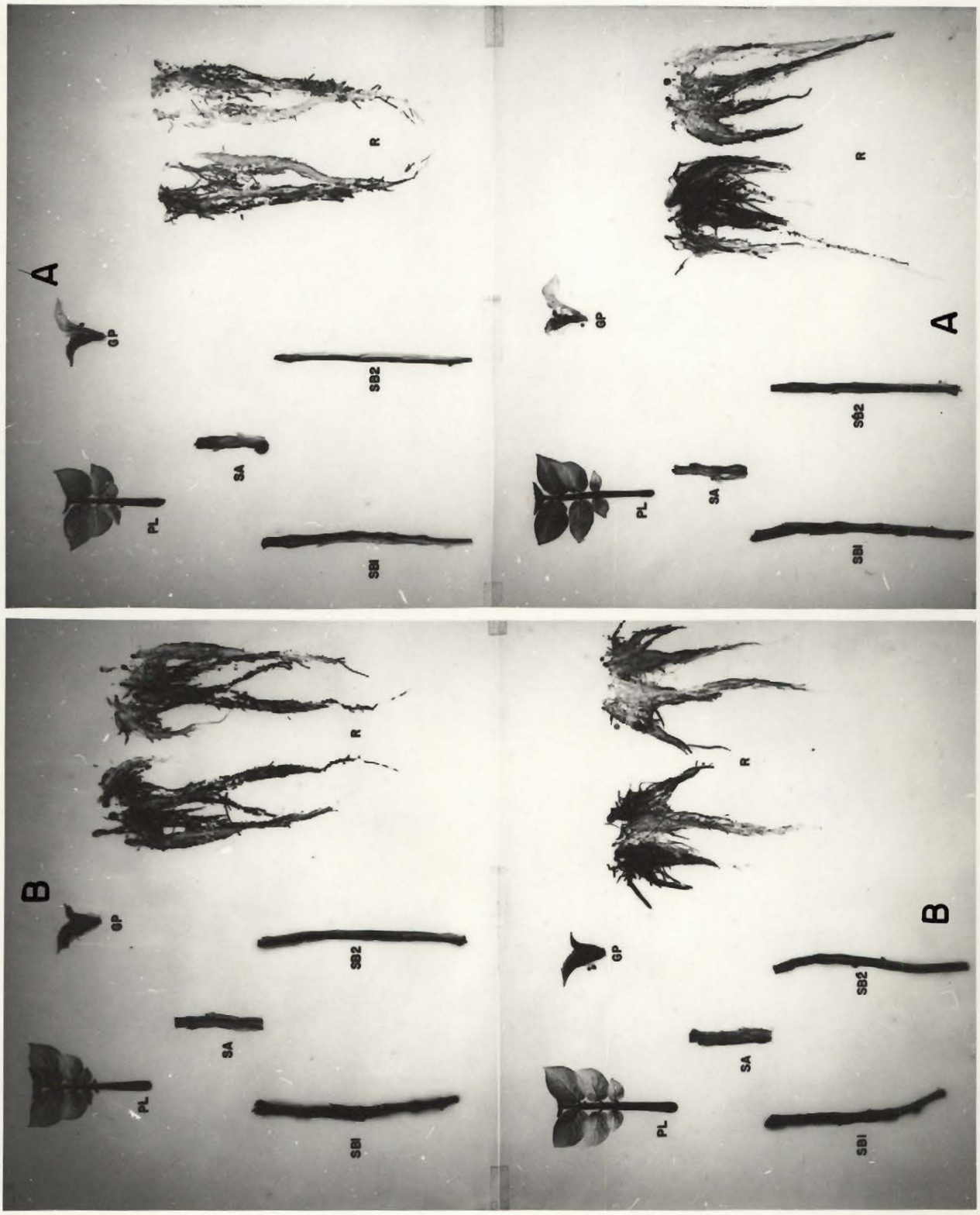
The experiments reported so far were not all performed at a fixed time during the day; some were performed following a period in the light, others following a period in the dark. The presence or absence of light preceding an experiment could possibly affect the distribution pattern of photosynthates, but the extent of such an effect, if any, is not known. Consequently, an experiment was performed with the hope of obtaining information on this problem.

The terminal leaflet of the presentation leaf of 2 plants that had been in the dark for 9 hours and 2 that had been in light for 14 hours were allowed to photosynthesize with $C^{14}O_2$ for 45 minutes, then the plants were left in ordinary air in the dark for 4 to 5 hours. At the end of this migration period, the plants were segmented, heat-killed and autoradiographed.

Autoradiograms prepared from these plants are presented in figure 22. In each of the plants that were in the dark for 9 hours (figure 22A), there is a similar concentration of radioactivity in the stem, roots and growing point. Also, in each of the plants that were in light for 14 hours (figure 22B), there is similar concentration of radioactivity

Figure 22.

Autoradiograms of: (A), 2 plants left in the dark for 9 hours; (B), 2 plants left in the light for 14 hours, before the terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The migration period lasted 4 to 5 hours. (PL), presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA), stem above the presentation leaf; (R), roots; (GP), growing point. The darker areas correspond to locations of higher radioactivity.


A
GP
PL
SA
SB1
SB2
R
B
GP
PL
SA
SB1
SB2
R

in the stem, the roots and the growing point. Further, there is a similar concentration of radioactivity in the stem and roots of all the plants studied. However, the concentration of radioactivity in the growing point of the plants in the dark for 9 hours is less than that of plants in light for 14 hours.

It is concluded from this study that the light regime preceding an experiment affects the amount of C^{14} in the growing point but not in the other plant parts.

H. THE DISTRIBUTION OF C^{14} IN PLANTS WHICH HAD EITHER THE ROOTS OR THE STEM ABOVE THE PRESENTATION LEAF REMOVED BEFORE THE MIGRATION PERIOD.

