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EFFECTS OF POWDERY MILDEW (*ERISYPHE CICHORACEARUM* D.C. EX MÉRAT) ON THE GROWTH AND DEVELOPMENT OF GROUNDSEL (*SENECIO VULGARIS* L.).

A thesis submitted to the Faculty of Science, University of Glasgow, for the degree of Doctor of Philosophy

by

Victor Dagogo Ben-Kalio

October 1976

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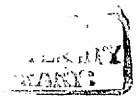

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To

My Mother, Madam Cheneboso Ben-Kalio

ACKNOWLEDGEMENTS

The work recorded in this thesis was carried out in the Department of Botany, Glasgow University, with facilities very kindly provided by Professor M. B. Wilkins, Regius Professor of Botany.

The area of research was suggested and supervised by Dr D. D. Clarke to whom I wish to convey my gratitude and appreciation for all his help.

I wish also to place on record my indebtedness to Drs C. G. Elliott, C. T. Wheeler and A. M. M. Berrie who have given their invaluable time for stimulating discussions and have given useful advice.

I offer my sincere thanks to all other persons who have contributed in various ways to make the work a success, especially Mr W. Burgess for his assistance in technical respects and Mr T. N. Tait for taking care of the photographic aspects; also Mrs C.C. Bennett for typing this thesis.

The Government of Rivers State of Nigeria kindly provided financial sponsorship during the period of research to which I offer my grateful acknowledgement.

SUMMARY

This thesis reports a study of the effects of powdery mildew infection on the growth and development of *Senecio vulgaris* L. with a view to finding the extent to which host plants in wild populations can tolerate the activities of organisms parasitic on them.

The causal organism of the groundsel powdery mildew has been tentatively identified as a member of the compound species, *Erysiphe cichoracearum* DC ex Merat, on the basis of its characteristics at the asexual stage. The ranges of the environmental conditions of temperature and humidity that permit the growth of the fungus were determined as a preliminary to infection studies. The fungus has temperature optimum between 18 and 28°C and humidity optimum between 90 and 98% RH, for growth and development. Germination occurred at all humidities tested.

A means of maintaining mildew-free plants for the comparative study was investigated and Benomyl (Benlate du Pont), at a concentration of 0.05% aqueous solution applied as soil drench, was found to be effective. At this concentration, it did not affect the growth of the groundsel and no phytotoxic effects were produced.

A diagrammatic scale for assessing the extent of mildew cover on the leaves was developed.

The analysis of the growth of infected plants in comparison with uninfected controls revealed that a 10% level of infection slightly increased the number of senescent leaves without affecting the overall growth and development of the host. A 75% level of infection, however, significantly reduced growth and development, although the distribution of dry matter amongst the various organs appeared to be unaffected. Heavily infected plants produced inflorescences and set viable seeds and thus indicates that groundsel has some tolerance to powdery mildew.

A study of the distribution of nitrogenous materials to the various organs revealed that infected plants contained more nitrogen per unit dry weight of plant material than the controls and the excess appeared to be present in the soluble, non-protein form. Results of one experiment only showed that the roots of infected plants contained more nitrogen per unit dry weight than those of the controls, suggesting that the translocation system of organic and inorganic nutrients might have been impaired. But this is at variance with the results of all other experiments.

CHAPTER 1

GENERAL INTRODUCTION

A parasite is an organism or virus existing in intimate association with a living organism from which it derives an essential part of the material for its existence (Anon 1973). The relationship between a parasite and its host is thus basically a nutritional one. The parasite, due to its feeding activities, constitutes a source of nutrient drain on its host and in this way alone can have an effect on the host's growth and development.

Besides deleterious effects due to this basic nutritional relationship, a parasite may also cause physiological disorders in its host by mechanisms other than through simple feeding activities. These effects are thus in principle quite separate from the effects caused by the depletion of materials used as nutrients.

The first group of activities may be classified as "parasitic" and the second as "pathogenic". (The term pathogenic activity is here used in the restricted sense to mean those activities other than food gathering which are thus of no direct benefit to the parasite.) Thus, the total effect of the activities of a parasite on its host will be in general terms the sum of the effects of each of these activities.

Basically, there are two ways whereby the pathogenic activities of a parasite can result in deleterious effects on the host. Firstly, the growth of the parasite can cause mechanical damage to the host tissue. For example, rust uredospores during development press against the host's epidermal tissue from inside and may thus rupture it. Also in certain wilt diseases, mucilagenous carbohydrate materials resulting from the degradation of the cell walls of the vascular tissue by enzyme action may, together with the physical mass of the causal organism itself, physically block the xylem vessels and thereby disrupt water movement (Wood, 1967

and Tarr, 1972).

Secondly, metabolites such as toxins and growth regulators produced by the parasite can induce changes in the overall metabolism of the host, the end result of which becomes manifest on the growth and development of the host and its final morphology. Obvious cases of toxin involvement in disease development have been shown with oats infected with Helminthosporium victoriae and with tobacco infected with Pseudomonas tabaci. Some of the physiological and morphological effects which result in the host in these and other cases have been reviewed by Crowdy and Manners (1971), Wood (1967) and Wheeler (1975) and these may be summarised as follows:-

(i) Effects on carbon assimilation.

(ii) Effects on translocation of organic and inorganic substances including water relations.

(iii) Effects on respiration.

(iv) Effects on growth regulatory mechanisms.

It is intended here to discuss these effects in broad terms limiting the discussion to a few examples that are thought to be of relevance in the context of this thesis.

Parasites have ~~also~~ been shown to reduce the rate of assimilation of living host tissue. Thus, Doodson et al. (1965) found that Puccinia striiformis, 14 days after inoculation, had reduced the rate of ^{14}C assimilation in wheat leaf to about 50% of that in the controls. Siddiqui and Manners (1971) investigated the more normal situation in which the whole plant was infected and they found that the assimilation process was affected in the same way although a higher percentage of assimilate accumulated in the infected presentation leaves.

During the early stages of infection, the rate of photosynthesis often rises but falls again in the later stages. Doodson et al. (1965) found that in yellow rust infected wheat plants, the rate of fall was greater

than the rate of decline in chlorophyll content indicating that the rate of assimilation per unit amount of chlorophyll had dropped. Allen (1942) similarly found the same trend in barley infected with Erysiphe graminis. Thus, the reduction in the rate of photosynthesis may in part be due to a reduction in the amount of chlorophyll and a reduction in the efficiency of the remaining photosynthetic apparatus.

Translocation of materials from the infected wheat leaf was also greatly reduced. Fourteen days after inoculation only 0.4% of the assimilates were leaving the presentation leaf of infected plants compared with 21% in the corresponding leaf of control plants. Infection also affected the distribution pattern of that proportion which was translocated. The percentage moving to the roots and tillers was drastically reduced and this was correlated with greatly reduced dry weight of these organs in infected plants.

Coffey et al. (1970) investigated the translocation pattern of labelled ^{14}C in tomato plants infected with the early blight fungus, Alternaria solani. They also found a significant reduction in the amount of assimilate exported from a diseased leaf but only at the early stages of infection. The pattern of distribution of the exported material was also altered. Three days after inoculation, the labelled carbon in the stem of infected plants was less than half that of the controls but that fraction in the roots was greater than that in the control. This investigation thus shows that necrotrophs can alter the translocation and distribution patterns in their hosts in ways very much similar to the effects produced by biotrophs. Livne and Daly (1966) and Thrower and Thrower (1966) provided evidence to show that Uromyces sp. infection on bean, in addition to reducing export from a diseased organ also promoted import to such organs. Doodson et al. (1965) and Siddiqui and Manners (1971), however, were unable to detect such movement into yellow rust infected mature wheat leaves. Some of these effects on

translocation and distribution which involve tissues other than those which are infected clearly appear to involve the action of toxin.

One of the commonest observations of the effects of a parasite on its host is that the respiratory rates are affected.

Studies on the respiratory response of cereals to infection by Puccinia striiformis and E. graminis have shown that they are no exception (Crowdy and Manners, 1971; Millerd and Scott, 1962 and Uritani and Akazawa, 1959). Within 48 hours of inoculating barley leaves with Erysiphe graminis, the rate of respiration increased by up to 300% (Allen, 1942; Allen and Goddard, 1938 and Scott and Smillie, 1963). On further examination, Bushnell and Allen (1962) found that the increased respiration was not wholly attributable to the respiration of the pathogen. They found that the increases occurred in the uninvaded cells adjacent to the periphery of the developing pustules and in the tissues immediately below. This observation suggests that the mildew parasite produced toxic substances which diffused into the underlying cells and there initiated the metabolic changes which resulted in the increased respiration. Allen (1942) reported that the period during which the respiratory rate of the infected leaf was high corresponded with the period when the assimilation rate was low. The net result was a depletion of the reserves of assimilate available to the host. P. graminis infection of wheat also produced similar effects in wheat (Daly et al., 1961).

Substances with growth regulatory activity may be produced by the parasite or induced to form in the host tissues by the parasite which alter the host's growth pattern and its final morphology. In rice, infection by Gibberella fujikuroi (Fusarium moniliforme) causes an overgrowth of the stem, a condition originally described as the bakanae or foolish seedling disease. The formation of abnormal structures in the host such as galls and tumours, e.g. swollen shoot of cocoa, maize

smut (Ustilago maydis) and potato wart (Synchytrium endobioticum) may be induced by metabolites produced in the same way. The abnormal growth utilises mutrients which would otherwise have been available for the development of normal structures and they are therefore acting as "metabolic sinks".

These are some of the ways, in addition to its feeding or parasitic activities that a parasite can affect the physiological and biochemical processes of the host plant ultimately leading to alterations in its growth and development. The problem now is how to assess for any host-parasite complex, the relative proportions of these pathogenic and parasitic effects in the overall responses of the host to infection.

The relative importance of parasitic and pathogenic effects on host plant growth

There are considerable difficulties in ascertaining the relative importance of the "parasitic" and the "pathogenic" activities of the invading micro-organisms in any host-parasite system. However, several studies of diseases on certain crop plants indicate that the pathogenic activities may be disproportionately large compared to the parasitic activities.

Last (1962) reported that although total dry weight of E. graminis infected barley plants continued to increase throughout the period of investigation, control plants developed 59% more dry matter than infected plants. The relatively greater reduction in root growth than of leaves in infected plants was perhaps the most unexpected feature of this host-parasite system, since powdery mildews are usually discussed in relation to their leaf environment with little thought of possible effects on the roots. The work of Fric (1975) offers an explanation of the phenomenal reductions in root dry weight of mildew infected barley plants. Thus, in addition to the higher respiration of infected leaves, he found that the export of labelled carbon assimilates from the infected leaves was

also significantly retarded. This meant that the infected leaves retained a greater proportion of their photosynthetic products and so the roots and sheaths received reduced amounts. Infection also reduced the number of tillers and their extension growth. The straw height of infected plants was 30% less than that of control plants. The loss of dry weight of tops showed a close relationship to loss in total leaf area which was attributable both to a decrease in the number of leaves and to the size of tillers per plant. The mean net assimilation rate of infected plants was reduced by about 27%. Ear development was similarly affected; the average dry weight per ear was 25 % less in the infected plants than in the controls.

The work of Harrison and Isaac (1969) is an outstanding example of an application of growth analytical techniques to plant pathological problems. They found that during the first 5 to 6 weeks of growth potato plants infected with Verticillium dahliae or V. albo-atrum were morphologically identical with the uninfected controls. However, during this period the growth rate of the plants was affected as was the distribution of dry matter between their various organs. It was also found that Verticillium impaired the photosynthetic efficiency of the leaf area which was itself greatly reduced by means of defoliation and stunting effects. The growth indices, unit leaf rate and relative growth rate, were also reduced by infection.

Doodson, Manners and Myers (1964) in a detailed quantitative study of the effects of Puccinia striiformis on the growth of wheat reported that attack reduced plant height by about 26%. The number of tillers as well as the length of leaves was reduced. Ear emergence and anthesis was delayed by about 14 days. In fully infected plants, the number of grains per ear was reduced by up to 42%. There was also a very striking reduction in root development - greater than that in the development of any other part of the infected plant. The mean root dry weight of fully

infected plants was reduced by 78%. This was probably related to the reduction in the amount of translocates moving to the roots from aerial parts of infected plants (Doodson et al., 1965), and also to the fact that the uninfected plants produced two to three times as many tillers as infected plants, each of which produced its own root system. The mean weight per grain was also reduced by 34%.

On the other hand, there are associations between hosts and parasites which do not lead to such adverse effects on the host but on the contrary may in some instances prove very advantageous to the host. For example in mycorrhizal associations between certain fungi and plant roots, the pathogenic activities of the fungal partner would appear to be minimal or even non-existent. As Harley (1971) pointed out, in addition to offering protection against invading organisms, some mycorrhizal fungi may also help to increase nutrient absorption by the host.

Much recent work has shown that plant growth may be improved by infection with vesicular-arbuscular mycorrhizal fungi and the growth response is normally associated with an improved supply of phosphorus from the soil (Mosse, 1973; Khan, 1975 and Cooper, 1975). This trend has been shown experimentally on a wide range of agricultural crops. Thus, Kleinschmidt and Gerdeman (1972) found that the dry weight of citrus plants in a fumigated field plot inoculated with Endogone mosseae were significantly greater than that of non-inoculated control plants. Ross and Harper (1970) obtained a 34-40% increase in the yield of soybean plants inoculated with Endogone spores. Vesicular-arbuscular fungal infection increased corn yield by 50% over the increases due to indigenous mycorrhizal fungi (Jackson et al., 1972). This was substantiated by the results of Khan (1975) who also found that maize inoculated with Endogone spores with at least 65% of the root length infected, increased the root/shoot fresh weight ratios by 50%, stimulated the rate of increase of leaf area and caused a twelve-fold increase in grain weight. Thus, Khan (1975)

suggested that mycorrhizal inoculation of crop seeds could be of great practical value in increasing grain production in those areas of the world where phosphorus limits plant growth and phosphorus fertilisation is not economical.

In all of these cases, plant growth was increased rather than being depressed by infection although the fungus might have obtained a large part of its carbon assimilation from its host. Thus, in theory, a host should be able to tolerate a parasite without its growth being greatly affected. This would occur if the host has sufficient extra assimilatory capacity over that required to support its own growth if the parasite does not produce toxins or other metabolites which cause marked disturbances in the host. Tolerance has been defined in different ways by different authors. However, the definition of Schafer (1971) given in his review of the subject, as"that capacity of a cultivar resulting in less yield or quality loss relative to disease severity or pathogen development when compared with other cultivars of that crop," seems quite appropriate in the context of the present work.

Caldwell et al. (1958) showed that with similar maximum levels of infection, the losses of wheat grain attributable to P. coronata were not significant on the variety Benton but were significant on the variety Clinton 59. Ellis (1954) reported local maize to possess a combination of both resistance and tolerance to Puccinia polysora compared with an imported hybrid although all were fully attacked by rust. He suggested that such tolerance indicates that plants do not express the full capabilities of their metabolism in their grain yield and that there may be a reservoir of unused yielding capacity available to be utilised by a parasite.

There is some evidence to show that compensatory growth can occur in an infected plant which can restitute the toll taken by a parasite. Thus, Livne (1964) found that in beans infected by Uromyces phaseoli,

the rate of assimilation in uninfected leaves of infected plants was up to 50% above that of corresponding leaves on healthy plants. It was also shown that greater amounts of photosynthates moved out of the uninfected leaves of infected plants than from leaves in the same position on healthy plants and this may have stimulated the increased photosynthesis in these leaves. Harrison and Isaac (1968) found that the leaf area of potato plants infected with Verticillium sp. continued to increase even after the development of disease symptoms due to the fact that the production of new leaves and their expansion continued at a faster rate than defoliation. In other words, the host was able to compensate for some of the pathogenic effects and thus also for some of the parasitic effects.

Tarr (1972) made the submission that long association between plant and pathogen would lead to the development of mutual tolerance. Plants severely injured by the pathogen, being unable to compete with those less severely injured, would be eliminated, so that the species as a whole becomes tolerant. Evolution would thus lead to a host-parasite relationship in which the host was little affected by infection and in which the parasite grew and reproduced relatively freely. Such a situation would be expected to lead to the maximum survival of both the host and the parasite.

It is likely that conscious and unconscious selection among crop plants for improved yielding capacities following the development of agriculture over the centuries has caused considerable disruption to the physiological balance that would have existed between these plants and their parasites. However, balanced host-parasite combinations would be expected still to occur in nature amongst wild populations of plant species. The extent to which a host plant can endure the activities of its parasite may then be best investigated using wild species which have not suffered such interferences through breeding

programmes as test plants. The results of such studies may bear considerable relevance to the potential value of tolerance as a breeding aim for controlling yield losses in crop plants.

The host-parasite system which has been selected for study is the Groundsel-powdery mildew combination. Groundsel is particularly suitable for this study because it is readily available and has a relatively short life cycle. This enables the production of several generations of plants from a seed stock per year. The flowers are self-fertilised and so populations of plants all of the same genotype can be used. The mildew parasite occurs readily on it, with heavily infected plants enduring the disease, growing up to maturity and producing viable seeds.

CHAPTER 2

SECTION I : THE CAUSAL ORGANISM OF GROUNDSEL POWDERY MILDEW

Introduction

The powdery mildews are characterised by the presence of a largely superficial mycelium on the host's surface together with masses of powdery conidia. The causal fungi are classified in one family - the Erysiphaceae, and separation into genera and even species delimitations is based largely on features of the cleistothecial stage.

There is some uncertainty about the identity of the causal organism of groundsel powdery mildew in this country because the cleistothecial stage of the fungus is not known. The conidiophores are morphologically relatively simple and in any case those of a single species may vary greatly in size and structure depending on the host and the prevailing environmental conditions (Peries 1960) and so they are of little use in the determination of the genus let alone the species.

The location of the mycelium on the host's surface again is of limited value because the mycelium of most genera remains as a superficial layer on the host. Only the genera Leveillula and Phyllactinia are characterised by having an internal mycelial system. Groundsel mildew clearly does not belong in either of these genera because its mycelium is entirely superficial.

Salmon (1900) in his studies of powdery mildews on plants from most parts of the world, grouped the powdery mildews on Senecio spp. in the compound species, Erysiphe cichoracearum. Blumer (1933), whose work was mostly concerned with powdery mildews on European plants, divided this compound species into a number of species, naming the one on Senecio spp. Erysiphe fischeri. Junnel (1967) in her taxonomical studies of the Erysiphaceae of Sweden accepted E. fischeri for the species that is

parasitic on Senecio.

Homma (1937) studied the Erysiphaceae of Japan and listed some Senecio spp. as host plants of members of the genus Sphaerotheca not Erysiphe. She did not discuss the species E. fischeri. It appears that the species E. fischeri has been recognised in certain parts of the continent of Europe but its occurrence has not been reported in the United Kingdom. Thus, Bisby and Mason's (1940) list of powdery mildews in Britain does not include E. fischeri. The Commonwealth Mycological Institute's description of pathogenic fungi (Anon, 1966) also does not include a description of E. fischeri but E. cichoracearum was described and listed as one that is commonly parasitic on wild composites.

Stone (1962) in a study of the possible alternate hosts of the cucumber powdery mildew reported that 27 out of 58 cucumber plants inoculated with groundsel mildew conidia developed a mildew which was indistinguishable from the cucumber mildew. Stone therefore suggested that groundsel might be an overwintering host for the cucumber mildew.

It is now well known that two powdery mildews can be parasitic on cucumber, namely Sphaerotheca fuliginea and E. cichoracearum and in the absence of cleistothecia their similar morphological features make it easy for them to be mistaken for each other (Kable and Ballantyne, 1963; Boerema and Kesteren, 1964; Hirata, 1966; Junnel, 1967; Kooistra, 1968 and Yarwood, 1973).

Thus a possible deduction one could make on the strength of the evidence available is that groundsel mildew could be incited by either a Sphaerotheca sp. or an Erysiphe sp. or even perhaps by both organisms occurring independently.

The genus Sphaerotheca produces only one large ascus in each cleistothecium but Erysiphe produces several asci, sometimes numbering up to 15. Both genera produce myceliod appendages on their cleistothecia which look like somatic hyphae in being flaccid and indefinite. The rare

formation of cleistocarps by the two species S. fuliginea and E. cichoracearum on cucurbits creates a problem in their differentiation (Hirata, 1966; Junnel, 1967; Ballantyne, 1963; Boereman and Kesteren, 1964; Alexopoulos, 1962 and Yarwood, 1973). This situation clearly rules out the possibility of differentiating by means of characters at the sexual stages, unless the formation of cleistocarps can be induced.

The factors affecting cleistocarp formation have been the subject of much experimentation and no specific features have been found that could apply generally to all species. Sexuality is of primary importance in cleistocarp formation. For example, many isolates of E. cichoracearum from different hosts are known to be heterothallic, that is to say, two compatible isolates (+ and -) need to be mated before cleistocarps can be formed. Thus single spored isolates of E. cichoracearum cultured on leaf discs obtained from Helianthus and Zinnia failed to produce cleistothecia but intercrosses between them resulted in cleistothecial formation (Morrison, 1961). Yarwood (1935) and Schnathorst (1959) found similar behaviour with the sunflower and lettuce isolates of E. cichoracearum. On the other hand, Homma (1933) sowed single conidia of S. fuliginea upon its host plant and the resultant mycelium produced cleistothecia. Hence, some isolates at least of S. fuliginea are homothallic (cited from Bessey, 1964).

Laiback (1930) also experimented on other conditions that permit cleistocarp formation and reported that this phenomenon was primarily dependent on the genotype of the host and to a lesser extent on the environmental conditions under which the host is grown, especially the water regime. Water deficiency appeared to favour their formation. Thus, the use of different isolates and manipulation of the environment might result in the induction and development of cleistothecia by the groundsel mildew. This would make the determination of the causal organism possible.

However, Yarwood (1957) expressed the desire for methods for the identification of the powdery mildews using the conidial stages since many species have never been found to produce cleistothecia on their hosts. Several workers from different countries in recent times have investigated methods of characterising and identifying powdery mildew species solely by means of features of the conidia and of the germ tubes formed when they germinate (e.g. Ballantyne, 1963; Boerema and Kesteren, 1964; Kooistra, 1968 and Clare, 1958). Yarwood (1973) proposed a key based on characters of the conidia and conidiophore to enable identifications of genus and species to be made in the absence of cleistocarps.

Conidia and conidiophores contain highly refractive particles known as "fibrosine bodies" or "corpuscles de Zopf". These bodies which were first reported by Zopf in 1887 are said to consist of a nitrogen-containing carbohydrate (Homma, 1937) and are located between the vacuoles. They are easily observed by a microscopic examination of conidia after clearing with dilute potassium hydroxide solution. Two types are known:-

(i) Well-developed fibrosine bodies.

(ii) Granular fibrosine bodies.

The well-developed fibrosine bodies are disc-shaped, conic or cylindrical. This type is common in the genera Sphaerotheca and Podosphaera. The granular fibrosine bodies are simple, minute particles of a rather regular consistency and this type occurs in the genus Erysiphe (Homma, 1937).

The shape of the germ tube and appressorium may be characteristic for certain species (Hirata, 1968; Zaracovitis, 1965 and Yarwood, 1973). S. fuliginea is unique in producing forked germ tubes but E. cichoracearum on the other hand, produces a long germ tube terminated with a well-differentiated club-shaped appressorium.

In this study, conidial and germ tube characters have been investigated in an attempt to further characterise the causal organism of groundsel mildew.

A. The fibrosine bodies of the conidia.

Methods

Conidia from heavily infected groundsel plants, grown in a growth chamber at a temperature of about 18°C and relative humidity of not less than 60%, were transferred to a clean glass slide using a camel hair brush. A drop of 5% potassium hydroxide solution was added to the conidia, a coverslip applied and left for about 10 minutes to clear. The conidia were then examined under the microscope at a magnification of 160 x. Conidia from infected groundsel plants were compared with those from other mildew infected plants - Rosa sp., (S. pannosa), Heracleum sphondylium (E. polygoni), Taraxacum officinale (S. fuliginea) and Plantago major (E. lamprocarpa).

Results

The fibrosine bodies in conidia from the groundsel mildew appeared as very minute grains of a regular consistency and were similar to those present in E. polygoni and E. lamprocarpa. The granular nature of the fibrosine bodies of groundsel mildew conidia became more apparent when compared with the fibrosine bodies contained in conidia from the powdery mildews, S. fuliginea and S. pannosa. Fibrosine bodies in conidia of these latter species appeared more coarse and quite distinct from those in conidia from the groundsel mildew.

B. Germ tube morphology

Methods

Conidia from the same host plants as used for the examination of conidial structure were placed onto coverslips and attached to a glass slide by means of vaseline jelly with the conidia-bearing surfaces uppermost. The whole system was then suspended on glass rings over about 5 cc of distilled water in petri dishes. The system was incubated at 20°C for 24 hrs in darkness. At the end of this period, the coverslips were inverted over a drop of

cotton blue in Lactophenol and the shape of the germ tubes produced were examined microscopically.

Results

The conidia from all plants produced germ tubes at the end of the incubation period and the shape of the germ tubes appeared to be characteristic of each species tested. The groundsel mildew conidia produced long germ tubes terminating in club-shaped appressoria (Plate I) and are thus quite distinct from the forked germ tubes produced by Sphaerotheca fuliginea from dandelion.

The results of this examination and that of the structure of fibrosine bodies are summarised in Table I.

Discussion

The evidence in the literature relating to the taxonomy of the powdery mildews indicates that the causal organism of groundsel powdery mildew could belong either to the genus Sphaerotheca or Erysiphe (Salmon, 1900; Blumer, 1933 and Junnell, 1967).

The results of Stone's (1962) work on the alternate hosts of cucumber mildew show that groundsel could be an alternate host and therefore might be attacked by the two species, Erysiphe cichoracearum and Sphaerotheca fuliginea which are the causal organisms of mildew on cucumber.

The non-formation of cleistocarps by these two species on cucumber and on a number of other common hosts is frequently reported in many countries. This may almost be regarded as a distinguishing characteristic. During the present period of study, no cleistothecia were found on any groundsel plant collections made. Thus, any attempt at identification has had to depend on features of the asexual stage. The two genera under consideration, Sphaerotheca and Erysiphe, have been separated by several workers through the characters of the asexual stage (Kooistra, 1968;

Hirata, 1966; Homma, 1937; and Zaracovitis, 1965). The facts that the conidia are borne in chains, contain granular fibrosine bodies and germinate to form long germ tubes terminating with well-differentiated appressoria indicate that the groundsel mildew is an Erysiphe not a Sphaerotheca. This combination of asexual characteristics is consistent with the description by other workers for isolates of E. cichoracearum on a wide range of hosts (Hirata, 1966; Zaracovitis, 1965; Kooistra, 1968; Homma, 1937; Ballantyne, 1963; Boerema and Kesteren, 1964 and Clare, 1958). Yarwood's (1973) identification key of the powdery mildews at the asexual stage also show these characters to be peculiar to E. cichoracearum. But no information is available about the conidia and germ tube characteristics of E. fischeri and it is impossible to compare its characteristics at the asexual stage with those of groundsel mildew in this country. Thus, it would seem safer to consider the causal organism of groundsel powdery mildew simply as a member of the E. cichoracearum complex (sensu Salmon, 1900). The status of the name of the fungus and its correct citation have been reported by Schmitt (1955).

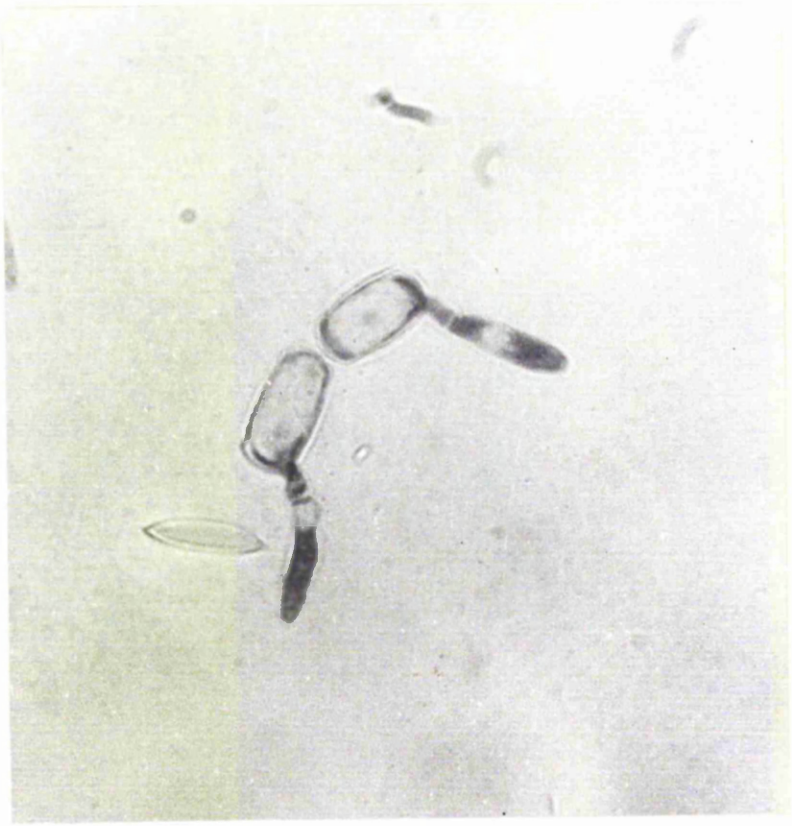


Plate 1. Photomicrographs of germinated conidia of groundsel powdery mildew showing the characteristic long germ tubes with well-differentiated, club-shaped appressoria.

Table I. Conidial and germ tube characters of some powdery mildew species.

	Mildew species	Host	Shape of germ tube with appressoria	Nature of fibrosine body
1	*E. polygoni*	*Heracleum sphondylium*	Short and lobate	Granular
2	*E. lamprocarpa*	*Plantago major*	Long and straight	Granular
3	*S. fuliginea*	*Taraxacum officinale*	Short and forked	Well-developed
4	*S. pannosa*	*Rosa* sp.	Long and straight	Well-developed
5	?	*Senecio vulgaris*	Long and club-shaped	Granular

CHAPTER 2

SECTION II : THE EFFECT OF ENVIRONMENT ON THE INFECTION OF GROUNDSEL BY E. CICHORACEARUM.

Introduction

To be able to provide the most suitable conditions for host infection by groundsel mildew in the growth room, a knowledge of the range of the various environmental factors affecting infection and parasite growth is necessary. This information would be particularly essential in order to manipulate these factors in the growth room to produce the levels of infection required for an investigation of the effects of different levels of mildew infection on the growth and development of groundsel.

The major environmental conditions affecting germination and growth of the powdery mildews are temperature and humidity. Light may also be of considerable importance. It is difficult to separate and account for the individual effects of these conditions on conidial germination and growth as in nature these conditions act in combination. It has therefore become common practice to discuss conidial response to environmental factors in combinations of factors, mainly temperature and humidity. The body of literature reporting studies on the environmental relations in powdery mildews shows that temperature relations have been studied more extensively than humidity or light. This may be because temperature is the easiest environmental factor to control.

Yarwood (1957) and more recently Schnathorst (1965) reviewed the general environmental relationships of the powdery mildews and more specificially Yarwood *et al.* (1954) reviewed temperature effects.

Optimum temperatures for conidial germination and growth in the powdery mildews tend to approach the optimum for the growth of their hosts. Thus, the cool-weather plant species such as lettuce are attacked

by mildews with relatively low optimum temperatures and conversely warm weather plant species, e.g. Grape, are attacked by mildews with relatively high optimum temperatures. For example, the optimum temperature for the lettuce mildew is 18°C (Schnathorst, 1960) and that for the grape mildew is 25°C (Delp, 1954).

There could, however, be considerable variation in optimal temperature requirements within a species as much as there could be between species. Erysiphe cichoracearum from cantaloupe and cucumber in warmer regions showed a temperature optimum of 25-28°C whilst a collection from squash from cooler regions showed a temperature optimum of 15°C (Yarwood et al 1954).

Humidity conditions are relatively difficult to control effectively in experimental work and this may have contributed to some extent to the inconsistencies of results in the literature relating to the direct effects of moisture stresses on conidial germination. Favourable effects of rain, dew, fog and sprinkler irrigation on powdery mildews have been reported (cited from Yarwood, 1957). On the other hand Boughey (1949) stated that the incidence of powdery mildews throughout the world decreases as rainfall increases. Schnathorst (1962) in a survey of the occurrence of the lettuce mildew in a certain locality in the U.S.A. found that the mildew was absent from the humid coastal areas but was frequent and severe in the drier interior.

However, results of many laboratory studies on the response of mildew conidia to varying moisture stresses measured in terms of percentage conidial germination and rate of elongation of the germ tube have established that free moisture is inhibitory to the development of certain powdery mildews. Corner (1935) found that 1-3 hours immersion of several species of mildew conidia in water killed them. Yarwood (1939), Delp (1954) and Perera and Wheeler (1975) reported the possibility of controlling powdery mildews by continually spraying with water. Perera

and Wheeler (1975) found that the effect of free water on the development of S. pannosa on Rose was most marked and persistent when leaves were wetted immediately after inoculation and the extent to which growth of the fungus was affected was directly related to the length of the wet period. Leaf wetness did not appear to have inhibited the production of the first germ tube. The most susceptible phase occurred some 6-8 hours after inoculation when processes leading to penetration of host tissue were being initiated.

So far, we have been discussing what may be regarded as general trends in the temperature and humidity requirements of the powdery mildews. It would be appropriate to consider specific examples of the response of some powdery mildew species to varying conditions of temperature and humidity.

Schnathorst (1960) studied the effects of varying temperature and moisture stresses on the lettuce powdery mildew fungus, E. cichoracearum. He reported that subjecting conidia to -5°C reduced their viability so that after 168 hours, only 3% of the conidia were still viable. Deslandes (1954) also found that subjecting conidia to -10°C for 24 hours did not affect their germinability. The coldest winter temperatures in the area of investigation was hardly as low as 0°C which implies that in natu the fungus has greater chances of surviving the winter conditions in the conidial state.

The minimum temperature that permitted germination of lettuce mildew conidia was found to be between 6° and 10°C, the maximum was 27° and the optimum for germination and growth was about 18°C. These results were obtained under moisture conditions of near 100% relative humidity. A humid atmosphere of 100% RH at 18°C inhibited germination. The highest rate of germination was obtained within the narrow range of 95.6 to 98.2% RH. This investigation was done on both detached leaves and on plain glass slides and the results showed that although a higher percent-

age of germination was obtained on detached leaves than on glass slides, the minimum, optimum and maximum ranges of temperature and humidity permitting germination were found to be similar in both cases. The stimulation of germination on leaves was suggested to be attributable to more conducive microclimatic conditions near the surface of the leaf and to the possible presence of some chemical stimulant peculiar to the leaf surface (Longree, 1939). Yarwood (1936) also remarked that the host's leaf surface could offer some protection for the fungus from desiccation which adverse atmospheric conditions might cause.

Delp (1954) similarly studied the temperature and humidity requirements of the grape powdery mildew, Uncinala necator, and the results he obtaineddeviated, as may be expected, from those of the lettuce powdery mildew. The optimum temperature for conidial germination and growth was about 25°C and at 33.5°C, germination did not occur. Little or no effect on infection and development was detected when the moisture stress was altered from 1-34 mm. - Vapour pressure deficit (VPD) (i.e. 98 to < 1% RH) as long as the temperature was near the optimum. (For a discussion of VPD see page 27).

Erysiphe polygoni on clover also showed a preference for low humidity (Yarwood, 1936). U. necator and E. polygoni represent the extremes of a group of fungi that show a tolerance to low atmospheric humidity.

On the basis of results from his tests and those of others on the conidial response to different moisture stresses, Schnathorst (1965) classified the powdery mildews into three groups:

1. Those that germinate only at low moisture stress, 0.5-6.0 mm VPD (75-95% RH) with an optimum of 0.5-1.0 mm VPD (96-99% RH) at an average temperature of 23°C, e.g. S. pannosa.
2. Those with an optimal germination at low moisture stress but with a small percentage of conidia capable of germinating at

high moisture stress even at 18-36 mm VPD (0% RH). The optimum is similar to group 1 but a sharp decline in germination could occur at 6 mm or more VPD (50-75% RH), e.g. E. cichoracearum and E. graminis.