In the preceding experiments there was a high concentration of radioactivity in the growing point and roots of the potato plant, suggesting that these organs are important centers of accumulation of photosynthates exported from a leaf. It is not known, however, whether the movement of photosynthates out of a leaf and subsequent distribution in the plant depends on the presence of the roots or upper shoot. If this is so, then the removal of one or the other should cause an alteration in the distribution of photosynthates exported from a leaf. Consequently a preliminary experiment was performed to investigate this problem.

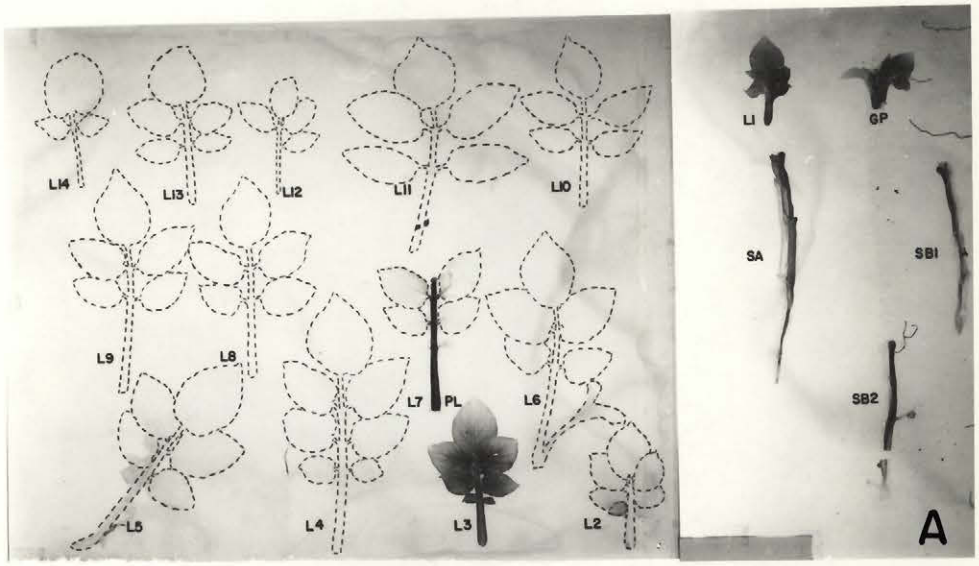
The terminal leaflet of the presentation leaf of each of 3 healthy potato plants was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. Immediately preceding a 4-hour migration period in ordinary air in the dark, the roots of one plant and the shoot above the presentation leaf of another plant were removed. The remaining plant was left untreated. At the end of the migration period the plants were heat-killed and autoradiographed.

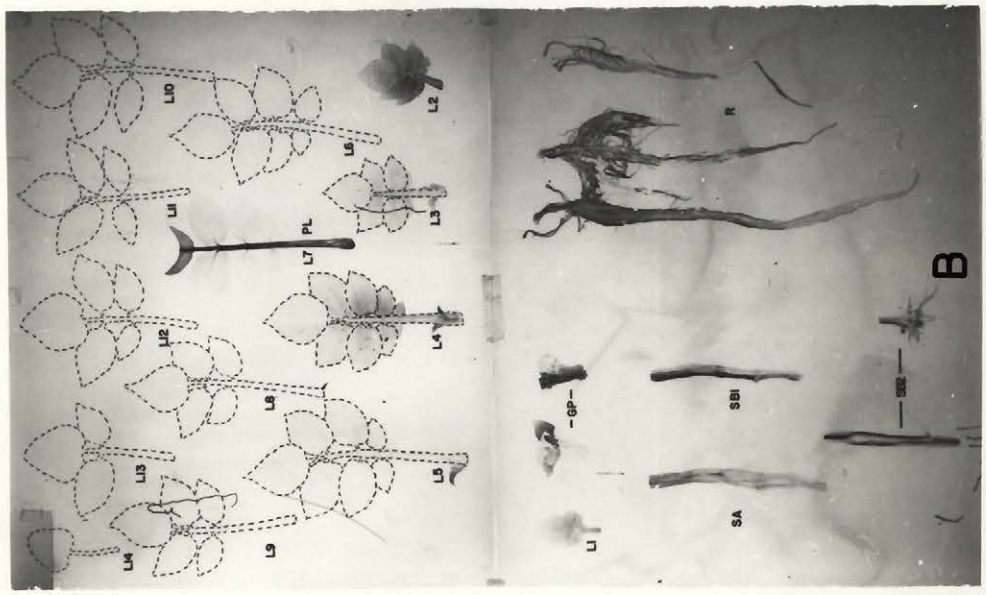
From the autoradiograms obtained (figure 23) it can be seen that, the distribution pattern of radioactivity following removal of the roots (figure 23A), is similar in the remaining segments to that in the untreated plant (figure 23B). Removal of the shoot above the presentation leaf (figure 23C), on the other hand, is accompanied by a lower concentration of radioactivity in the stem and roots than in corresponding segments of the uncut plant but, not by a change in the distribution of radioactivity to these segments.

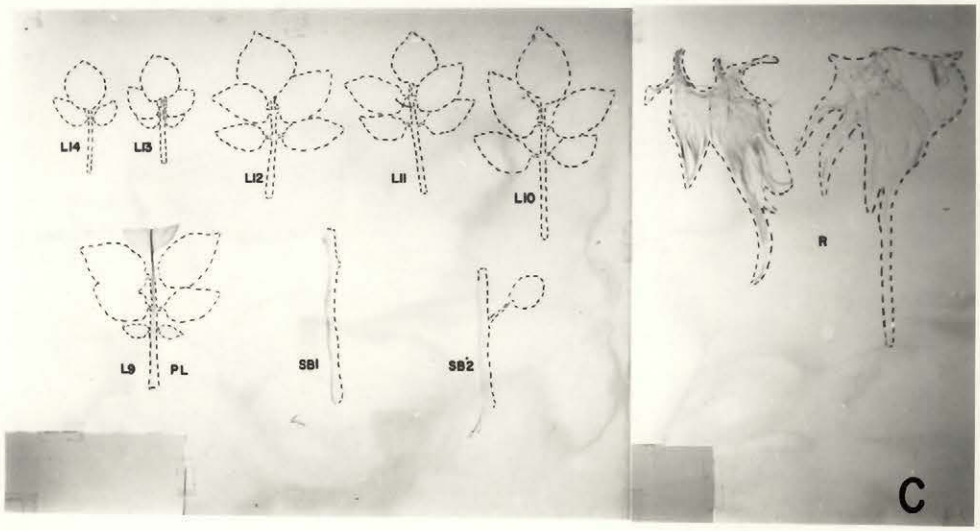
It is concluded that the distribution and concentration of C^{14} migrating from a leaf is not greatly affected by removal of the root but the concentration is affected if the shoot above the presentation leaf is removed.

Figure 23.

Autoradiograms of segments of a plant: (A), with the roots removed before the migration period; (B), left intact; (C) with the shoot above the presentation leaf node removed before the migration period. The terminal leaflet of the presentation leaf of each plant was allowed to photosynthesize with $C^{14}O_2$ for 45 minutes. The subsequent migration period lasted 4 hours. (PL), presentation leaf with most of the terminal leaflet removed; (SB), stem below the presentation leaf; (SA) stem above the presentation leaf; (R), roots; (GP), growing point; and leaves (L1, L2, L3 etc.). The darker areas correspond to locations of higher radioactivity.


L14
L13
L12
L11
L10
L9
L8
L7
PL
L6
L5
L4
L3
L2
L1
GP
SA
SB1
SB2
A


L10
L2
L9
L1
L3
PL
L7
L12
L4
L8
L13
L5
L14
L6
R
GP
SB1
SB2
SA
B


L14
L13
L12
L11
L10
L9
PL
SB1
SB2
R
C

SECTION II. STUDIES WITH DETACHED LEAVES.

In the preceding experiments attempts have been made to determine the effect of disease on the distribution of C^{14} translocates in whole plants. Evidence was obtained indicating that infection causes accumulation of C^{14}-labelled translocates in tissues surrounding lesion sites. It was not known whether or not these tissues could fix $C^{14}O_2$ at an enhanced rate. However, it was known that this occured with other diseases of other plants. For example, infected excised leaves, of barley and wheat, exposed to $C^{14}O_2$ accumulate radioactivity around infection sites (Shaw and Samborski,1956). This has not been shown conclusively with potato leaves infected with P. infestans. It would be difficult to determine the rate of fixation in these tissues if the leaf was allowed to remain attached to the plant because translocation of C^{14}-labelled photosynthates out of the leaf would occur. Also, there is the possibility of reintegration of some C^{14} into the leaf. Experiments were therefore performed with detached leaves to determine the effect of infection on the intra-leaf distribution of C^{14} following administration of $C^{14}O_2$; (a) in the light and (b) in the dark.

A. THE DISTRIBUTION OF C^{14} IN HEALTHY AND INFECTED LEAVES AFTER EXPOSURE TO $C^{14}O_2$ IN LIGHT.

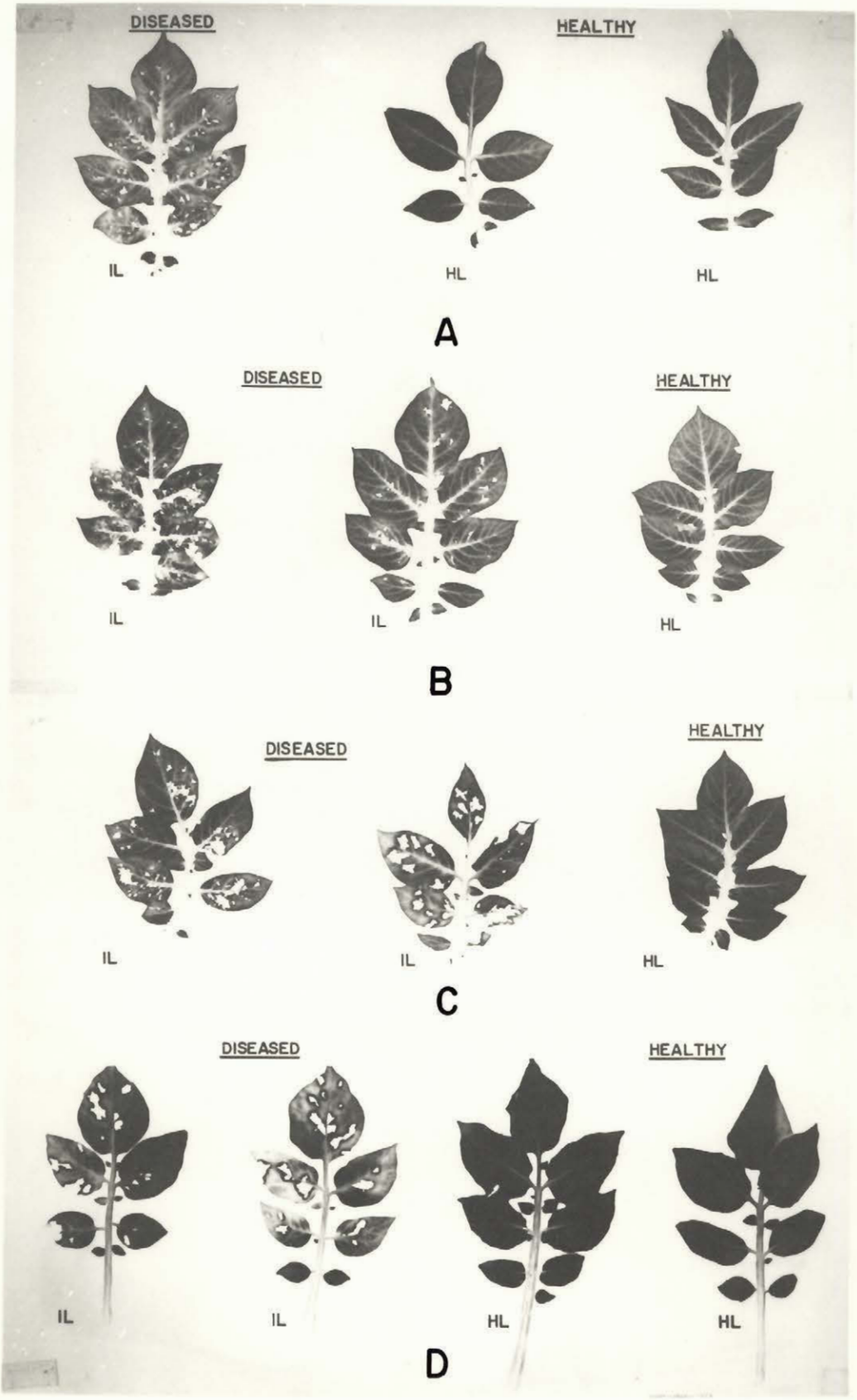
Infected leaves taken from diseased plants 3 days following inoculation and comparable leaves from healthy plants were used. The leaves were excised from the plants a few minutes before the exposure to $C^{14}O_2$. The leaves were set up in a bell jar in the manner described previously in the section, Materials and Methods. Leaves were exposed to $C^{14}O_2$ in light for 10, 15, 20, and 45 minutes. At the end of each experiment the leaves were heat-killed then autoradiographed.