3. Those that germinate well throughout a wide range of moisture stress, from 0.5-36 mm VPD (0-99% RH) at about 23°C. In this group, differences in germination in response to a wide range of moisture stresses would not be significantly different and the major differences in germination would be expected to be due to temperature effects.

One of the most extensively studied species of the powdery mildew fungi is E. graminis and the reports concerning the humidity requirements for its growth have been conflicting. Yarwood (1936) found that E. graminis f. sp. avenae germinated at 0% RH but E. graminis f. sp. hordei gave almost no germination even at 100% RH. Clayton (1942) also obtained poor germination with E. graminis f. sp. hordei even at humidities over 90% and none at low humidity. Cherewick (1944), however, observed good germination at 1% RH with formae hordei and avenae. Brodie (1945) similarly stated that conidia of many powdery mildews including formae of E. graminis would germinate at low humidities and he claimed that further studies on other mildews would show that they behaved in the same way. In contrast, Grainger (1948) reported that Scottish collections of E. graminis f. sp. avenae required 100% RH for germination and Nour (1958) found that germination of E. graminis f. sp. hordei steadily decreased below 80% RH.

In an attempt to resolve the differences in results obtained by their predecessors, Manners and Hossain (1963) compared the response of conidia of the three formae of E. graminis on wheat, barley and oats to varying temperature and humidity conditions. No significant differences were found between the three formae in their rate of germination,

latent period of germination and elongation of germ tube at any of the temperatures and humidities tested. The optimum temperature for conidial germination was 20°C and optimum RH was 100% in the absence of free water. Some germination, however, was found to have occurred even at 0% RH. The optimum conditions for elongation of germ tubes for all three formae speciales were 25°C and 100% RH. They ascribed the differences in results reported by previous authors largely to differing experimental conditions, the use of differing germination criteria and the use of inocula of different viability and history.

Yarwood in his review (Yarwood, 1957) attempted to explain the source of disagreement amongst some workers regarding the generalisation that powdery mildews could thrive under dry atmospheric conditions. He suggested that this disagreement may be attributable to previous misconceptions, to differences in mildew strains and species investigated or to differences in method and misinterpretation of correct observations. He stated that the belief that all spores require moisture for germination might have influenced the judgement of some investigators. However, he made the submission that most foliar pathogens do require free moisture or high humidity at least during certain stages of their life cycle but the difference between powdery mildews and other groups of fungi is that the former carry a very high percentage of water in them which they require for germination. Schnathorst (1965) gave considerable merit to the above suggestion.

Criteria for assessing the response of powdery mildew conidia to varying atmospheric conditions

There are usually a lot of unforeseen problems involved in the assessments of conidial response to varying environmental conditions. Besides the direct effects which the environmental conditions under investigation may exert, several other factors such as the state of the

conidia, the physiological state of the host plant from which the inoculum was derived (Schnathorst, 1960) and the methods of investigation may influence conidial germination. The state of the conidia, whether turgid or shrunken, and the age and history of inoculum may be important to the extent that a delay in the emergence of the first germ tube and a decrease in the rate of elongation of the germ tubes may be caused.

There are no set criteria that are suitable for all kinds of investigation but most investigators have employed percentage germination and the length of germ tube as appropriate measures of response. Tomkins (1932) suggested that percentage germination taken after an arbitrary period of incubation could not be a sufficiently accurate measure of such variable environmental factors as temperature, humidity, pH or fungicidal or fungistatic activities of chemicals on the viability of the inoculum. He stated that percentage germination used alone as the criterion could bring about variation between results. Three criteria were suggested which when used together or individually depending on the nature of investigation could yield consistent results, viz.:

1. The latent period of germination, i.e. the average time for germ tubes to appear.
2. The final percentage germination, not one taken after some arbitrary time.
3. The rate of elongation of the germ tubes.

For the purposes of determining the effects of environmental conditions on the germination of spores, Tomkins expressed the opinion that the final percentage number of conidia which germinated and the latent period of germination are of most interest.

In addition to percentage germination and the mean length of germ tube, Nour (1958) used the size of conidia to determine the effect of humidity including free moisture on the germination of several species of powdery

mildew conidia. Manners and Hossain (1963) also shared the view that percentage germination alone could not yield an accurate assessment of mildew conidial response to environmental conditions. The rate of germination and elongation of the germ tube were suggested to be more sensitive measures.

There is some confusion about when a conidium may be regarded to have germinated. Some workers consider a conidium to have germinated on the first appearance of a visible germ tube whilst others do not consider germination to have occurred unless a germ tube of substantial length has been formed. Manners and Hossain (1963) considered a germinated conidium to be one which produced a germ tube, the length of which exceeded the breadth of the conidium. This condition may not be applicable to general situations since conidia of different mildew species invariably produce germ tubes of varying sizes and shapes. For example the germ tubes of E. polygoni are short and lobate, those of S. fuliginea are also short but forked and those of S. pannosa are long and slender.

Relative humidity (RH) and vapour pressure deficit (VPD)

Some investigators have shown a preference for expressing moisture stress in terms of mm of vapour pressure deficit (VPD) rather than percentage relative humidity (RH) (Delp 1954; Schnathorst, 1960). Anderson (1936) and Stevens (1916) have reviewed the use of VPD and the concept of atmospheric humidity.

RH is not a direct measure of any absolute quantity of water vapour in an atmosphere but it is merely a ratio (expressed as a percentage) between the actual moisture content of the atmosphere and the amount that could exist under the same conditions without condensation. The capacity of space to hold water vapour increases rapidly with an increase in temperature so that atmospheres with the same RH but at different

temperatures contain different amounts of water vapour and therefore have different VPDs. On the other hand VPD expresses the difference between the amount of water vapour actually present and the amount that could exist without condensation. It thus gives a direct measure of the atmospheric moisture content independent of temperature.

VPD can be determined from psychometric readings or can be calculated from the formula, VPD = (1-RH)E, where E is the vapour pressure at saturation at a given temperature (Schnathorst 1965).

The facilities available did not permit the direct measurement of VPD. In any case, it was considered sufficient for this study to express the moisture conditions as percent RH as long as the related temperatures are stated.

Controlling relative humidity

The importance and demand for methods of controlling RH in small closed spaces for biological studies have been aptly emphasised by Winston and Bates (1960). Two methods which are commonly used to control RH are:

(a) Saturated salt solutions (Winston and Bates, 1960).

(b) Concentrations of sulphuric acid (Stevens, 1916; Wilson, 1921 and Hepburn, 1927).

Salt solutions appeared to be more frequently used than sulphuric acid for studying the effects of humidity on fungal spore development. Salt solutions do not normally change in vapour pressure to any appreciable extent with changes in temperature and it is relatively easy to set up a series of solutions to control the desired relative humidities. Saturated salt solutions also have the added advantage over sulphuric acid solutions of not being corrosive and they keep well for long periods especially if they are not hygroscopic.

A more sophisticated apparatus, the controlled atmosphere wind tunnel

which allowed a direct reading of humidity and temperature conditions around the conidia was used by Delp (1954) to study the relation of humidity and temperature to mildew development on single attached grape leaves. However, this apparatus is not widely used. Although one would assume that it would yield accurate results and thus be quite suitable for critical studies, the apparatus has not displaced the use of the more common sulphuric acid and saturated salt solutions and these solutions have proved reasonably reliable for investigations aimed at determining the humidity requirements for the development of fungi.

Experimental Work

Temperature effects on conidial germination

For the determination of the response of conidia to different temperatures, petri dishes in place of screw-capped jars were used because only 5 cc of distilled water was required to maintain a constant humidity.

To avoid using conidia which might have lost their viability, the infected plants from which conidia were to be taken were shaken and blown across the previous day to remove as many conidia as possible. This procedure allowed a fresh crop of conidia of comparable age to develop and these were removed for investigation using a camel hair brush and were placed on circular cover slips. Four such cover slips were placed on a glass slide held in position by means of Vaseline jelly with the conidia bearing surfaces uppermost. The conidia were suspended over about 5 cc of distilled water by means of glass rings and incubated at 6, 12, 18, 21, 25 and 34°C in the dark.

At the end of 24 and 48 hours of incubation, the cover slips bearing the conidia were inverted over 2% cotton blue in lactophenol to kill and fix the conidia and thus prevent further germination and growth. A total of at least 400 conidia were counted and the percentage number of germin-

ated conidia was calculated. The length of 20 germ tubes was measured by means of an eyepiece micrometer. When taking measurements of germ tube length only those that produced the characteristic club-shaped appressoria were taken into account. The proportion of germinated conidia without the characteristic appressoria in relation to those with appressoria was also determined. However, it was not possible to make measurement of the abnormally long germ tubes because some of them were of curved shapes and had got entangled. The results are given in appendix Tables 1A and 1B and summarised in the text figures 1 to 3.

Humidity effects on conidial germination

Relative humidity regulating solutions were prepared from chemically pure concentrated sulphuric acid as tabulated by Stevens (1916) (see Table 2a) and from saturated salt solutions as tabulated by Winston and Bates (1960) (see table 2b).

Screw-capped jars of approximately 250 cc volume were used as humidity chambers in preference to petri dishes which have been used by other workers. They allowed for the use of large volumes of the humidity regulating solutions and thus may improve the effectiveness of the solutions in maintaining the desired humidity.

Conidia were placed on cover slips as described for the tests on temperature effects. The slides were suspended about 3 cm above the humidity regulating solutions on glass supports. The lids were screwed down on the jars and were sealed with parafilm sealing tape. They were incubated in darkness at the desired temperature for varying periods as is specified in the tables and figures summarising the results.

After incubation, the conidia were fixed and stained with Cotton Blue in lactophenol as described before and the percentage number of germinated conidia and the length of the germ tubes were determined. The results are summarised in Figures 4 and 5.

Table 2a. Concentrations of sulphuric acid for regulating different relative humidity.

% H_2SO_4	82	66	54	44	38.03	33.4	23.5	11.6	0 (Water)
% RH	1.5	10.5	29.5	49	60.7	70.4	85.7	95.6	100%

Table 2b. Saturated salt solutions used for regulating relative humidity (after Winston & Bates, 1960).

% RH	Salts
0	Calcium chloride (anhydrous)
5.5	Sodium hydroxide
9	Phosphoric acid
33	Magnesium chloride
44	Potassium carbonate
55.5	Calcium Nitrate
65.5	Ammonium Nitrate
76	Sodium Nitrate
80.5	Ammonium Sulphate
85	Potassium chloride
93	Ammonium monophosphate
98	Potassium dichromate
100	Distilled water

Results

A. The effects of temperature

It was observed that some conidia produced germ tubes which were of abnormal length and which did not bear the characteristic club-shaped appressoria (Plate 2). The appressorium is the organ with which the organism appresses itself to the host's surface at the initiation of an infection process. It is therefore considered to be an indispensable organ. Thus, the assessment of temperature effects is based mainly on germinated conidia terminated with appressoria.

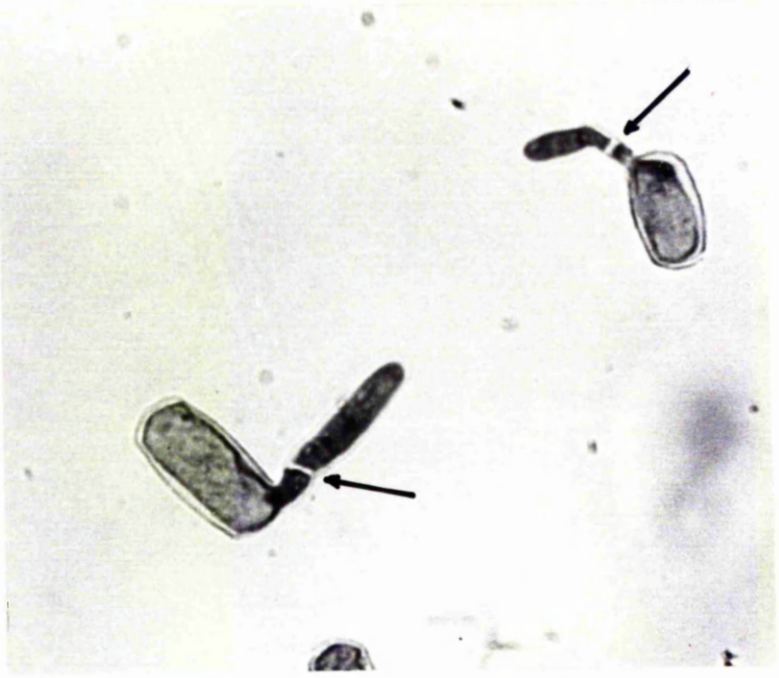
In Fig. 1, lines C and D represent total percentage germination after 24 and 48 hours respectively and lines A and B represent percentage germination after 24 and 48 hours respectively when only germ tubes terminated with appressoria were considered. Most germination occurred in the first 24 hours of incubation with very few more germinating during the next 24 hours (Fig. 1). The proportion of germ tubes without appressoria was found to increase with increasing temperature amounting to 32% of the total at 28°C after 48 hours of incubation (Fig. 3).

The effects of different temperatures on germination and germ tube length measured after 24 and 48 hours of incubation are presented in Appendix Tables 1A and 1B and the means are plotted graphically in Figs. 1 and 2. The minimum temperature tested 6°C permitted some germination but 34°C, the maximum tested, did not permit any germination at all. Conidia at 34°C were shrivelled and appeared dead.

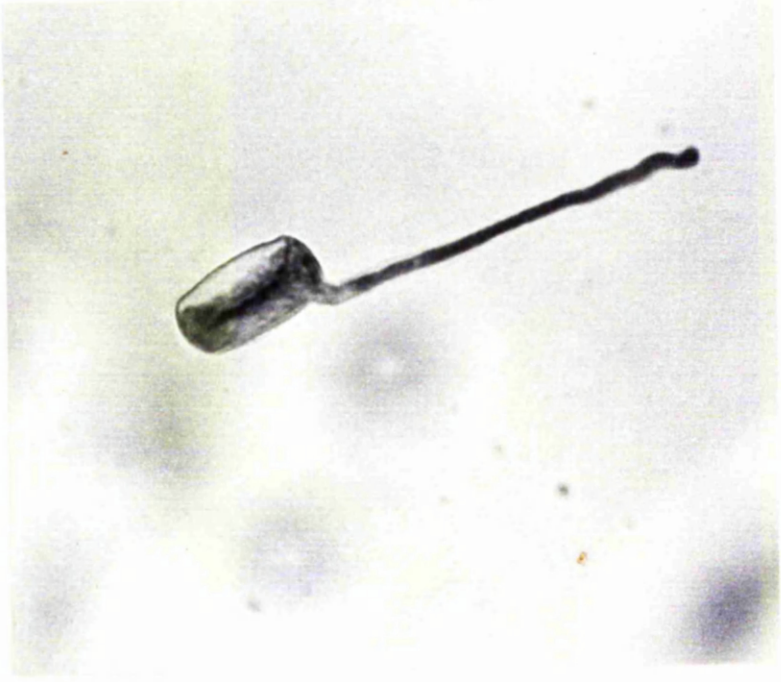
The optimum temperature range for germination was between 18 and 28°C (Fig. 1). That for germ tube elongation was between 12 and 28°C (Fig. 2) and was thus slightly wider than that which promoted germination.

B. The effects of humidity

Germination was obtained at all humidities from 0-100% RH irrespective of whether humidity was controlled by saturated salt solutions or by con-



(A)



(B)

Plate 2. Photomicrographs illustrating normal (A) and abnormally elongated (B) germ tubes of E. cichoracearum conidia grown on plain glass slide. Note the septum (arrowed) and the characteristic club-shaped appressorium in (A); both features are absent in (B).

FIG. 1

Percentage germination of groundsel powdery mildew conidia incubated at different temperatures.

A 0——0 after 24 hours excluding germ tubes without appressoria.

B 0—·—·0 " 48 " " " " " " "

C +——+ " 24 " including " " " " "

D ●——● " 48 " " " " " " "

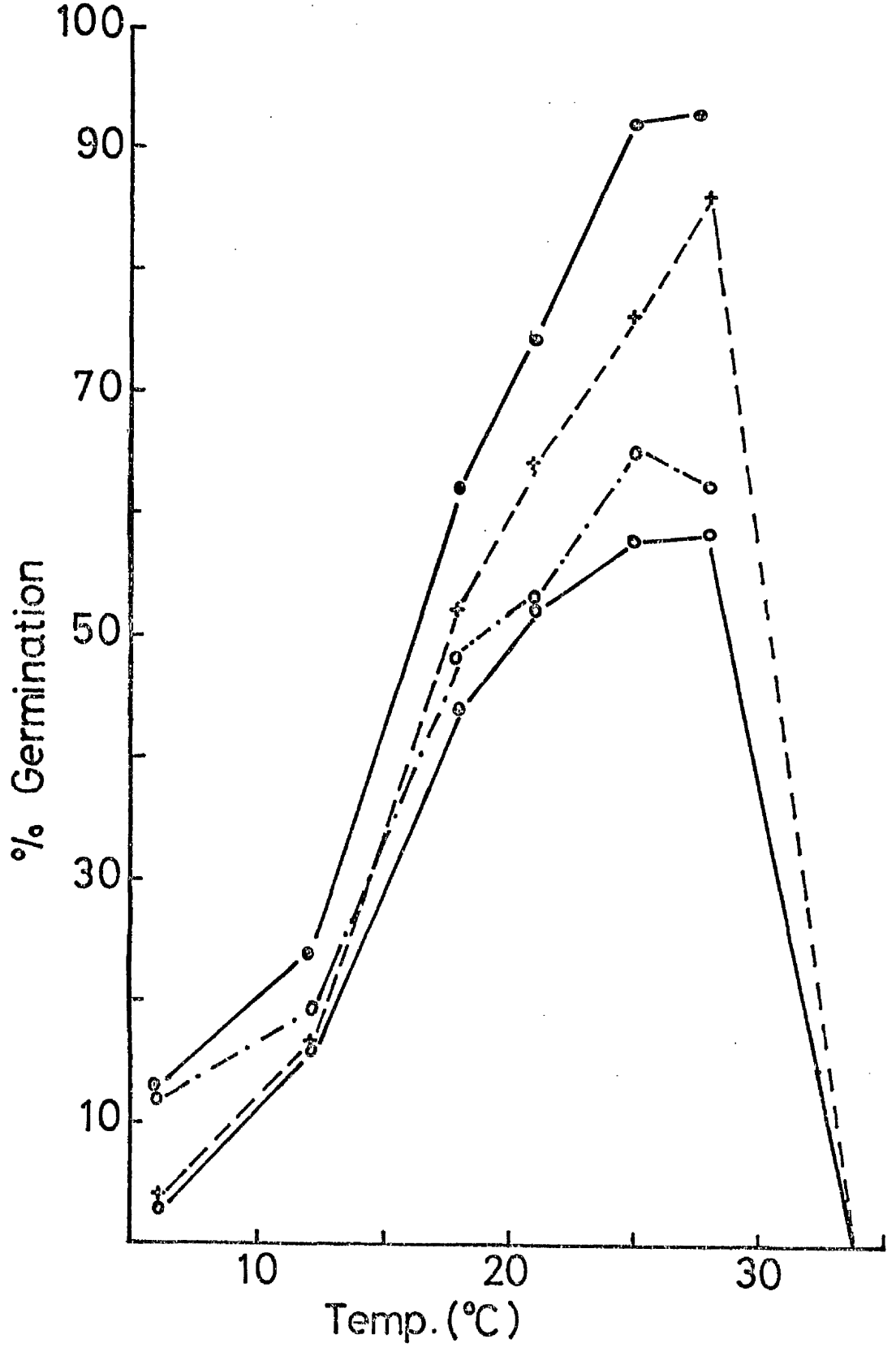

100
90
70
50
30
10
% Germination
10
20
30
Temp. (°C)

FIG. 2

The length of germ tubes of groundsel powdery mildew conidia measured after 24 hours (O——O) and 48 hours (●-·-●) of incubation at different temperatures.