Figure 24 illustrates the autoradiograms of the leaves used in the experiments. There is accumulation of radioactivity in tissues around lesions in all cases, the accumulation increasing with increasing time. On the other hand radioactivity is uniformly distributed in healthy leaves. In addition, after 20 minutes (figure 24C) and 45 minutes (figure 24D) there is a lower concentration of radioactivity in the uninvaded tissue of the infected leaves than in comparable regions of healthy leaves.

It is concluded from this experiment that accumulation of C^{14}, following photosynthesis with $C^{14}O_2$, is greater in a zone around the lesions than in more removed parts.

Figure 24.

Autoradiograms of healthy (HL) and infected (IL) excised leaves, which were exposed to $C^{14}O_2$ in light for: (A), 10; (B), 15; (C), 20; and (D) 45 minutes. Infected leaves were exposed to $C^{14}O_2$, 3 days after inoculation. Darker areas represent higher radioactivity.


DISEASED
HEALTHY
IL
HL
HL
A
DISEASED
HEALTHY
IL
IL
HL
B
DISEASED
HEALTHY
IL
IL
HL
C
DISEASED
HEALTHY
IL
IL
HL
HL
D

B. THE DISTRIBUTION OF C^{14} IN HEALTHY LEAVES AND IN DISEASED LEAVES AFTER EXPOSURE TO $C^{14}O_2$ IN THE DARK.

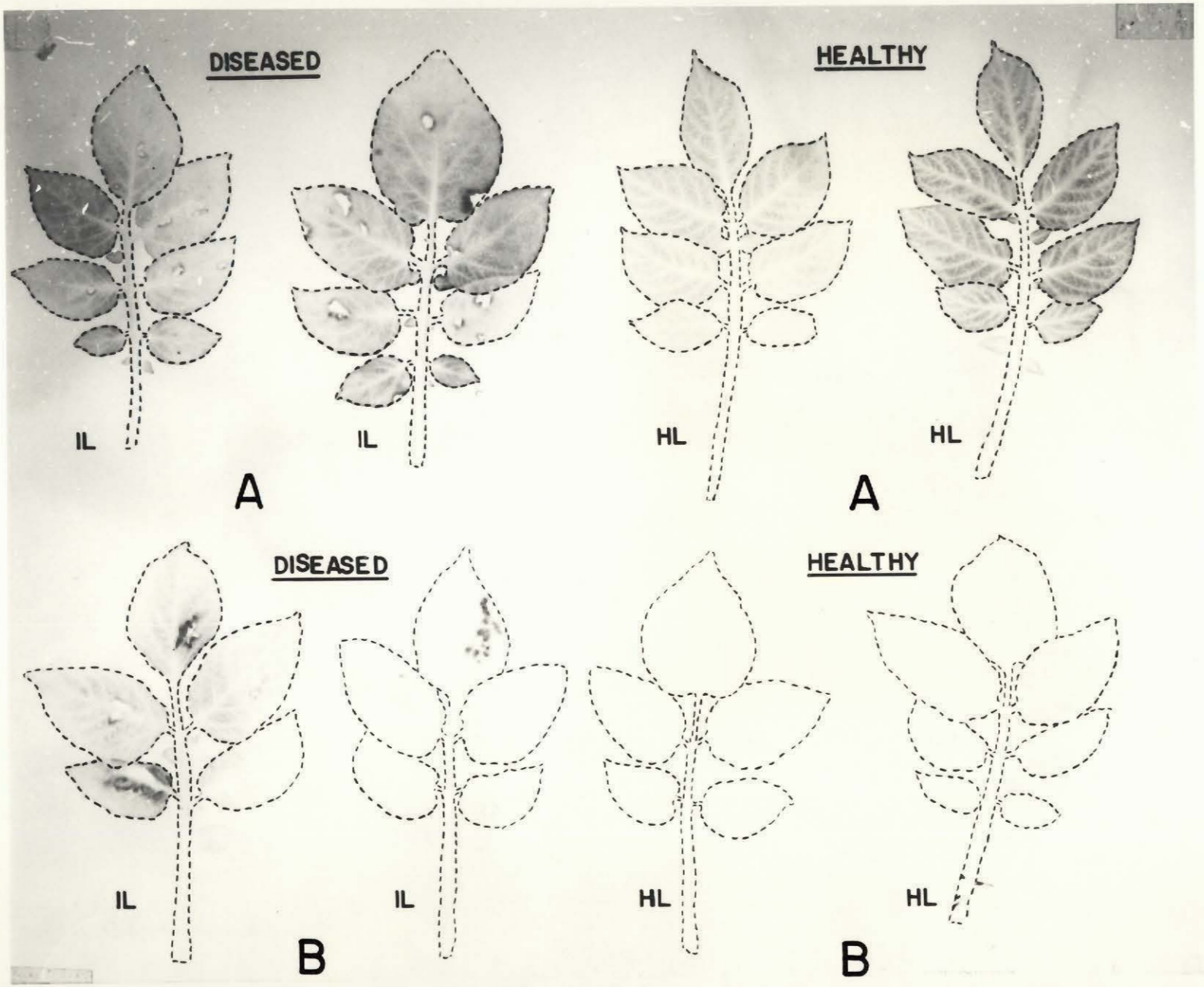
In this study 2 experiments were performed using infected and healthy leaves, set up as described in the preceding section. In each experiment, 2 healthy and 2 infected leaves were exposed to $C^{14}O_2$ in the dark for 2 hours. In one experiment the leaves were taken from plants which were in continuous light for 14 hours, and in the other leaves were taken from plants which were in the dark for 9 hours. At the end of the exposure period the leaves were heat-killed then autoradiographed.

Figure 25 illustrates the autoradiograms of the leaves used in the experiments. There is accumulation of radioactivity around infection sites of infected leaves from plants in light for 14 hours (figure 25A), and from plants in dark for 9 hours (figure 25B). On the other hand activity is uniformly distributed in uninvaded tissue of infected leaves, and throughout comparable healthy leaves.

From the results of these experiments it is concluded that there is in the zone around the lesions a greater accumulation of the C^{14} of dark-fixed $C^{14}O_2$.

Figure 25.

Autoradiograms of healthy (HL) and infected (IL) leaves from plants: (A), in light for 14 hours; (B), in dark for 9 hours, before the leaves were detached for exposure to $C^{14}O_2$ in the dark for 2 hours. Infected leaves were exposed to $C^{14}O_2$ 3 days after inoculation. The darker areas correspond to locations of higher radioactivity.


DISEASED
HEALTHY
IL
IL
HL
HL
A
A
DISEASED
HEALTHY
IL
IL
HL
HL
B
B

SECTION III. THE DISTRIBUTION OF STARCH IN HEALTHY AND IN INFECTED LEAVES.

In the preceding experiments it has been shown that, in leaves, radioactivity accumulates around infection loci of P. infestans. It was shown for other diseases that synthesis of carbon compounds can be accelerated by infection (Shaw and Samborski, 1956; Uritani and Akazawa, 1959; Wang, 1961). For example, Shaw and Samborski (1956), demonstrated starch accumulation around lesions of rust infected sunflower leaves and TMV infected Nicotiana glutinosa leaves, and, even more striking, around stem rust lesions on wheat leaves which normally do not contain starch. The distribution of starch in potato leaves infected with P. infestans had not, however, been studied. Tests were, therefore, performed to determine the effect of infection on the distribution of starch in potato leaves.

Heat-killed leaflets from infected and healthy leaves below the presentation leaf of plants used to study the distribution of C^{14}, were tested for starch by extracting the pigments in boiling 80 percent alcohol and then immersing in iodine solution (0.2 percent iodine + 2.0 percent potassium iodide). The presence of a blue colour in any part of the leaf was taken as an indication of the presence of starch.

This test was applied on several healthy and infected leaflets.

Figure 26, illustrates the distribution of starch in an infected leaflet (D), and a healthy leaflet (H). Starch is present in a zone around the infection lesions and not in the remaining uninvaded tissue. On the other hand starch is uniformly distributed in the healthy leaflet.

It is concluded that infection of a potato leaf with P. infestans prevents the accumulation of starch in the uninvaded tissue of the leaf while allowing accumulation in a zone around the infection lesions.

Figure 26.

Healthy (H), and infected (D), leaflets of potato plants after clearing and staining with iodine. A blue color indicates the presence of starch which is uniformly distributed in the healthy leaflet, but present only in a zone around lesions in the infected leaflet.

V. DISCUSSION.

The effects of P.infestans infection on the distribution of assimilates in the host will be emphasized in this discussion. But first it is necessary to consider some of the limitations of this work which are mainly due to the environment, the experimental material, and the techniques which were employed.

The plants used in the experiments were grown in culture solution and in controlled growth chambers. In view of the important influence of environmental conditions on translocation (Swanson, 1959), it is probable that the results are different from those which would be obtained in the greenhouse and in the field.

One susceptible potato variety reacting with one virulent race of the pathogen, was used in all experiments. It is not known if the results would be similar with other host-parasite combinations.

There was considerable variation in the rate of growth, size and shape - and presumably the physiological condition - of plants used in the experiments. This variation probably is responsible to some extent for the variability of the results of replicate experiments.

The leaves were handled and rubbed during the application of the inoculum; comparable leaves on control plants were left untouched. Thus effects ascribed to disease could be due, in part, to injury of the leaves associated with handling during inoculation. However, most of the experiments were performed 3 to 4 days after inoculation of the leaves. By that time the effect of slight injury during inoculation should have been overcome.

The gross autoradiographic technique which was used throughout, indicates only the pattern of distribution of radioactivity and - for reasons indicated by Dugger (1957) - is quantitative only to a limited extent. However, for the purpose of this work it seemed sufficiently accurate. Also, the distribution of radioactivity following a given migration period gives no direct information about the mechanism of translocation.

Notwithstanding these experimental limitations, the distribution pattern of radioactivity obtained from preliminary experiments; different migration periods; heat-girdling of petioles; different conditions of illumination; removal of the shoot and removal of the roots are useful. They give some indication of the direction and pathways of organic solute transport in the potato plant, and will be discussed briefly here.