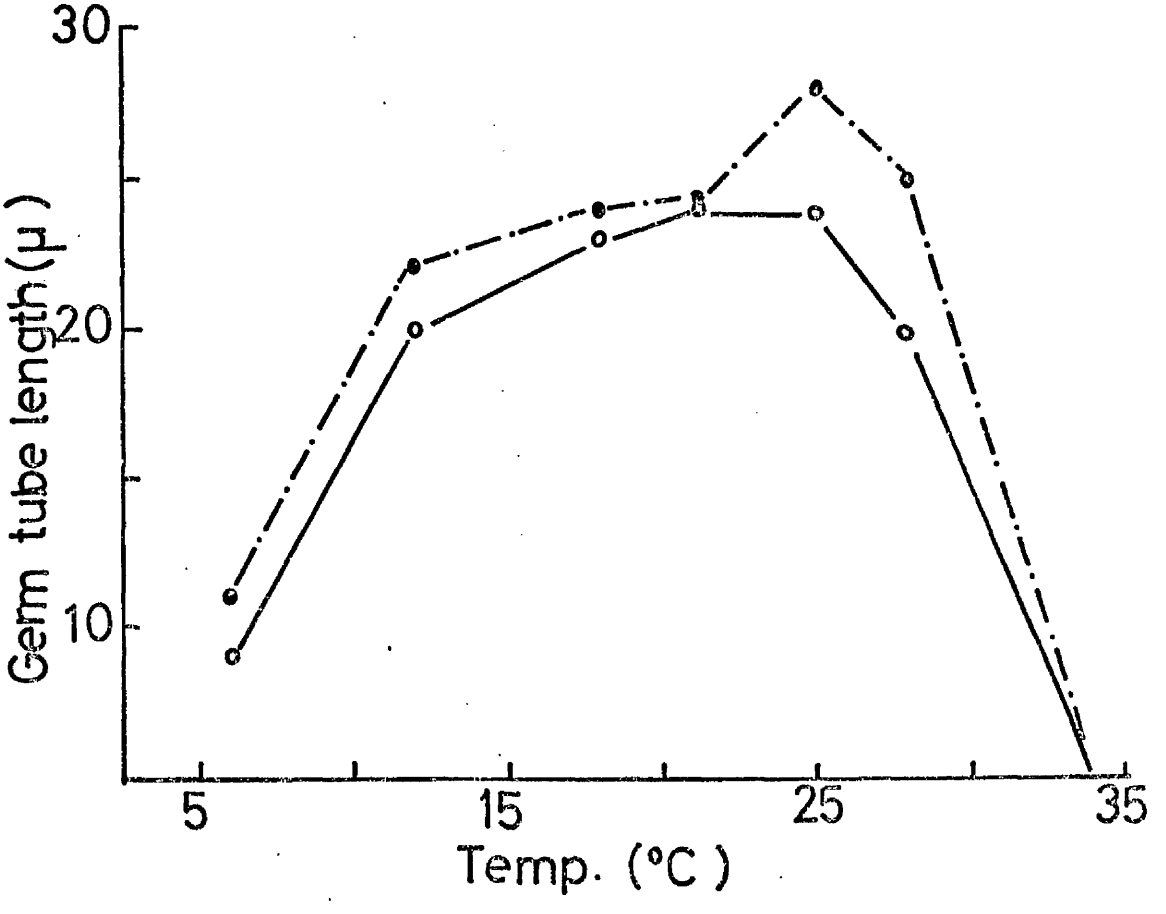


FIG. 3

Proportion of germinated groundsel powdery mildew conidia without appressoria expressed as percentage of total number of germinated conidia, after 24 and 48 hours of incubation at different temperatures.

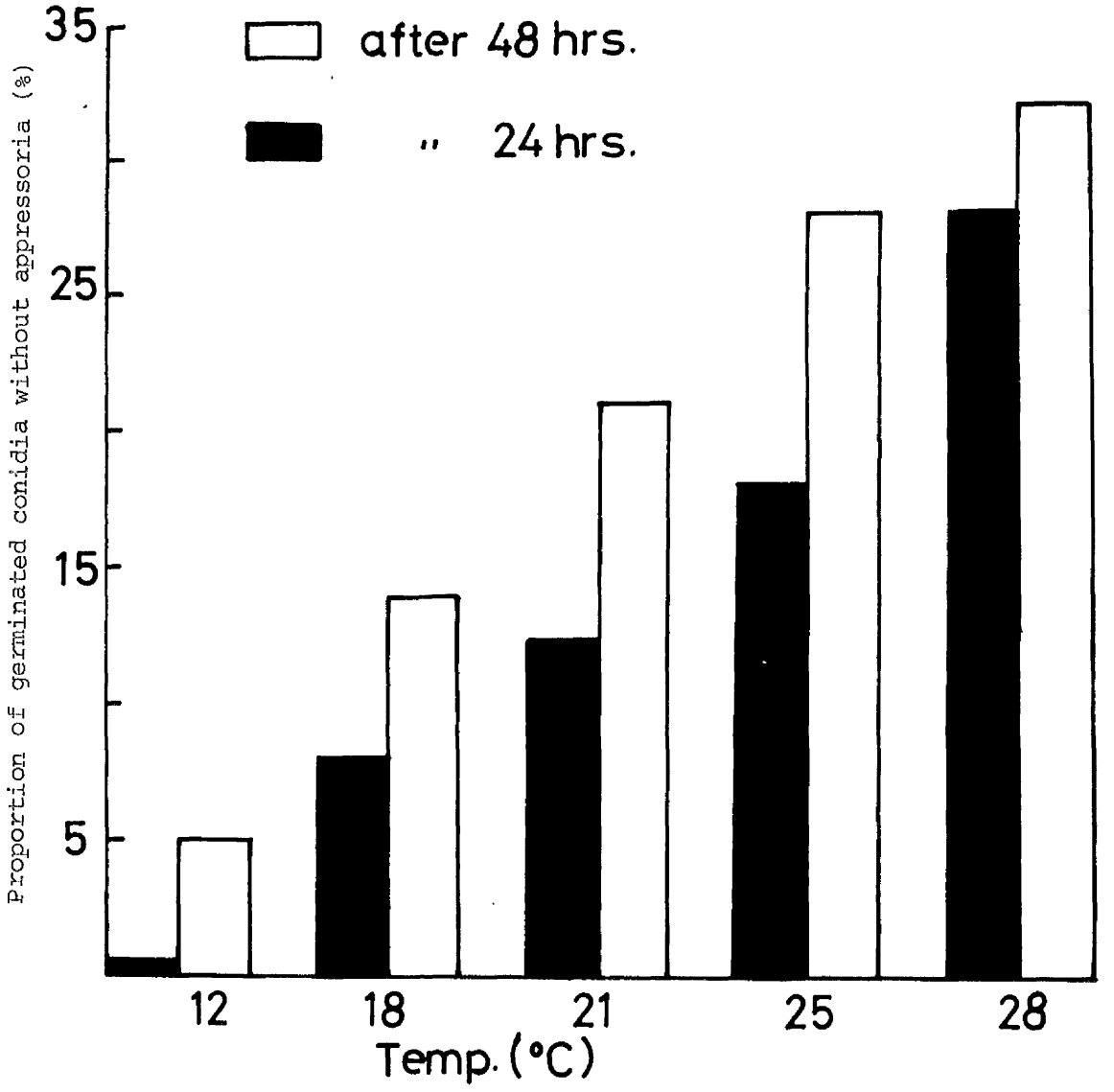

35
25
15
5
after 48 hrs.
" 24 hrs.
Proportion of germinated conidia without appressoria (%)
12
18
21
25
28
Temp. (°C)

FIG. 4

Effect of different relative humidities on the germination of groundsel powdery mildew conidia using saturated salt solutions or concentrations of sulphuric acid to control humidity.

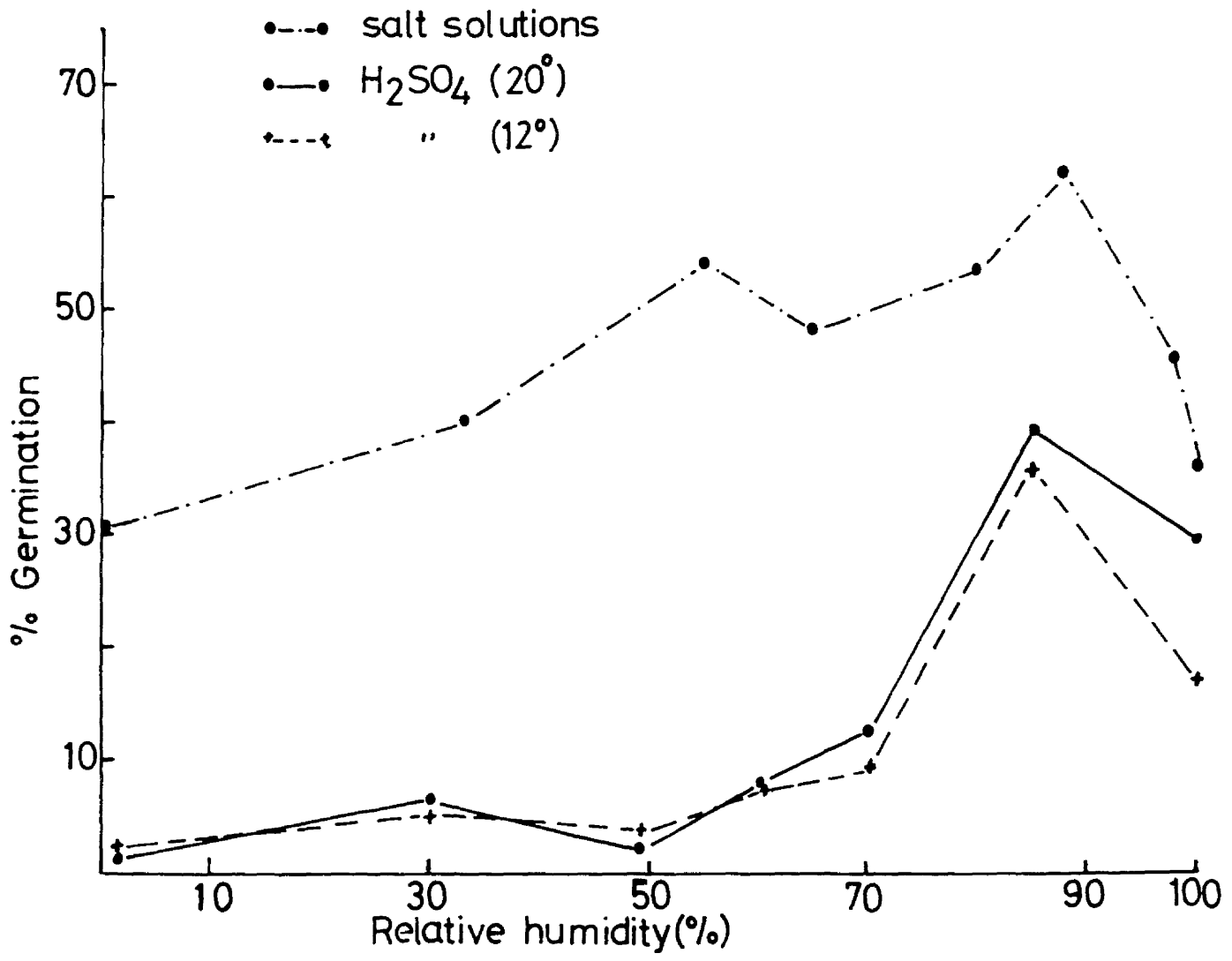
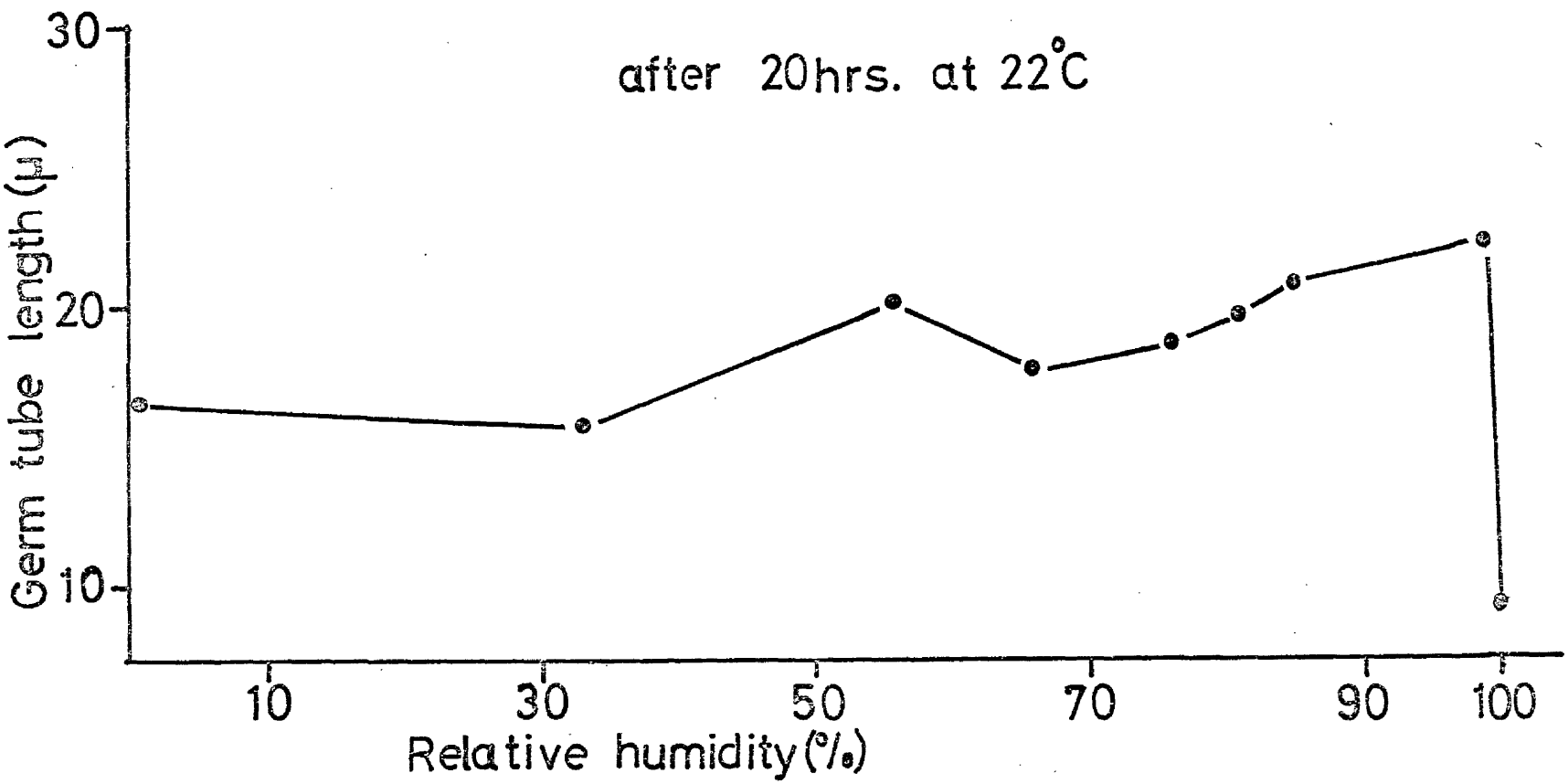

salt solutions
H_2SO_4 (20°)
" (12°)
70
50
30
10
% Germination
10
30
50
70
90
100
Relative humidity(%)

FIG. 5

Length of germ tubes of groundsel powdery mildew conidia after 20 hours of incubation at 22°C and at different relative humidities. (Humidity was controlled by means of saturated salt solutions.)



centration of sulphuric acid. However, a higher germination percentage was obtained with saturated salt solution than with concentration of sulphuric acid (Fig. 4).

Percentage germination and germ tube length increased with RH reaching a peak at about 90% RH (Fig. 4). Best growth as determined by length of germ tube was recorded at 98% RH (Fig. 5).

Two peaks of germination were observed with both saturated salt solutions and sulphuric acid, the one occurring at relatively low humidity (30-50% RH) and the other at relatively high humidity (80-98% RH). The phenomenon was also observed when conidial response was expressed in terms of the length of the germ tube (Figs. 4 and 5).

Discussion

Percentage germination and the length of germ tube, determined after 24 hours incubation appear to be suitable measures of the response E. cichoracearum conidia to the environmental conditions because only a small increase in percentage germination occurred after the first 24 hours.

Of the two solutions, saturated salt solutions and concentrations of sulphuric acid, used for regulating relative humidity, the former gave a higher germination percentage. The reason for this difference is not known but powdery mildews are known to be sensitive to sulphur and it is thus possible that traces of sulphur dioxide may have been present in the sulphuric acid containing chambers and thus resulted in the inhibition of germination.

It is apparent from a comparison of Figs. 1 and 2 and 4 and 5 giving the data on response to different temperatures and humidities that it is temperature rather than humidity that is more important for the development of the fungus. The results show that germination occurred at all humidities from 0-100% RH, but temperatures below 6°C and above 28°C appear

to be non-conducive to the development of the fungus. Thus, in the absence of free water, temperature may be the limiting factor to the development of the fungus.

Its behaviour in relation to relative humidities indicate that groundsel form of mildew may be grouped in category two of Schnathorst's classification. This classification is based on response to moisture stress and category two includes those species with an optimal germination at high humidity but with a small percentage of conidia capable of germinating at low humidity even at 0% RH (Schnathorst, 1960, 1965).

Its relation to temperature indicates that it is a warm weather mildew. Although young seedlings of Senecio vulgaris may be found all the year round, seedlings remain stunted and undergo little vegetative growth during the winter season and most growth occurs during the warmer months. It could be concluded from the results that the groundsel form of E. cichoracearum is the warm weather type with a relatively high optimum temperature range of 18-28°C. Thus, this investigation substantiates the general concept that the optimum temperatures for the growth of powdery mildew fungi tend to approach the optimum for the growth of the hosts (Yarwood 1954 and Schnathorst, 1965).

Since the appressorium is an essential structure in the infection process, in the present investigation, a conidium was regarded as having effectively germinated only when a germ tube terminated with an appressorium had been produced. Some germ tubes were observed to be of abnormal length and these did not bear appressoria (See plate 2). It is clear from Fig. 1 that when germ tubes lacking appressoria were included in the determination of germination percentage, the optimum temperature range permitting germination was narrower, 25-28°C (Fig. 1, lines C and D), than the range 18-28°C found when only conidia bearing appressoria were considered (Fig. 1, lines A and B). These results clearly demonstrate reasons for some of the apparent inconsistencies

between results in the literature when the concept of germination is not always clearly defined.

Corner (1935) observed a similar type of abnormally long germ tube development by E. graminis. These produced erect germ tubes when germinated on water surfaces which according to his description looked like "a forest of telegraph poles". It was this observation which led Corner to advance the theory that powdery mildews require a high oxygen tension and a low carbon dioxide tension (negative chemotropism to CO_2) in their hyphae, hence the mycelia of most genera of powdery mildews are compelled to grow epiphytically on the host's tissue and are usually incapable of entering stomata.

Because the host plants are penetrated directly, the appressorium is an indispensable organ in the process of infection. Thus, in so far as the appraisal of the response of powdery mildew conidia to environmental conditions in relation to infection is concerned, the absolute length of the germ tube is of minor importance compared to whether or not an appressorium is formed.

It is difficult to say whether the "telegraph pole-like" germ tubes described by Corner are similar in morphology to the abnormally long germ tubes observed in the present study since the latter grew dia-thigmotropically on glass slides and the former grew negatively thigmotropically on water surface. The important relationship, however, is that they were both of abnormal form and their occurrence may bear some relationship to exposure to some adverse conditions.

The occurrence of two peaks on the conidial germination and germ tube length curves at different humidities was a consistent phenomenon in this investigation. This phenomenon could be the result of a particular type of metabolism within the organism such that it has both a low and a high optimum humidity. Another possible explanation is that two different populations of mildew fungi occurred, the one having

a low optimum humidity and the other having a high optimum humidity. Numerous observations on isolates of the fungus from groundsel did not reveal characters which are only specific to any other genus such as Sphaerotheca or indeed any species other than E. cichoracearum. However, it is possible that different isolates of E. cichoracearum exist having different optima. Further work is required to determine which, if either of the possibilities is correct.

CHAPTER 3

SECTION 1 : DETERMINING MILDEW INTENSITY.

Introduction

An accurate method for quantifying the levels of fungal infection at any stage of a host's growth is an essential prerequisite for a study of the effects of that fungus on the growth and development of its host. Several other motivating reasons for measuring amount of disease and the relation of this to crop loss have been given by Chester (1950), Large (1966) and Tarr (1972). This chapter describes a method which has been developed for measuring the intensity of mildew infection on groundsel.

Effort was made as far back as the late 19th century to quantitatively appraise diseases on different crops in order to determine their effects on yield. Unfortunately, the science of disease measurement did not make much progress until recently although contributions from a few individuals have been of significant importance.

In Britain interest in disease assessment was first stimulated by the Plant Pathology Committee of the British Mycological Society at a Symposium it organised in 1933 on the measurement of plant diseases. Some years later the committee set up a sub-committee to develop assessment methods for recording disease prevalence and intensity in relation to locality, season, soil and other relevant factors. By 1941 tentative quantitative methods had been proposed for assessing several diseases with special attention to six that were important during war time including loose smut of wheat, blight and virus diseases of potato, virus yellows and downy mildews of sugar beet and brown rot of apple (Moore, 1969; Moore, 1943). The assessment key for potato late blight (Phytophthora infestans) was further tested by a number of observers; its

practical usefulness was satisfactorily established and the key was finally presented in its revised form (Anon, 1948). This potato blight key has passed into general use in many parts of the world.

Large (1966) and Moore (1969) have reviewed progress made in disease assessment and many more contributors have added to the body of the literature discussing the subject, e.g. Tarr (1972), F.J. Moore (1969), Preece (1971) and Chiarappa (1971), etc. But by far the most valuable contribution on this subject was a treatise by Starr Chester (1950). This was a comprehensive review of progress made in the subject of plant disease/loss appraisal including most of the world literature and a classical detailed treatment of the various aspects of plant disease measurement. The principles and techniques for determining plant disease intensity, methods of relating this to yield loss and analysis and summation of disease intensity-loss relationship data were extensively discussed.

The generally accepted requirements of any assessment methods are that they should enable reasonably accurate assessments of disease incidence to be recorded numerically; they should be objective and be so defined as to produce comparable results from one worker to another and, whenever possible, should provide a basis on which estimates of crop loss can be made. Each disease/host complex presents a different problem in assessment. Large (1966) therefore suggested a "strategy of investigation" which involves a detailed knowledge of the progress of the disease on the one hand and the growth of the healthy plant on the other hand. This would then make it possible to work out a suitable assessment method for a particular disease/host complex. The problem of assessment is relatively easy when the diseased plant or plant parts are total losses, e.g. damping off or fruit rots. Counts of diseased plants or affected parts and conversion of the counts into percentages can give accurate measurements of disease intensity. But problems of assessment become more complex when localised parts of the plant or crop only are

affected to varying degrees, e.g. foliage diseases, rust, mildew, potato blight. For such disease assessments methods involving the identification of grades of infection are required. Two methods are widely used to estimate such diseases, descriptive scales and standard diagrams.

1. Descriptive scales

These summarise disease intensities delimited by a grading system. Descriptive scales have been used to differentiate between disease grades and if these disease intensity grades are properly defined the scales should represent a reliable method of appraisal. Scales based on subjective estimates and arbitrary gradings such as "light", "moderate", and "severe" are meaningless and they in no way conform with the requirements for any approved assessment methods. A very good example of a descriptive scale is that for the assessment of potato blight developed by the British Mycological Society (Anon, 1948). Large (1954) developed a similar field key for cereal mildew but his key was most relevant for plants at growth stages between heading and before ripening, the period which accounts for most of the yield of the crop. The key describes percent levels of infection usually found on the top four leaves, in respect of total leaf area of each of the four leaves. A diagrammatic key illustrating different levels of infection was also included. Large and Doling (1963) used this key in a series of trials to determine the relationship between percentage infection and yield loss.

II. Standard diagrams are series of drawings showing disease symptoms of different intensities which can be used to estimate disease intensity on plants by comparing the area occupied by pustules on the plant with what is represented on the standard diagrams. The first example of a standard diagram for a plant disease was that of Nathan Cobb published in 1892 for assessment of leaf rust of wheat in Australia. It showed 5 grades of rust intensity ranging from 1% to 50% leaf coverage by pustules. The original scale of Cobb has been modified in various ways in different places notably

the USA and Russia. It has been emphasised that the following points be taken into consideration whilst drawing up standard diagrams (Large, 1966; Moore, 1969; Chester, 1950; Tarr, 1972):-

(a) The grades between 1% (or the minimum rating) and 100% (or the maximum rating) illustrated on the standard diagram should not be too numerous and they should be clearly distinguishable by eye.

(b) Logarithmic scales are preferable to arithmetical scales for the reasons that pathogens and pests tend to multiply geometrically as time progresses arithmetically and such behaviour is best recorded by using a log. scale. Moreover, the human eye is so adapted that it perceives differences of equal spread on a log. scale with more or less equal ability but not differences of equal spread on an arithmetical scale.

(c) The unaided eye tends to overestimate disease intensity around the 50% level. Below 50% the eye "sees" the diseased tissue but above 50% it "sees" the healthy tissue. Smith et al. (1969a) provided evidence in support of this when they found that eye estimates of tomato leaf mould overestimated disease intensity by about 12% when the leaves were 50% infected. But below and above 50% disease intensity, the eye overestimation decreased to 1% at 0% disease intensity and 3% at 100% disease intensity.

Diagrammatic scale for assessing Groundsel mildew

An attempt has been made to prepare a diagrammatic scale for assessing powdery mildew intensity on Groundsel, on the basis of the principles discussed above. Since this fungus grows epiphytically on the surface of the host, it is possible to measure disease intensity in terms of the proportion of the total surface area of the host which is covered by

mildew colonies.

The leaf area was determined by means of a planimeter (see Chapter 5) and the mildew pustules were set out in a pattern resembling as far as possible those occurring naturally. The areas occupied by the colonies were also determined and presented as percentage mildew cover. For the purposes of the present work, a diagrammatic scale seems more appropriate than a descriptive scale because it is easier to use in practice and makes for rapid assessment. The scale (Figure 6) provides for six disease gradings and more than this would overload the scale and include grades that are not readily distinguishable by eye. Pustules on the stem have been purposely left out in the assessments since stem area constitutes a negligible portion of the total photosynthetic area of the plant particularly in the rosette stage of growth.

Surface area of a single plant occupied by mildew colonies (Z) can be calculated by using the following formula:-

$$Z = \frac{X_1 + X_2 + X_3 \text{ ---- } X_n}{Y} ,$$

where X is the intensity on leaf 1, 2 ---- n respectively as estimated by use of the diagrammatic scale and Y is the number of leaves assessed. The average intensity of infection in a population can be obtained by dividing "Z" by the number of plants assessed in the population. In the growth analyses to be described later, all fully expanded leaves which were included in leaf area measurements were also included for disease assessment.

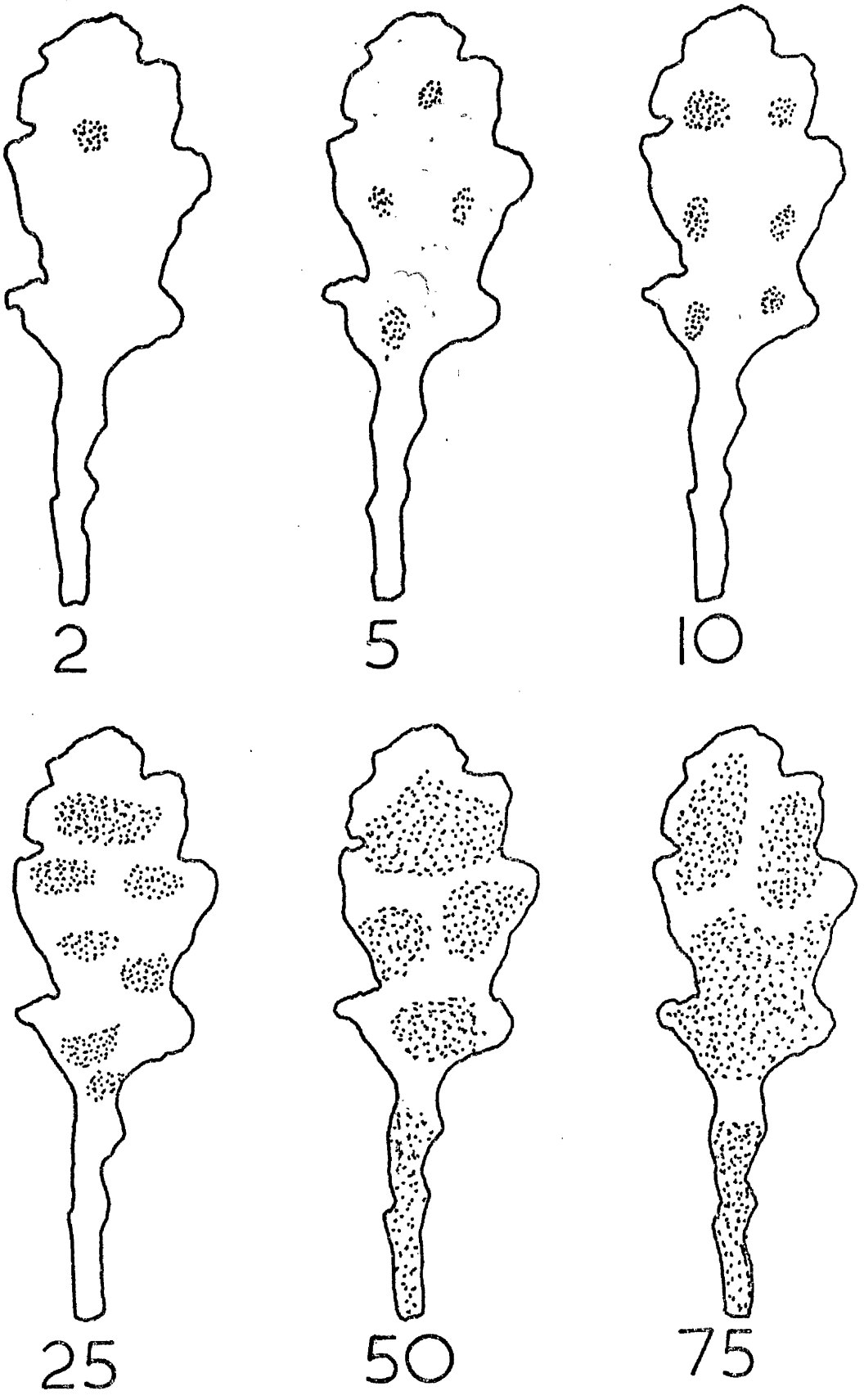


FIG 6. Diagramatic scale for appraising mildew intensity on groundsel leaves

SECTION II : COMPARATIVE OBSERVATIONS ON DISEASE DEVELOPMENT IN THE FIELD AND IN THE ENVIRONMENTAL GROWTH ROOM

All the critical investigations on the effects of powdery mildew on the growth of groundsel were carried out in the controlled environment. A survey of disease development in the field was carried out in order to compare disease development under natural conditions with that which obtains in a controlled environment.

Methods

A population of 15 plants were grown in 4" pots in the environmental growth room. The temperature was approximately 19°C during the 14 hour photoperiod. During the dark period the temperature dropped to about 17°C. Trays containing water were placed amongst the plants to increase the relative humidity which was maintained at approximately 75%. Heavily infected plants were introduced to provide a high load of inoculum in the growth room. In general, plants were allowed to become infected from the airborne inoculum distributed by air currents produced by the fan system. However, some plants were inoculated by manual transference of inoculum from the infected plants using a camel hair brush so as to be able to observe how long it takes for macroscopic colonies of mildew to develop, visible to the unaided eye.

The progress of disease intensity was observed throughout the growth period from germination up to death of the plant.

Results

The artificially inoculated plants in all cases developed mildew colonies visible to the naked eye within six days of inoculation.

Seedlings at the cotyledonary stage also developed infections and surprisingly some of these seedlings were able to grow to maturity still heavily infected.

At the time when the seedlings were transplanted, 28 days after germination, no mildew was observed on the plants which had been left to get infected by airborne inoculum.

Observations on plants which were left to get infected naturally in the growth room are summarised in Table 3 and Figure 7. The first signs of mildew colonies appeared 42 days after germination when 9 out of 15 plants were found to have mildew on the cotyledons and on the first pair of leaves. Generally, mildew colonies first appeared on the lower leaves but later developed on the upper ones as well. At the first appearance of mildew, on average only 4 leaves per plant had fully expanded. In the next 29 days, all 15 plants became infected and the mean mildew intensity had progressed to about 5%. After another 10 days mildew intensity reached 25% and the mean number of leaves was 24. At 96 days of age almost the entire aerial parts of the plants were covered by mildew colonies including the inflorescences and stems and about 19 days later 20% of the plants had died.

This kind of severe mildew development was observed only on plants grown in the environmental room where the temperature and humidity conditions which earlier experiments had shown to be ideal for the full development of the fungus.

During a survey of mildew occurrence in the field it was observed that mildew intensity was hardly as high as 75%. It was sometimes difficult to distinguish infected plants from uninfected ones especially after a few days of rainfall which washed off much of the superficial mycelium. But symptoms quickly redeveloped when suitable rainless weather ensued. Thus, symptom expression shows a marked relationship with weather conditions, a wet period being unfavourable to mildew growth.