Preliminary experiments indicate that radioactivity is detectable only in the stem, root, growing point and very young leaves, after a migration period of 3, 5 and 12 hours. Thus most of the detectable radioactivity becomes localized, in certain parts of the plant, within the shorter migration period and the pattern does not change afterwards. Therefore it appears that slight differences in migration times exceeding 3 or 4 hours would not cause serious differences in the distribution pattern.

Preliminary experiments also show that accumulation of radioactivity in stem, growing point and root is greater on the side of the presentation leaf than on the opposite side. This asymmetric distribution indicates that the vascular interconnections of the supply leaf can affect the distribution of radioactivity (Jones et al., 1959).

The distribution pattern following different migration periods varies in a complex way. In general, the initial movement of C^{14} from the presentation leaf is predominantly downward; which suggests that the phloem is the pathway for solutes moving out of a leaf (Biddulph and Cory, 1957).

Heat-girdling of petioles of either healthy or diseased leaves prevents export of C^{14} but does not affect its import. This suggests that the export of solutes depends on

the presence of living tissues, presumably the phloem (Colwell, 1942). It also suggests that import is in non-living vessels of the xylem and that disease does not affect the path of translocation although it may alter the distribution pattern (see below).

The C^{14} concentration in the growing point is higher in plants kept in the light before photosynthesizing $C^{14}O_2$ than in plants kept in the dark. This is an indication that there is enhanced movement of solute to the growing point in light, which is probably due to enhanced transpiration.

Removal of the shoot above the presentation leaf for the migration period reduced the concentration of C^{14} in the roots and the remainder of the shoot. This result indicates that solute export from the leaf is retarded by this treatment. Mechanical injury, changes in hydrostatic pressure in the injured plant, and alterations in the auxin supply from the growing point, are factors which could explain, in part, the results obtained. At any rate it appears that the presence of the upper part of the stem is necessary for normal solute export from a leaf.

Removal of the roots for the migration period did not cause a detectable alteration in the concentration or the distribution of C^{14} in the remaining plant parts. This suggests

that solute export from a leaf is not wholly dependent on the presence of the roots, and is in contrast to the preceding observation in which it is indicated that the presence of the upper stem is important in solute export. Also, to some extent, this conclusion is contrary to that of Aronoff (1955), and Nelson and Gorham (1959b). These workers indicate that export from a bean leaf is determined by a "pull" exerted in the roots. It appears from this present result that export may also be determined by a "pull" exerted in the stem. The pressure flow hypothesis may account for this "pull" mechanism (Crafts, 1961; Swanson, 1959). However, in view of the studies of Mokronosov and Bubenshchikova (1961), on translocation of assimilates in the potato plant, it is possible that some of the solutes are also exported from the leaf by a metabolically dependent mechanism (Kursanov, 1961).

Some of the results and conclusions of the aforementioned experiments contribute to an understanding of the effects of P. infestans infection on the distribution of C^{14} - labelled assimilates, to be discussed presently.

Infection of 2 leaves below the presentation leaf did not alter the gross distribution among, and the concentration of radioactivity in, the different uninfected organs of the plant. However, the infected leaves had a higher concentration of radioactivity than comparable leaves from healthy

plants, and this radioactivity accumulated mainly around lesions. Therefore, it seems that infection, on leaves other than the presentation leaf, does not affect the movement of food to uninfected organs. This conclusion should, however, be regarded with caution. First, it is important to emphasize that this observation relates to movement from an uninfected leaf only and does not give information as to the concurrent behaviour of other leaves. The observation that the concentration of radioactivity is reduced in uninfected organs as a result of infection on the presentation leaf is evidence that the rate of export of organic solutes by different leaves on a plant is not the same. Furthermore, each leaf on a plant exports a different proportion of the food synthesized (Shiroya et al., 1961); infection notwithstanding.

The relatively high concentration of radioactivity in infected leaves indicates that movement of assimilates, via translocation, to those leaves is accelerated by infection. This acceleration can be due to either increased transpiration, or increased metabolic activity in infected leaves, or both. It is possible that accelerated transpiration is the cause of the high concentration of radioactivity in infected leaves because treatment to reduce transpiration reduces the concentration of radioactivity in those leaves. Further, following different migration periods, radioactivity appears in an infected

leaf before it appears in an adjacent uninfected leaf. In addition the results of heat-girdling experiments indicate that C^{14}-labelled assimilates move to a leaf in the xylem. Accelerated transpiration could also cause accumulation of assimilates at infection sites in the same way that it causes accumulation of dyes (Harvey, 1930; Yarwood, 1947). Assimilates like dyes move in the xylem transpiration stream.

On the other hand selective accumulation can be a result of an increase in metabolic activity. The accumulation of radioactivity in IAA treated petioles suggests that this may be so. In this case increased transpiration is not the reason for the accumulation observed because the petioles of the control plants have no similar accumulation. However, it is known that IAA treatment of the plant tissues stimulates their metabolic incorporation of C^{14} (Galston and Purves,1960; Sen Gupta and Sen, 1961). Thus, it may be that radioactivity observed in IAA treated petioles is accumulated by an active process in the same way as it is at infection sites.

Selective accumulation of organic and inorganic solutes has been reported for rust and mildew infected wheat leaves (Shaw and Samborski, 1956; 1961), and rust and mildew infected bean leaves (Yarwood and Jacobson, 1955). These workers concluded that selective accumulation is due to metabolic incorporation of solutes at infection sites.

If it is assumed that P. infestans (non-obligate parasite), affects the host in the same way as obligate parasites, then it may be that selective accumulation of C^{14} at late blight leaf lesions is due, in part, to enhanced metabolic incorporation of translocated organic solutes. In this connection it is possible that the starch seen around lesions is a product of synthesis associated with enhanced assimilation of translocates at infection sites. Indeed, the observation by Allen (1942), that rust infected wheat leaves accumulate starch around lesions, which is most abundant at the time of maximum respiration, may be taken as evidence in support of this view.