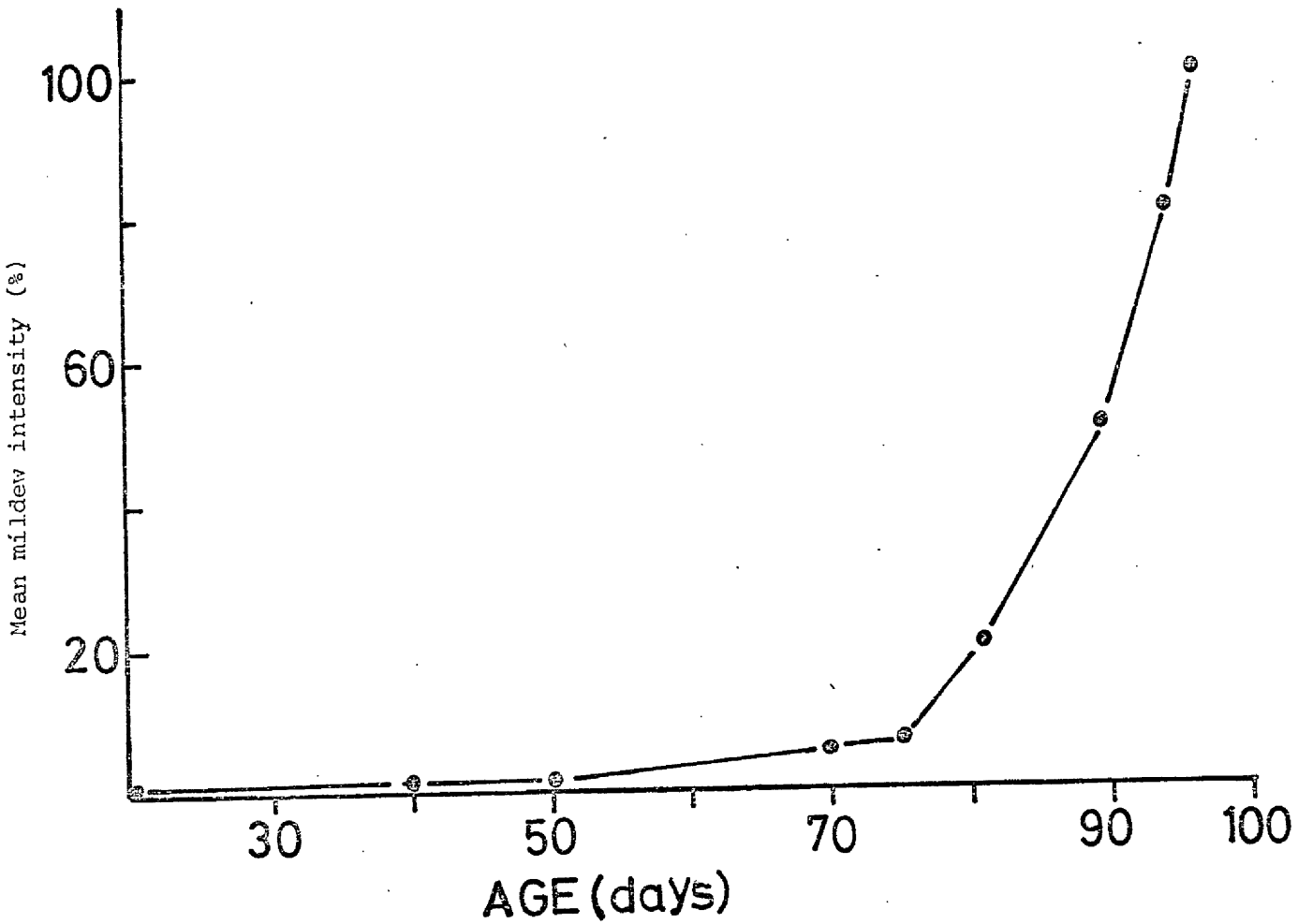
Table 3. Showing mildew development during the life of groundsel.

	Age (days)								
	42	50	71	75	81	89	94	96	115
No. of plants showing symptoms	9/15	9/15	All						
Mean disease intensity	<1%	< 1	5	5	25	50	75	>75	20% of plants dead.
No. of fully expanded leaves/ plant	4	5	10	18	24	No further counts made			

In the field, young plants were not usually found to be diseased and most infected plants were at or near their flowering stage of growth. It was possible to deduce from the number of leaves and the presence of flower initials on the infected plants that infection occurs late in the life of the plant, at maturity, when the reproductive phase of growth has commenced. It would appear therefore that young plants possess some characteristics that confer juvenile resistance.

FIG. 7

Mildew development during the life of groundsel plant.



CHAPTER 4

THE PRODUCTION OF MILDEW FREE PLANTS

Introduction

An experimental evaluation of the effects of powdery mildew on the growth of groundsel requires the production of mildew-free plants for comparison with the infected plants. It was originally intended to grow the test plants in an enclosed system ventilated with filtered air and thus ensuring a mildew-free environment.

The enclosed system was designed with a partition so that infected plants could grow in one compartment and uninfected plants in the other compartment. Both compartments were otherwise exposed to the same environmental conditions. However, no adequate ventilation system was available and as would be expected, a temperature build-up occurred in the compartments due to the enormous amount of heat emitted by the tungsten mercury lamps which were used as a source of supplementary light. The use of this system was therefore abandoned.

The use of small propagation units which were ventilated with filtered air was investigated but they were found to be of no practical use because the height of the plants produced exceeded the height of the chambers. Moreover they could contain only a limited number of plants at a time. In the absence of such a system, the more conventional method of using a fungicide to keep control plants free from infection was resorted to.

Comparing growth and yield of diseased plants with those kept free from disease by means of fungicides is one of the most widely used methods for evaluating crop losses due to disease. It is according to Tarr (1972), generally accepted as a reasonably accurate method. However, the use of fungicides can be questionable if the chemical is capable of influencing the growth of the plant in ways other than through its direct effect on the

parasite.

Basically, the effects of fungicides on plant growth can either be harmful or beneficial. One harmful effect is that of phytotoxicity. Fungicides may also radically alter the composition of the soil and leaf surface microflora (Tarr, 1972; Martin, 1950; Kreutzer, 1960 and Munnecke, 1972) and thus indirectly affect the host's plant growth. Thus, the elimination of one soil-borne pathogen may encourage the occurrence of either another more virulent pathogen or perhaps other beneficial micro-organisms in its place and such alterations in the composition of the soil microflora may prove to be harmful or beneficial to the plant. For example Garrett (1970) stated that death and autolysis of some of the microbial population as well as most of the soil fauna caused by partial sterilisation of soil may result in a release of nitrogen, phosphorus and other nutrients from their cells. This could amount to a substantial contribution to the nutrient status of the soil and thus may enhance plant growth. On the other hand, soil treatment with pentachloronitrobenzene for the control of Rhizoctonia accentuated the severity of the attack of sugar beet seedlings by Pythium ultimum and P. aphanidermatum (Kreutzer, 1960). Thus care is required in the interpretation of results.

Of the fungicides available, Benomyl (Benlate du Pont) was the first one selected for testing. It is generally non-phytotoxic and is reported to give a good control of mildew (Tarr, 1972; Crowdy, 1971; Edgington et al., 1972 and Brooks, 1970).

The use of benomyl to produce mildew free plants

Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate) (Fig. 8) is a systemic fungicide based on benzimidazole with a broad spectrum of activity. The literature on systemic fungicides has been reviewed by Wain and Carter (1967) and Erwin (1973).

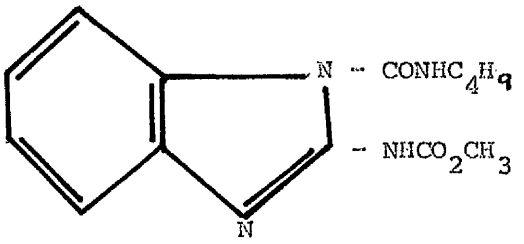


Fig. 8 Benomyl (Benlate du Pont)

The term systemic fungicide implies a material which is translocated in the plant and which is directly active against the pathogen (Dimond and Horsfall, 1959; Howard and Horsfall, 1959; Wain and Carter, 1972). A number of workers (Peterson and Edgington, 1970 and 1972; Ben Arie, 1975; Siegel and Zabbia, 1972 and Crowdy, 1971), have investigated the movement of Benomyl within the plant and their results appear to be consistent. It was found that Benomyl is readily absorbed by intact roots and is translocated to other organs of the plant through the xylem (apoplastic movement). It moves acropetally within the leaves resulting in the accumulation of the material at the margins or tips. Its transport pattern is said to be controlled mainly by physical factors and its accumulation in the different plant organs is governed by the rate of transpiration of that organ. Thus fruits and seeds of dwarf pea retained little or none of the material even when the roots were continuously in the presence of the fungicide (Siegel and Zabbia, 1972). In post-harvest treated pears, greatest residue concentration was retained in the fruit peel with a declining gradient toward the core (Ben-Arie, 1975).

As systemic compounds enter the plant they encounter a variety of active biochemical systems which could alter their chemical composition. In solution, Benomyl breaks down and forms an active metabolite which has been identified as a methyl ester of 2-benzimidazolecarbamic acid and the systemic fungicidal activity of Benomyl has been attributed to

this compound (Clemons and Sisler, 1969).

Wensley and Huang (1970) showed that Benomyl retarded the growth of muskmelon temporarily when used at rates that controlled wilt. Wensley (1972) reported that when applied as a soil drench, it retarded growth during the first four days after treatment but then the rate of growth frequently increased. He reported that phytotoxicity was expressed as chlorosis and tissue breakdown in leaves, and the development of characteristic growth patterns. Similarly, Reyes (1975) reported that Benomyl produced phytotoxic effects on cultivars of crucifers, the extent depending on the age of the seedlings, the concentration of the chemical and the presence or absence of a surfactant. Thanassoupoulos et al. (1970), however, found that it did not produce any phytotoxic effects on tomato and watermelon when applied as a soil drench at rates of up to 10,000 ppm of active ingredient.

Thus, benomyl may affect the growth of plants adversely by causing physiological disorders through phytotoxic effect, or by causing structural abnormalities. It may also affect the growth of plants favourably by enhancing some metabolic processes and thereby stimulate growth. Little is known as yet about these aspects of Benomyl and, as it was rightly pointed out by Tarr (1972), the occurrence of such effects ought to be investigated in experiments involving the comparison of fungicide treated and non-treated plants.

Thus, as an essential preliminary to the studies on the effects of powdery mildew on the growth and development of groundsel, an investigation was carried out of the effects of Benomyl on some primary values of growth of the host plant.

Effect of Benomyl on the pathogen

0.05% benomyl solution was applied to a group of ten 3-week-old plants and another group was left untreated. Both sets of plants were

placed adjacent to each other in a growth chamber infested with powdery mildew inoculum. The ability of benomyl to control mildew and the longevity of its fungicidal action was assessed.

Effect of benomyl on the growth of Groundsel

Materials and Methods

The experiments were carried out in an environmental growth room maintained at a temperature of about 20°C and a relative humidity of about 40% with a 14 hours photoperiod. These conditions were within the range conducive for mildew development as indicated by results of the experiments described in Chapter 2, Section II. A dinocarp smoke bomb was used to disinfect the growth room prior to the commencement of the experiments.

In a preliminary experiment, groundsel seedlings were raised in a seed tray, in Levington compost. 48 seedlings of similar vigour were selected 4 weeks after germination and transplanted at a spacing of 5 x 5 cm in two seed trays. After a further two weeks of growth the seedlings in one tray were treated with 0.05% aqueous benomyl solution applied as a soil drench. The control seedlings in the other tray were merely watered. Seedlings in both trays were watered thereafter only when found necessary.

One and two weeks respectively after treatment, 8 plants from each group were selected at random and harvested by carefully removing the whole plant complete with roots from the soil medium. At both harvests the root systems of the individual plants were not too extensive and they could be uprooted with ease without interfering with the remaining plants. After the second harvest, the remaining plants were watered with 2% aqueous benomyl solution.

The root systems were washed clean of adhering soil particles and the seedlings were separated into root and shoot by cutting off at the soil level. The plants were blotted dry between tissue paper and after

determining their fresh weights, they were packed in aluminium foil containers and dried to constant weight in an oven at 80°C. Their dry weights were then determined.

The experiment was repeated under slightly different conditions. Seedlings were raised as before in a seed tray and 3 weeks after germination, they were transplanted into 4 inch pots. The seedlings were allowed to grow for five additional weeks before those of uniform vigour were selected and separated into two groups of 10 plants each. One group was treated with 0.05% aqueous Benomyl solution and the other group of plants served as untreated controls. Four plants from each group were harvested two and four weeks respectively after treatment. Thus the plants were 10 and 12 weeks old at harvest. Fresh and dry weights of leaves, stem and root were determined. Leaf area was also determined as described in the next chapter.

Results

The test of the effectiveness of benomyl against mildew showed that a single application of 0.05% solution controlled mildew throughout the life of the plants, a period of up to 10 weeks, whilst the non-benomyl treated plants developed the disease, abundantly. The applied dosage closely approximates to the officially recommended rate.

The results of the experiments on effects on growth are recorded in Appendix Tables 2A and 2B, and are summarised in the text tables 4 and 5. Statistical analysis of both fresh and dry weight and leaf area data showed no significant differences between benomyl treated and non-treated plants. Leaf senescence, chlorosis or other symptoms of phytotoxicity were not observed. Thus, benomyl applied at a concentration of 0.05% had no effect on the growth of groundsel at any stage of growth.

However, when 2% solution was applied to nine week old plants, symptoms of phytotoxicity expressed as chlorosis were observed within

seven days of application. It may thus be concluded that benomyl applied at high concentrations can produce harmful phytotoxic effects on groundsel but not at the low concentration of 0.05%. These results therefore warrant the use of benomyl as a fungicide to maintain mildew-free plants in experiments designed to compare the growth and development of infected and non-infected groundsel plants.

Table 4 Effect of Benomyl on the development of Senecio vulgaris

	7 weeks		8 weeks	
	Benomyl treated	Control	Benomyl treated	Control
Root fresh wt. (g)	0.021	0.019	0.044	0.048
Shoot " " "	0.0207	0.0195	0.049	0.0545
Root dry wt. (g)	0.016	0.015	0.025	0.022
Shoot " " "	0.0115	0.0122	0.0341	0.0316
No. of fully expanded leaves	5	5	7	7

Figures are means of 8 plants

Table 5 Effect of Benomyl on some primary values of growth of *Senecio vulgaris* at 10 and 12 weeks of age.

	Age 10 weeks		Age 12 weeks	
	Benlate treated	Control	Benlate treated	Control
Stem fresh weight (g)	1.0969	1.2203	2.0942	2.1909
Root " " "	1.8381	1.7939	3.5822	3.5532
Leaf " " "	5.5378	5.2414	10.4056	10.3907
Stem dry weight (g)	0.0665	0.0708	0.1483	0.1410
Root " " "	0.0856	0.0876	0.1836	0.1704
Leaf " " "	0.3425	0.3219	0.6826	0.6483
Leaf Area (sq. dm)	1.5770	1.5210	2.6571	2.719

Figures are means of 4 plants. None of the differences between treated and control were significant at $p = 0.05$.

CHAPTER 5

HOST-PARASITE RELATIONSHIPS

The host: Groundsel (Senecio vulgaris L.)

Groundsel is a ubiquitous, herbaceous annual weed which occurs as a first colonist of loose, newly tilled soil. It does not appear able to compete with other plants and as a result becomes less frequent when other plants become established within the stand.

It is usually self-fertilised, producing numerous small seeds which are easily disseminated by wind. It occurs in various forms; plants may be profusely branched or may have a slender single unbranched or little branched axis. They are usually green but some types with red stems and midribs have been recorded. These forms may be controlled genetically but the fertility or other environmental factors of the site of occurrence may exert some influence.

Harper and Ogden (1970) observed that there can be considerable variation in the pattern and timing of development of the various organs. They recognised three fairly distinct phases of growth; the first phase characterised by a high growth rate and a development of a leafy rosette; the second by a decline in growth rate, the occurrence of stem elongation and branching, bud development and flower opening and the third phase corresponding to a period of seed maturation and shedding with a steep decline in leaf area due to death of leaves.

The aim of this study was to investigate the effects of mildew infection on the growth of the host through all phases of growth from seedling to maturity. For this purpose, methods of growth analysis were used (Evans, 1972) and so this section begins with a discussion of growth analysis with particular emphasis on those aspects most applicable to this study.

Growth analysis

Growth in plants may be defined in various ways depending on the nature of study involved. Wilkins (1969) defined plant growth as the irreversible increase in plant volume and Sestak et al. (1971) alternatively defined it as the increase in dry weight of the plant. In the present work, the later definition is more appropriate since measurements of dry weight changes are an important feature of the analyses used.

Growth analysis makes it possible to follow the formation and accumulation of biomass resulting from the interactions between external environmental factors and the internal factors of the plant. Fresh and dry weight of the various organs, the size of the assimilatory apparatus (green area), protein and chlorophyll content are some of the primary attributes which are often employed in the analysis of growth. From these primary values, various indexes and growth characteristics can be calculated. These describe the growth of the plant and its various organs as well as the relationship between the assimilatory apparatus and dry matter production and also the pattern of dry matter distribution in the plant.

Investigations involving growth analysis date back to 1906 when Nolll put forward the concept of "substanz quotient" which he obtained by determining the amount of dry matter of a plant at regular intervals and relating each weight thus obtained to the previous one by dividing the former by the later. Obviously this quotient is a function of both the rate at which the plant is growing and of the length of time which has elapsed betwen the two determinations of dry matter. Also "substanz quotients" obtained from a growing plant over different periods of time do not bear very simple relationships to each other. Thus, the usage of this concept was discontinued.

Blackman (1919) developed another concept on the basis of the well established view that the increase in dry weight in plants follows the

compound interest law. Thus,

$$W_2 = W_1 e^{R(T_2-T_1)}$$

where W_2 and W_1 are dry weights at times T_2 and T_1 respectively and R is a constant. Blackman termed R the Efficiency Index and considered it a measure of dry matter production.

Briggs, Kidd and West (1920a,b) found that collected data did not conform with the view that R, the Efficiency Index, is a constant. They thus abandoned the term Efficiency Index and were the first to use the term Relative Growth Rate as an integrated measure of all the processes bringing about an increase in dry weight in the plant. They defined Relative Growth Rate during any given week in the life cycle of a plant as the amount of dry matter which 100 gram of dry matter taken at the beginning of the week adds during that week. The relative growth rate is not constant for more than very short periods because of the ontogenetic changes which take place during the growth period of the plant. The problem then facing them was how to distinguish between the effects of ontogenetic drifts on the relative growth rate from the effects of environmental factors such as weather.

It is worthwhile, at this stage, to consider what ontogenetic drift means and examine how it could affect plant form. Ontogenetic drift in a plant may simply be described as developmental changes of the various parts which ultimately determine the final form of the plant. As a plant grows, the structures and functions of the various organs are continually changing. Different growth attributes in a plant show different growth progressions. For example, there are some which may increase throughout the life of the plant, e.g. dry weight, some may rise to a maximum and remain constant e.g. stem height and others reach a maximum and then decline more or less as the plant ages e.g. leaf area (Evans, 1972). Furthermore,

different organs may have different lengths of life, e.g. stems may be longlived and leaves may be relatively shortlived. Thus the pattern of ontogenetic changes could easily vary from organ to organ in more complicated ways that the simple progressions mentioned above. These are examples of ontogenetic changes in morphology during the vegetative life of the plant but marked physiological ontogenetic changes may also occur in all aspects of the overall metabolism of the plant, e.g. in photosynthesis, respiration, nutrient absorption, etc.

In order to be able to distinguish between the effects of the environment on plant growth from the effects due to inherent ontogenetic changes, Briggs et al. (1920a, b) separated the overall growth index, RGR, into two constituent parts, leaf area ratio (LAR) and unit leaf rate (ULR) by expressing the rate of dry weight increase on the basis of photosynthetic area; leaf area representing the size of the photosynthetic surface. Thus,

$$RGR = LAR \times ULR$$

The definitions of these growth indexes are as follows:-

RGR over a time interval is the increase in dry matter per unit of dry matter present initially ($g\ g^{-1}\ week^{-1}$).

ULR is the increase in dry weight per unit leaf area per unit time ($g\ dm^{-2}\ week^{-1}$).

LAR is the ratio between leaf area and total plant dry weight ($\frac{La}{W}$).

It can be seen from the above definititions that LAR is a morphological index. From analysis at an early stage of growth of maize, Briggs et al. (1920a,b) found that LAR showed an ontogenetic drift broadly parallel to that of RGR with ULR showing a less marked drift. In consequence they found it much easier to analyse the effects of environmental factors on the basis of ULR than on RGR and the two are related through LAR as shown in the above equation. The size of the assimilatory apparatus (leaf area)

determines the drift in ULR. For example a slow development of young leaves on a seedling will result in a very low value of ULR but when the leaves expand and new ones are formed, ULR is likely to increase.

In his works published from 1926 onwards Gregory preferred to use the term Net Assimilation Rate (NAR) as synonymous with ULR. As a result of the enormous contributions he and his associates made in the study of a wide range of Agricultural and horticultural problems and due to the great body of published work which emerged from their studies, the term NAR became widely used in the literature. Recently, however, Coombe (1960) and Evans (1972) elucidated the implications in the use of both NAR and ULR in terms of their comprehensive definitions and established that ULR is a more appropriate measure of plant metabolic production.

Leaf area ratio is a complex characteristic describing the relative size of the assimilatory apparatus. Its value at particular moments in time is determined by several internal correlation mechanisms such as those which control the proportion of new assimilates that are translocated to the sites of development of new leaves and the area which these new leaves assume in relation to the dry matter which they contain. Hence, LAR shows a marked ontogenetic drift.

To further simplify analysis of LAR, this growth characteristic has been split into simpler ratios namely, specific leaf area (SLA) and leaf weight ratio (LWR). Thus,

$$LAR = SLA \times LWR$$

SLA is the ratio between leaf area and leaf dry weight $(\frac{La}{Lw})$. It usually reflects leaf thickness and the relative proportions of assimilatory and conductive or mechanical tissues in the leaves. It is said to have a marked ontogenetic drift (Evans, 1972).

LWR is the ratio between leaf dry weight and total plant dry weight $(\frac{Lw}{W})$. In analytical terms, LWR represents the

average fraction of the plant's total stock of organic material divided between the photosynthetic systems and the rest of the plant. It is little affected by changes in the environment. Thus, it could be used to disentangle the interrelations of plant responses to the environment from those imposed by ontogenetic changes. Unlike ULR, these ratios can be determined at a specific time, that of harvest.

Computation of unit leaf rate (E)

In the literature much discussion has been devoted to ways of calculating ULR (Williams, 1946; Coombe, 1960; Evans, 1972; Whitehead and Myerscough, 1962; and Radford, 1967). Several equations have been formulated and their application depends on the relationship between plant dry weight, leaf area and the time course as discussed below. Evans (1972) and Sestak et al. (1971) have reviewed the derivation and application of the different formulae and here mention only need to be made of the formulae being adapted and the circumstances under which each could be applied.

Assuming that leaf area increased linearly with time and dry weight has a quadratic relationship with leaf area, then

$$E = \frac{2W_2 - W_1}{(_2La - _1La)(T_2 - T_1)} \quad \ldots (1)$$

(Coombe, 1960 and Evans, 1972)

where W_2 and W_1 are total plant dry weight and $_2La$ and $_1La$ are leaf area at times T_2 and T_1 respectively.

But if leaf area increases exponentially with time, then RGR would be expected to be constant and the formula would then be

$$E = \frac{W_2 - W_1}{T_2 - T_1} \cdot \frac{\log_e {}_2La - \log_e {}_1La}{{}_2La - {}_1La} \quad \ldots (2)$$

This equation would also be applicable if dry weight was linearly related

to leaf area.

However, Coombe (1960) has shown that so long as the leaves do not change in area during the period between harvests by more than a factor of 2, the error produced by the wrong choice of formula for calculating E does not exceed 4%, provided that the relationship between leaf area and dry weight lies within the range from linear to quadratic.

Comparison of growth and development of E. cichoracearum infected and non-infected groundsel.

Introduction

Two sets of experiments are described which determined the effects of light (less than 10%) and heavy (75-100%) mildew infection on various parameters of host plant growth.

Experiment 1 : This was done in a greenhouse without temperature controls. The temperature ranged from 18-30°C and the relative humidity was less than 35% with a 14 hour photoperiod. Such environmental conditions were expected to limit mildew development. The maximum mildew intensity reached during the period of the experiment was approximately 10%.

Experiment 2 : This was carried out in an environmental growth room maintained at about 19°C, a relative humidity of about 75% and a 14 hour photoperiod. To increase the relative humidity, trays were filled with water and left amongst the plants. Disease intensity towards the later stages of the experiment was between 75-100%.

General Methods

Production of plant material : Seedlings were raised in Levington compost in a seed tray. Three weeks after germination, those of equal vigour were selected and transplanted into Levington compost in 4" pots. They were separated into two groups of 40 plants each.

Inoculation procedure : Plants in one group were inoculated when three weeks old with E. cichoracearum conidia transferred by means of a camel

hair brush from a heavily-infected groundsel, grown in the growth room. Mildew intensity was determined by means of the diagrammatic key described in Chapter 3.

Maintenance of mildew-free plants: Freshly prepared 0.05% aqueous Benomyl solution was applied as a soil drench to keep control plants free from mildew.

Harvests: Five plants per treatment were harvested at weekly intervals and 4-5 harvests were made.

Post-harvest treatment: The root systems were carefully rinsed under running water to remove any adhering compost particles. The plants were blotted dry between tissue paper and then separated into their constituent parts - root, leaves and stem. The inflorescences were included with the stems.

Measurement of leaf area: The plant assimilatory apparatus is usually understood to mean the green surface area which provides the site for photosynthetic activities. Leaf area, stem area and chlorophyll content are the features very often employed to represent the assimilatory apparatus in various investigations.

For this particular study, however, only leaf area has been determined to represent the entire plant assimilatory surface because the stem, petioles and green floral parts ~~may~~ contribute negligibly as sites for photosynthetic activities.

It is clear from the previous section that the size of the assimilatory apparatus is an important attribute of growth from which various growth characteristics and indexes can be calculated. But determination of the size of the assimilatory apparatus is usually the most tedious procedure in growth analytical studies.

Several methods are now available for assessing leaf area and the most convenient for extensive studies appears to be the photoelectric method. For the present study, no photoelectric equipment for measuring leaf area was available and so after comparing the suitability of other manual/mechanical methods a revised planimetric method was adopted.

Leaves from the individual plants were excised from the stem and blotted dry between tissue paper. They were then placed flat between two sheets of glass and copies were made with a Xerox machine. The areas within the outlines were measured by a hand planimeter (type Allbrit 39875). At least two measurements per leaf were made. Sestak et al. (1971) put the maximum error that can be obtained by planimetering at 3%.

The study involved measuring large numbers of leaves and the destructive method of growth analysis used warranted a leaf area assessment method which would permit rapid assessment in order to avoid errors due to shrinkage by the loss of cellular water. The method used met these requirements and in addition provided copies of leaf outlines which are available for future reference.

Dry weights. After determining fresh weights, the plant organs were wrapped in aluminium foil envelopes, dried to constant weight at 80°C and their dry weights were determined after a cooling period of about 20 mins in a desiccator.

Growth analytical methods. Growth of the plants was assessed by calculating the growth characteristics discussed in the introduction of the present chapter.

Seed weight and viability. Seeds from each of 10 infected and control plants were collected and after cleaning them, the weight per 100 seeds

was determined and their viability was tested by placing 50 seeds on wet filter papers in petri dishes and left on the laboratory bench for 6 days. This test was repeated using seeds surface-sterilised with 0.1% mercuric chloride for 5 mins and then washing with several changes of sterile water. Neither of these tests gave a satisfactory number of germinated seeds. Two further attempts were therefore made by germinating seeds on non-sterile Levington compost, in the growth room.

Results

Effects of low levels of E. cichoracearum infection on the growth and development of groundsel.

The results are recorded in Appendix Tables 3A-3D and summarised in Text Tables 6 and 7.

The mean values of the various parameters were compared using the student t-test. The differences between the means of the fresh and dry weights of the different organs of infected and control plants were not significant. The growth characteristics, specific leaf area, leaf weight ratio, leaf area ratio and unit leaf rate of infected and non-infected plants did not appear to differ significantly (Table 7).

However, the number of senescent leaves at each harvest was consistently higher in infected plants than in controls. During the ninth, tenth and eleventh weeks of growth, the differences in number of senescent leaves between infected and control plants were significant ($p = 0.05-0.01$).

The difference in the rate of senescence of the lower leaves was the only feature apart from infection which differentiated plants with low level of infection from non-infected plants. The mildew colonies were only present on the lower leaves, presumably where the microclimate was conducive to the development of the fungus. These infected leaves were

Table 6 Primary values of growth of infected (i) and non-infected (control) plants at 5% level of infection and at later stages of growth. Figures are means of 5 plants.

		Height (mm)	Fresh Weight (gms)			Dry Weight (gms)			No. of senescent leaves	Total no. of green leaves	Leaf area (sq. dm.)	Total dry weight (g)
			Stem	Leaf	Root	Stem	Leaf	Root				
1st Harvest 8 weeks	(i)	23.40	0.0971	0.4063	0.0709	0.0058	0.0284	0.0094	0.4	8	0.1540	0.0434
	(c)	21.60	0.0706	0.3367	0.0504	0.0041	0.0262	0.0048	0	9	0.1492	0.0356
	't'-test	NS	NS	NS	NS	S	NS	NS	-	NS	NS	NS
2nd Harvest 9 weeks	(i)	40.0	0.2876	0.9817	0.3158	0.0238	0.1164	0.0270	3	11	0.5115	0.1671
	(c)	39.0	0.3144	1.5680	0.3301	0.0200	0.1176	0.0250	0	12	0.5716	0.1625
	't'-test	NS	NS	NS	NS	NS	NS	NS	S p=0.02	NS	NS	NS
3rd Harvest 10 weeks	(i)	58.6	0.8292	3.0455	0.9064	0.0699	0.2703	0.0712	4	13	0.9598	0.4114
	(c)	67.2	0.9336	3.5845	1.2031	0.0878	0.290	0.0845	2	15	1.0270	0.4623
	't'-test	NS	NS	NS	NS	NS	NS	NS	S p=0.05	NS	NS	NS
4th Harvest 11 weeks	(i)	107.75	1.4813	4.3438	2.2580	0.0940	0.3180	0.1147	7	15	1.5175	0.5266
	(c)	106.6	1.6222	5.575	2.2824	0.1077	0.3777	0.1260	2	16	1.6712	0.6114
	't'-test	NS	NS	S (p=0.1)	NS	NS	NS	NS	S p=0.01	NS	NS	NS

NS = not significant at p=0.05 S = significant at p=0.05

Table 7 Showing some growth characteristics E. cichoracearum infected and non-infected groundsel at 5-10% disease intensity.

		Age (weeks)			
		8	9	10	11
Total dry wt. (g)	(i)	0.0434	0.1671	0.4114	0.5266
	(c)	0.0356	0.1625	0.4623	0.6114
SLA ($dm^2\ g^{-1}$)	(i)	5.4225	4.3943	3.5509	4.7720
	(c)	5.5880	4.8605	3.5414	4.4247
LWR(gg^{-1})	(i)	0.6544	0.6966	0.6570	0.6039
	(c)	0.7500	0.7237	0.6273	0.6178
LAR(sq.dm g^{-1})	(i)	3.5484	3.0610	2.3330	2.8817
	(c)	4.1910	3.5175	2.2216	2.7334
		A	B	C	
ULR ($g\ dm^{-2}wk^{-1}$)	(i)	0.8722	1.4923	0.7412	
	(c)	0.7382	1.8708	0.7194	

A = Growth interval between 8th and 9th week of growth

B = " " " 9th " 10th " " "

C = " " " 10th " 11th " " "

observed to become chlorotic, later becoming necrotic and eventually dying much more rapidly than the lower leaves of the uninfected leaves.

Effects of heavy infection by E. cichoracearum on the growth and development of groundsel.

Disease intensity during the course of this study was much higher than that which is usually found to occur under natural conditions. Colonies covered all aerial parts of the infected plants including the inflorescences (see Plate 3).

The results are presented in Appendix Tables 4A-E and summarised in Text Tables 8 and 9. It was obvious without statistical analysis of the data that the growth of the infected plants was unfavourably affected by the fungus.

Stem height (Fig. 9) : Regression analysis of stem height against age of the plant showed a significant linear relationship in both infected and control plants during the period, seven to eleven weeks after germination. During the same period, the infected plants were shorter by 25-30%. An analysis of variance showed that the differences in height between infected and control plants were highly significant ($p = 0.001$).

Age (weeks)	Mean stem height (mm)				% Loss
	Control	S.E.	Infected	S.E.	
7	32.6	1.503	24.2	0.629	25.8
8	74.0	4.506	57.6	3.234	22.6
9	100.0	2.236	84.0	1.673	16.0
10	138.0	5.14	102.6	3.544	25.7
11	175.0	9.359	125.2	7.344	28.5

Fresh weight (Table 8) : Infection adversely affected fresh weights of the different organs. Stem fresh weights of infected plants were between

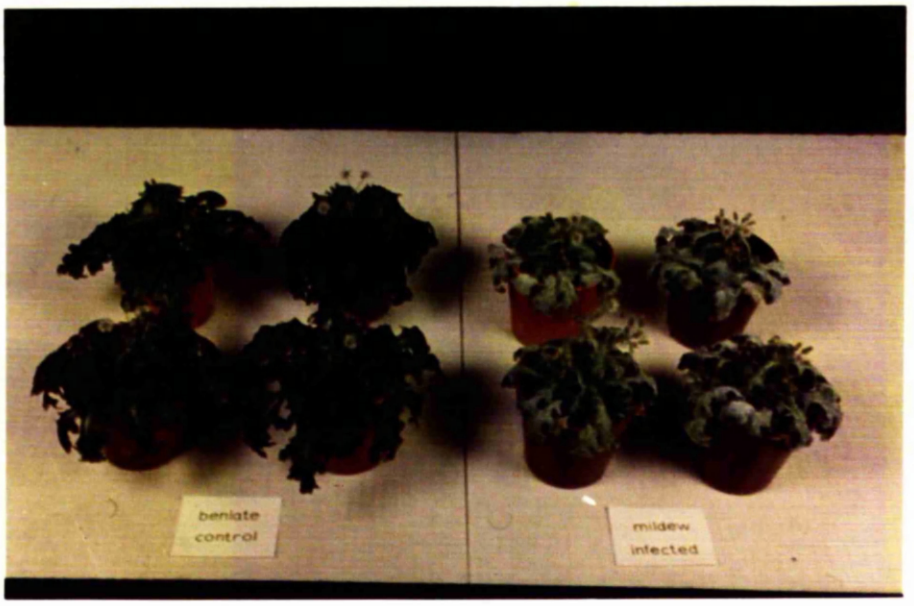
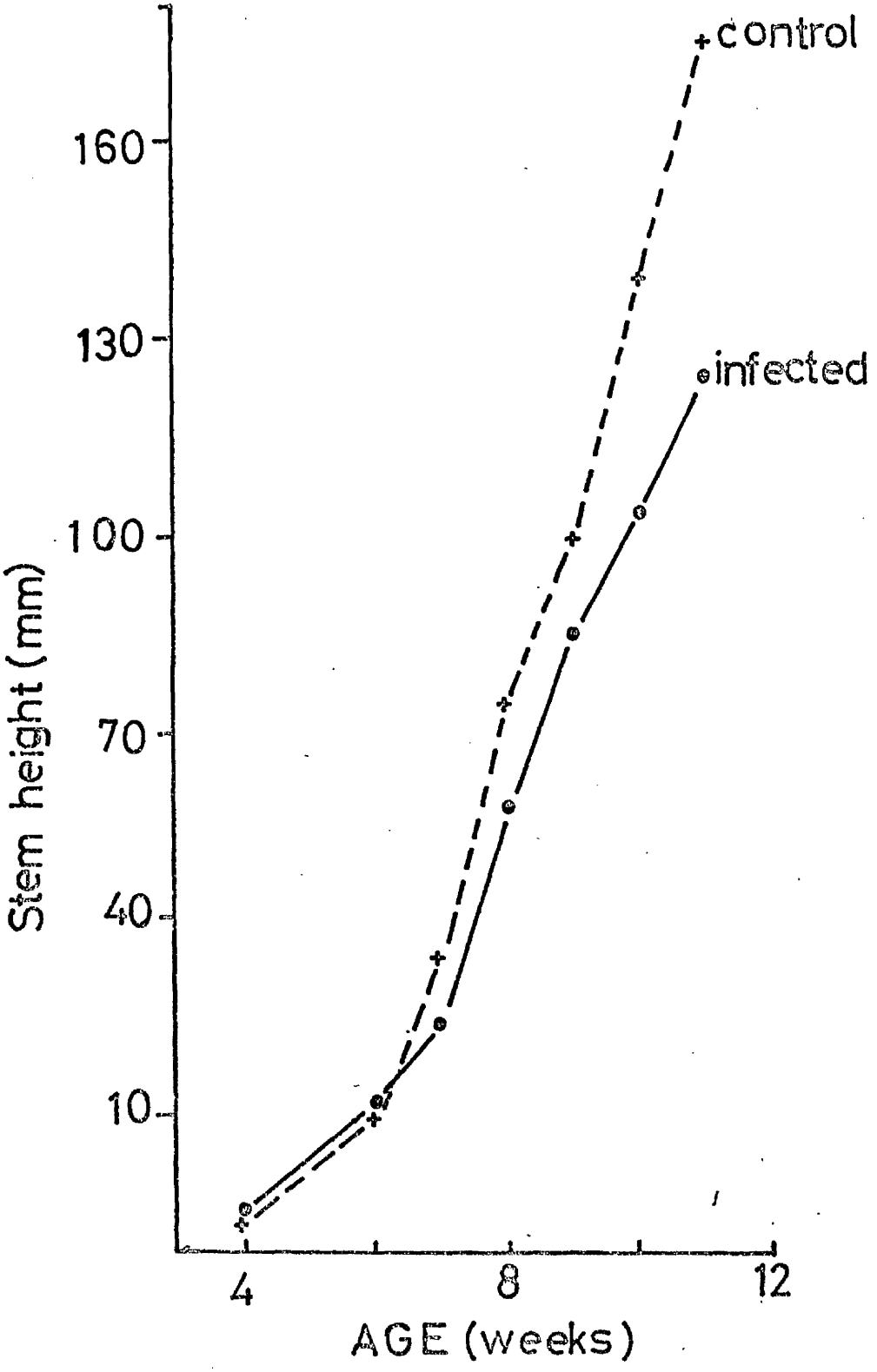


Plate 3. Heavily E. cichoracearum infected (more than 75% mildew intensity) and benlate treated groundsel plants at a growth stage, during flowering.