From the foregoing it appears that accelerated transpiration and enhanced metabolic activity can play a part in determining the concentration and distribution of assimilates in infected leaves. It is possible that this occurs in the following way: The movement of C^{14}-labelled assimilates in the xylem transpiration stream, to the infected leaves, is accelerated by increased transpiration. Subsequently, the assimilates that move to infected leaves are utilized and metabolically incorporated at infection sites at an enhanced rate; resulting in selective accumulation.

The fact that the distribution of assimilates after translocation to potato leaves infected with P. infestans is

similar to rust-infected wheat, sunflower and bean leaves (Shaw and Samborski, 1956; Yarwood and Jacobson, 1955), should be emphasized. Indeed Shaw and Samborski (1956), observed with obligate parasites that: "The rapid transport of radioactive substances toward and their accumulation at infections is probably similar to the accumulation of radioactive substances in meristematic or other rapidly metabolizing regions". Perhaps infected potato leaves behave in a similar way.

Evidence that infection lesions correspond to zones of relatively high metabolic activity is indicated by the accumulation of translocated C^{14} and starch around lesions, mentioned previously; and the accumulation of C^{14} following dark fixation of $C^{14}O_2$, to be discussed presently.

The relatively high concentration of C^{14} around lesions following dark $C^{14}O_2$ fixation by infected detached leaves suggests that dark CO_2 fixation is faster in the neighborhood of lesions than in more remote areas. Dark fixation of CO_2 in plant tissues is of fairly general occurrence (Ranson and Thomas, 1960; Saltman et al., 1956; 1957; Sen and Leopold, 1956; Thomas, 1949; Thomas and Ranson, 1954). It is known, for example, that in the formation of malic acid from a product of glycolysis carbon dioxide is metabolically fixed (Thomas and Ranson, 1954). The high rate of dark fixation of $C^{14}O_2$ at potato leaf infection sites is striking and is

probably similar to that found in tobacco leaves (Kunitake, et al., 1959), Bryophyllum leaves (Saltman et al., 1957), and Kalanchoë leaves (Kunitake et al., 1957; Thomas and Ranson, 1954), in which the fixed C^{14} is mainly in malic acid. C^{14} is fixed to a lesser extent in other organic and amino acids - including, citrate, isocitrate, succinate, glutamate and aspartate - which are directly or indirectly associated with the Krebs tricarboxylic acid cycle (Saltman et al.,1957). In the light of this analogy one would expect that malic acid and other acids would be present in relatively high concentration, around late blight infection sites. Evidence that this might be so comes from the observation that rust infected wheat leaves contain a relatively high concentration of malic, succinic, and citric acid - important intermediates of the Krebs cycle - as well as aspartic and glutamic acid (Daly and Krupka, 1962). However, in these authors' work, the possibility that a high concentration of organic and amino acids in infected tissues may be due, in part, to enhanced translocation from other parts of the plant (Jain, 1958), should not be overlooked.

Enhanced dark fixation of $C^{14}O_2$ at infection sites is also in agreement with the theory that the auxin concentration - which increases metabolism (Galston and Purves, 1960) - is increased. An increase in the auxin concentration at infection sites has been found for wheat rust and powdery

mildew (Shaw and Hawkins, 1958), for safflower rust (Daly and Inman, 1958), and for bean rust (Pilet, 1953), but it is not known for P. infestans infected potato. However, the observation of Sen Gupta and Sen (1961) that IAA increases CO_2 fixation into malate and other intermediates of the Krebs cycle appears significant because this could mean that enhanced $C^{14}O_2$ fixation at P. infestans infection sites is related to an increased concentration of auxin.

It was mentioned previously that the concentration of radioactivity in other organs is reduced following exposure of an infected leaf to $C^{14}O_2$. This suggests that infection causes a reduction in the export of organic solutes from a leaf. This reduction in solute export is probably due to a reduction in food synthesis in the infected leaf, and/or an increase in the utilization of the food synthesized.

The relatively low concentration of radioactivity in infected detached leaves following exposure to $C^{14}O_2$ in light indicates reduced photosynthesis. Further, the uneven distribution of radioactivity in those leaves suggests that the rate of photosynthesis is different in different parts of an infected leaf. Thus the relatively low concentration of radioactivity in the uninvaded part of the leaf indicates that the assimilation rate in this area is low. On the other hand, the relatively high concentration of radioactivity around the

infection lesions indicates that the assimilation rate in this area is high.

It is unlikely that the high assimilation rate around lesions compensates for the reduction in assimilation in the uninvaded and in the necrotic leaf tissues. Furthermore, the assimilates around lesions are probably utilized at an enhanced rate as a result of enhanced respiration.

The uneven rate of photosynthesis in different sites of infected leaves may account for the distribution of starch observed in them. On the other hand an increase in the rate of utilization of food reserves could also explain this. For example, the absence of starch in the uninvaded tissues suggests that food reserves are utilized more rapidly than they are supplied by assimilation. In this respect infected leaves differ substantially from healthy leaves in which there is always an abundance of starch which is uniformly distributed in the leaf. Perhaps, infected potato leaves, like infected leaves of other plants, respire more rapidly than healthy ones. This abnormally high respiration could explain, in part, the depletion of starch in the uninvaded tissues. As indicated earlier, starch around lesions could be due to enhanced photosynthesis or enhanced metabolism in this area.

At any rate, it is evident, that the net result of infection is a reduction in the amount of exportable solutes from a leaf.

All of the foregoing discussion, relating to the effect of P. infestans infected leaves on the distribution of C^{14}- labelled solutes in the potato plant, leads to one general conclusion: The host is starved. Starvation is evidently brought about in two main ways. First, food assimilation in the infected leaf is reduced and its utilization is increased; so that less food is available for export to other organs, which need it for normal growth and metabolism. Second, an abnormal amount of food is imported to infected leaves. Infection stimulates transpiration which in turn stimulates the movement of assimilates - in the xylem transpiration stream - to infected leaves, where there is accumulation at infection sites. So that food which normally goes to other organs is imported to infected leaves.

Infected leaves, therefore, play a dual role: they deprive the other organs of food and at the same time compete with them for that which is available in reduced concentration. Indeed, it seems logical to conclude that infected leaves are parasitic on their hosts and thus cause starvation.

On the basis of this hypothesis one would expect to find symptoms of starvation in the uninfected organs or tissues of a plant having a number of leaves infected with P. infestans. Furthermore, alterations in the metabolism of these uninfected organs may occur even if diffusible substances, such as toxins - produced at infection sites as a result of the host-parasite reaction, e.g. Müller's phytoalexins (Müller, 1959; Müller and Behr, 1949) - did not become systemic.

VI. SUMMARY.

The effect of Phytophthora infestans infection - as well as a number of other treatments - on the distribution of C^{14} in whole plants, and in detached leaves was investigated. In experiments with whole plants, a terminal leaflet of one leaf (presentation leaf) of each plant was exposed to $C^{14}O_2$ in light for 45 minutes, followed by a migration period of variable duration in ordinary air in the dark, then segmented, heat-killed, and autoradiographed. Detached leaves, on the other hand, were exposed to $C^{14}O_2$ in light for up to 45 minutes, or in dark for 2 hours; then heat-killed and autoradiographed.

Preliminary experiments with healthy plants indicate a relatively high concentration of radioactivity (C^{14}), in the presentation leaf, roots, growing point and stem, and some in very young leaves. It is seldom detectable in older leaves.

Infection of 2 leaves below the presentation leaf causes an abnormal increase in the concentration of C^{14} in them. There are indications that this abnormal accumulation of C^{14} in infected leaves - which accumulates around lesions - is due, at least in part, to increased transpiration. There

are no detectable alterations in the concentration and distribution of C^{14} among the uninfected plant parts.

Infection of the presentation leaf causes a decrease in the concentration of radioactivity in the plant, but does not alter its distribution.

Heat girdling of petioles of leaves of healthy and diseased plants prevents export of C^{14}, but does not affect its import, via translocation, to them.

The distribution pattern following different migration periods varies in a complex way. However, in general, C^{14} moves towards the root before it moves to the growing point, and appears in an infected leaf before it appears in an uninfected one.

Application of indoleacetic acid to the tips of debladed petioles stimulates the accumulation of C^{14} in these petioles and prevents accumulation in buds in their axils.

C^{14} concentration in the growing point is higher in plants kept in light before photosynthesizing $C^{14}O_2$ than in plants kept in the dark.

Removal of the root for the duration of the migration period does not cause any detectable alteration in the

concentration or distribution of C^{14} in the remaining parts. But removal of the shoot above the presentation leaf causes a reduction in C^{14} concentration in other parts.

Infected detached leaves, following fixation of $C^{14}O_2$ in light and in dark, contain a relatively high concentration of C^{14} around lesions. C^{14} is uniformly distributed in comparable healthy leaves.

Starch in infected leaves is present around lesions only; in a zone coinciding with that of accumulation of C^{14}. But, comparable healthy leaves contain more starch, which is uniformly distributed.

It is concluded that leaf infection can cause starvation of uninfected parts and in this way bring about some, at least, of the other systemic physiological alterations known to occur in plants with localized infections.

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VIII. APPENDIX.

Appendix 1a

Ranks of the autoradiograms obtained from different segments of healthy and diseased potato plants, when segments of each plant are placed in order of decreasing optical density. Results are given for different times after 2 of the leaves below the presentation leaf had been inoculated with *P. infestans*.

Plant Segment	Expt.	Rep.	Ranks					
			3 Days		4 Days		5 Days	
			H	D	H	D	H	D
Presentation leaf (PL)	I	1	2.5	3.5			1	1
		2	1	2			1	4
	II	1	1.3	1.3	1	1	1	1
		2	1	1.5	1	1	1	1
Stem above (PL)	I	1	5	5			4.5	5
		2	4	4.5			4	5
	II	1	5	5	5	5	5	5
		2	5	5	3.3	5	5	5
Stem below (PL)	I	1	4	3.5			2.5	3.5
		2	2.5	4.5			2.5	3
	II	1	1.3	1.3	3	3	2.3	2
		2	2	1.5	3.3	3.5	2.3	2.5
Growing point	I	1	2.5	1.5			4.5	3.5
		2	5	1			2.5	2
	II	1	4	4	4	4	2.3	3
		2	4	4	3.3	3.5	2.3	4
Roots	I	1	1	1.5			2.5	1
		2	2.5	3			4	1
	II	1	1.5	1.5	2	2	2.3	4
		2	3	3	1	2	2.3	2.5
Leaf 1	I	1	6.5	7			5.5	6.5
		2	6.5	5			5.5	6
	II	1	6.5	6	6.5	6	6	6.5
		2	6.5	6	4.5	6	6	6
Leaf 2	I	1	6.5	6			5.5	6.5
		2	6.5	5.5			5.5	7
	II	1	6.5	7	6.5	7	7	6.5
		2	6.5	7	4.5	7	7	7

Appendix 1b.

Sum of the ranks (A) of autoradiograms presented in Appendix 1a.

	Sum of Ranks (A)					
	3 Days*		4 Days*		5 Days*	
Plant Segment	H	D	H	D	H	D
Presentation leaf(PL)⊙	5.8	8.3	2	2	4	7
Stem above PL	19	19.5	8.3	10	18.5	20
Stem below PL	9.8	10.8	6.3	6.5	9.6	11
Growing point	15.5	10.5	7.3	7.5	11.6	12.5
Roots	8	9	3	4	11.1	8.5
Leaf 1#	26	24	11	12	23	25
Leaf 2#	26	25.5	11	14	25	27

* Number of days following inoculation with P. infestans.

Leaves below presentation leaf; infected ones on diseased plants.

⊙ Ranks based on optical density of leaf petiole.

See figure 9 for illustration of the different segments.