FIG. 9

Stem height of heavily E. cichoracearum infected and non-infected groundsel plants measured at weekly intervals.



18-25% of that of the controls in the seventh to eleventh week growth period. Leaf fresh weights were only 7-47% of the controls and root fresh weights were 22-48% of the controls.

Dry weight (Table 8): An analysis of variance of the data showed significant differences between harvests in control plants but no significant differences were found in dry weights of infected plants between the eighth and eleventh weeks of growth. Thus, the infected plants did not add any significant amount of dry matter after the eighth week stage (Fig. 10).

The dry weight of infected plants showed a 38% reduction over the controls by the seventh week and a reduction of 82% by the eleventh week.

Leaf area : An analysis of variance of the progression of leaf area between harvests showed significant differences in both infected and control plants ($p = 0.01$). Leaf area in both groups continued to increase but that of infected plants started to decline after the 9th week of growth, a week earlier than in the controls (Fig. 11). At the last harvest, after eleven weeks of growth, the leaf area of infected plants was reduced by 90.4% of that of the controls. By the 12th week all leaves on infected plants had lost their chlorophyll and the plants appeared dead. Most of the leaves of control plants of the same age however, were still green.

Age (weeks)	Mean leaf area (sq. dm)				% Reduction
	Control	S.E.	Infected	S.E.	
7	1.310	0.071	0.803	0.066	38.7
8	2.129	0.094	1.011	0.098	52.5
9	2.122	0.269	1.041	0.028	52.9
10	3.356	0.178	0.484	0.052	85.6
11	2.903	0.178	0.280	0.036	90.4

FIG. 10

Total plant dry weight of heavily E. cichoracearum infected and non-infected groundsel plants determined at weekly intervals.

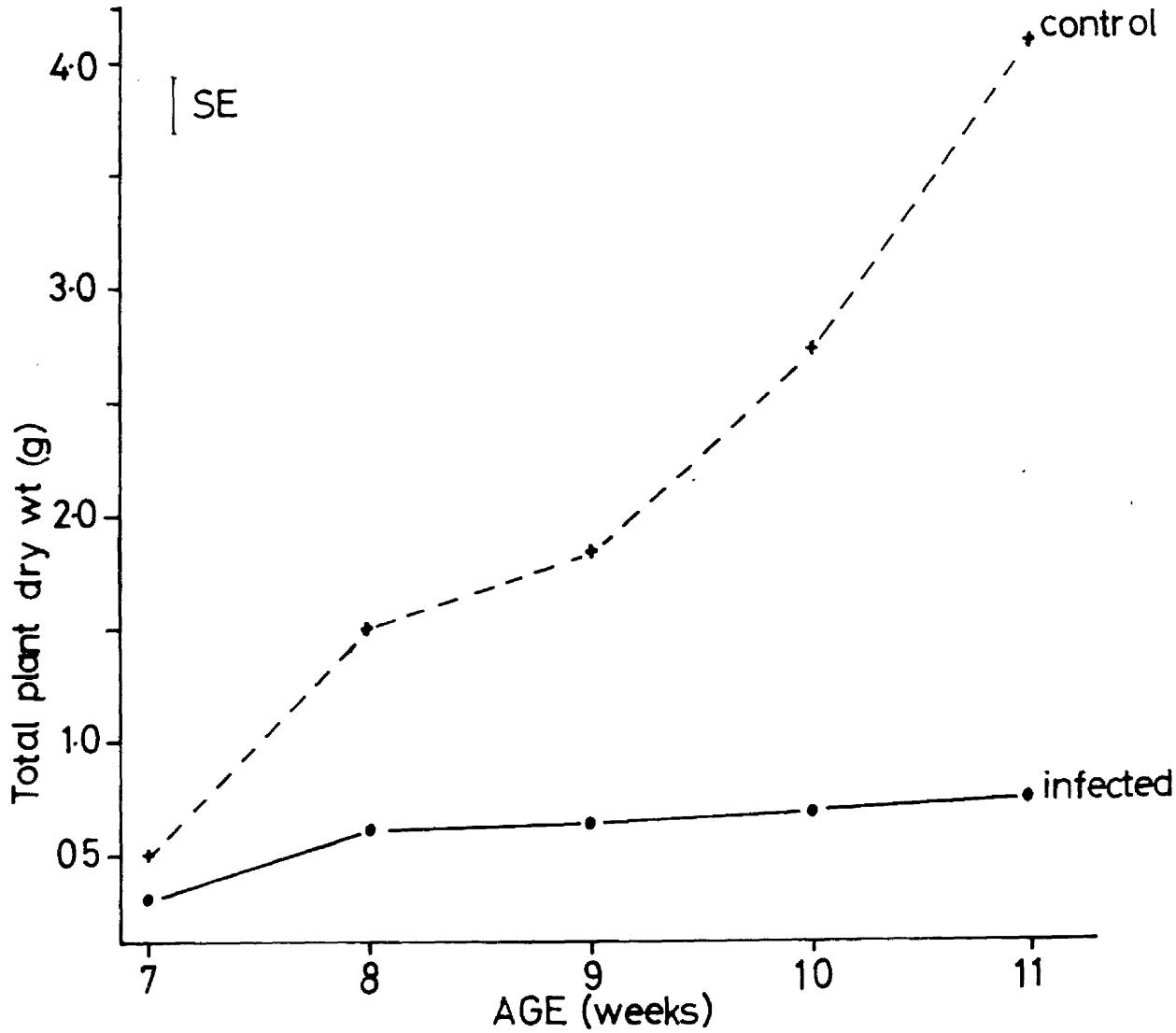
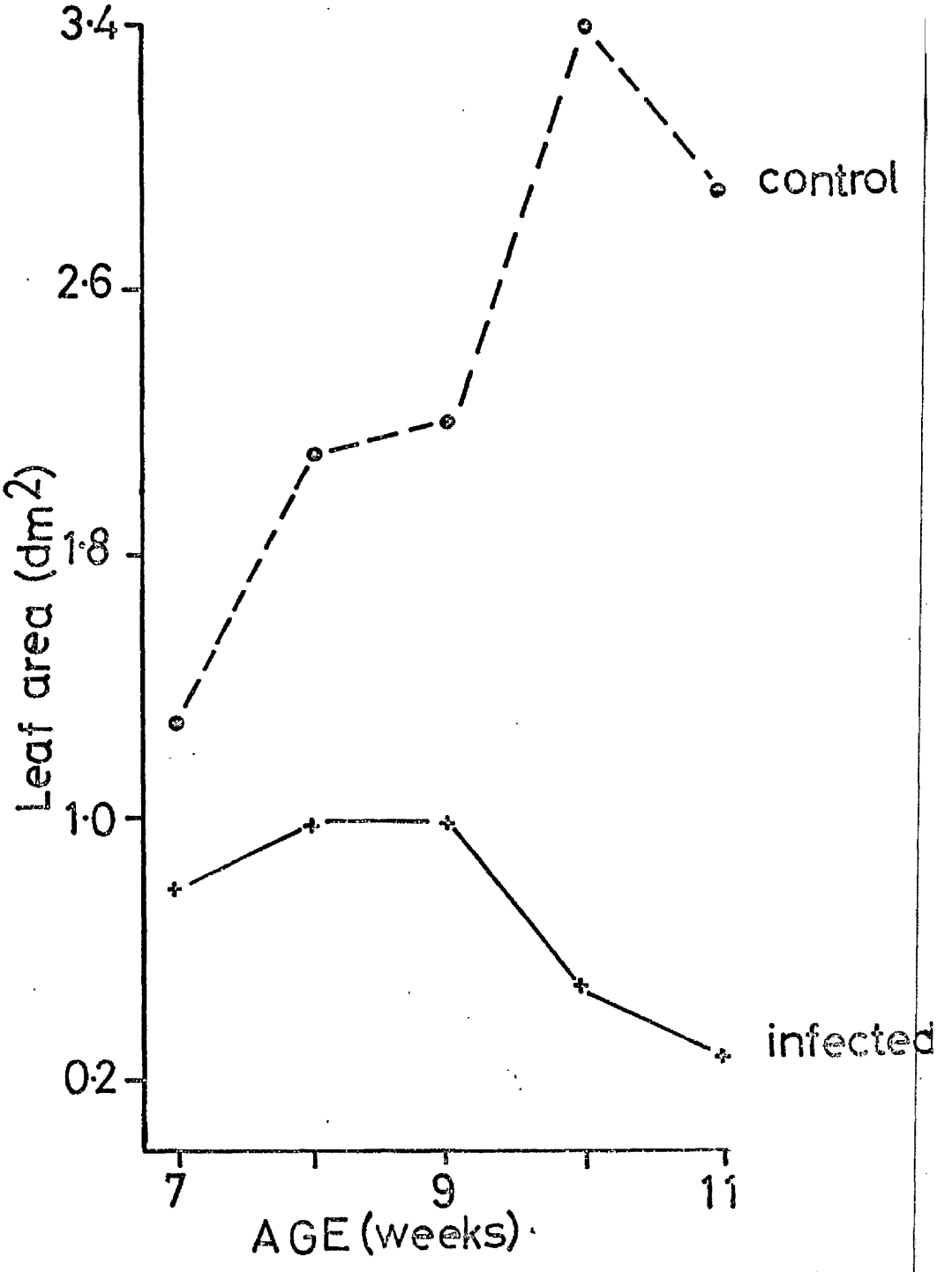

4·0
3·0
2·0
1·0
05
SE
Total plant dry wt (g)
control
infected
7
8
9
10
11
AGE (weeks)

FIG. 11

Leaf area development of heavily E. cichoracearum infected and non-infected groundsel plants.



Number of senescent leaves (Table 8) : The results showed that leaf senescence was the only sensitive indicator of the presence of the fungus at low level of infection. With 75% level of infection, the number of senescent leaves was at least three times as many compared with plants of equivalent age with low levels of infection. Senescent leaves were first found during the eighth week of growth on both infected and control plants, but by the tenth week infected plants had about four times as many senescent leaves as the control plants.

Number of branches (Table 8) : A count of the number of branches on eleven weeks old plants showed that infected plants had produced only half the number of branches produced by the control plants.

Number of flowers (Table 8) : The first visible flower heads appeared during the seventh week of growth in both infected and control plants. Eight week old control plants had about twice as many flowers as infected plants of equivalent age and about three times as many after nine and ten weeks of growth. It is remarkable, however, that in spite of the debility in growth of infected plants, they were still able to produce flowers (Plate 3) and set viable seeds.

Seed weight and viability

	Weight (g) per 100 seeds from each of 10 plants					
	(1)	(2)	(3)	(4)	(5)	(6)
Infected	0.0147	0.0167	0.0179	0.0177	0.0158	0.0153
	(7)	(8)	(9)	(10)		
	0.0158	0.0170	0.0146	0.0176	Mean = 0.0163	
Control	(1)	(2)	(3)	(4)	(5)	(6)
	0.0237	0.0247	0.0233	0.0234	0.0237	0.0213
	(7)	(8)	(9)	(10)		
	0.0225	0.0227	0.0213	0.0186	Mean = 0.0225	

Table 8 Primary values of growth of infected (i) and non-infected (c) plants. Infection was early and was of high intensity (75-100% level of infection). Figures are means of 5 plants.

	Fresh weights (g)			Dry weight (gm)			No. of senescent leaves	No. of flowers	No. of leaves	No. of side branches
	Stem	Leaf	Root	Stem	Leaf	Root				
1st Harvest (i)	0.3275	2.8017	0.7270	0.0244	0.2240	0.0539	0	-	26	-
(7 weeks) (c)	0.7074	5.8645	-	0.0521	0.3697	0.0634	0	-	36	-
2nd Harvest (i)	1.8553	4.9080	1.2184	0.1488	0.3634	0.0870	11.6	19	-	-
(8 weeks) (c)	3.5896	11.4763	2.5235	0.3554	0.8728	0.2227	2.4	41	-	-
3rd Harvest (i)	2.3057	1.1088	1.1088	0.2234	0.2959	0.0973	23	26	-	-
(9 weeks) (c)	6.6774	12.0179	3.2599	0.6801	0.8559	0.2520	6	73	-	-
4th Harvest (i)	3.1877	1.6917	1.0772	0.3418	0.2102	0.0947	26.2	39	-	-
(10 weeks) (c)	13.0195	17.6298	3.5128	1.2160	1.2084	0.3047	7	126	-	-
5th Harvest (i)	4.2448	1.3730	1.0910	0.4800	0.1745	0.0874	-	-	-	10
(11 weeks) (c)	23.0523	21.1962	4.9002	2.2240	1.5064	0.3458	-	-	-	20

Table 9 Growth characteristics of E. cichoracearum infected (i) and non-infected (c) groundsel taken at weekly intervals during the growth period from the 7th to the 11th week.

Growth characteristics	Treatment	Age (weeks)				
		7	8	9	10	11
Specific Leaf Area ($dm^2 g^{-1}$)	(i)	3.5848	2.7820	3.5180	2.3039	1.6045
	(c)	3.5444	2.4392	2.5844	2.7772	1.9271
Leaf Weight Ratio $g\ g^{-1}$	(i)	0.7409	0.6064	0.4798	0.3250	0.2352
	(c)	0.7619	0.6015	0.4786	0.4427	0.3694
Leaf area ratio $dm^2 g^{-1}$	(i)	2.6557	1.6870	1.6879	0.7487	0.3773
	(c)	2.7004	1.4671	1.2368	1.2294	0.7118
		A	B	C	D	
Unit Leaf Rate $g\ dm^{-2}\ wk^{-1}$	(i)	0.3289	0.0170	0.0509	0.2551	
	(c)	0.5720	0.1555	0.3455	0.4407	

A = growth period between the 7th and 8th weeks
B = " " " " 8th " 9th "
C = " " " " 9th " 10th "
D = " " " " 10th " 11th "

The weight of seeds from the heavily infected plants was significantly reduced ($p = 0.001$).

Viability tests conducted in petri dishes with surface sterilised and non-sterilised seeds did not yield any worthwhile results because the seeds germinated only sparsely. However, one attempt using Levington compost yielded results which showed a mean viability of 72% of seeds from infected plants and 33% of seeds from the controls. These figures may not categorically infer higher viability of seeds from infected plants than from controls, but they show that heavily infected plants yielded seeds that are of high viability.

Distribution of dry matter along the plant axis : The ratios of the dry weights of stem, leaf and root to total dry weight are given in Table 10. The results show that between the seventh and ninth weeks of growth, the proportion of dry matter contained in the leaves was higher than that contained in the stem or the roots in both infected and control plants. The situation reversed from the tenth week when the stem accumulated more dry matter than the leaves. The proportion of dry matter in the roots, however, appears relatively constant throughout the growth period in both infected and control plants. The ratios of dry matter in the various organs show very little differences between infected and control plants. It, thus, would appear that although infection reduced dry matter production, it did not affect its distribution and all the major organs were stunted in proportion.

Leaf weight ratio, specific leaf area and leaf area ratio : These growth indices have been plotted against total dry weight (Fig. 12) in accordance with the suggestion by Evans (1972) that because they are calculated from total dry weight, plotting against this would minimise the effects of accidental variations. For example, for LWR, leaf dry weight enters into

Table 10 Dry matter distribution on the plant axis calculated from mean dry weights of plant organs and mean total plant dry weight.

Age		Dry weight ratios (%) Stem	Root	Leaves	Mean total plant dry weight (g)
1st Harvest	(i)	8.07	17.83	74.10	0.3023
(7 weeks)	(c)	10.74	13.07	76.20	0.4852
2nd Harvest	(i)	24.83	14.52	60.65	0.5991
(8 weeks)	(c)	24.50	15.35	60.16	1.4509
3rd Harvest	(i)	36.23	15.78	47.99	0.6166
(9 weeks)	(c)	38.04	14.09	47.87	1.7880
4th Harvest	(i)	52.85	14.64	32.50	0.6467
(10 weeks)	(c)	44.56	11.17	44.28	2.7291
5th Harvest	(i)	64.70	11.78	23.52	0.7419
(11 weeks)	(c)	54.57	8.48	36.95	4.0771

both numerator and denominator so that any accidental increase in leaf dry weight will increase both LWR and total dry weight. Thus a sort of correlation system is built into the curves. However, for the purposes of comparison, these growth characteristics have also been plotted against time (Fig. 13).

In general, all three growth characteristics showed a negative correlation with increasing total dry weight (Figs. 12) and with time (Fig. 13) in both infected and control plants. The downward trend of leaf weight ratio indicates that as the plants got older and as total plant biomass increased, the amount of dry matter that accumulated in the leaves diminished. It has already been shown that the proportion accumulating in the stem increased during the later stages of growth. This is to be expected because a diversion of dry matter to the stem apices would be necessary for flower and seed production. (In the analyses, the inflorescences have been regarded as part of the stem.)

A decline in specific leaf area suggests that a relatively higher proportion of the assimilates of the leaves were retained within the leaves. Whether they are retained within the cells as starch or other storage materials or utilised to form new cells was not determined. The specific leaf area of infected plants declined faster than that of controls which suggests that the leaves of infected plants were maturing faster than those of the controls.

Since leaf area ratio was also determined from leaf area data as was specific leaf area, it also shows a similar negative correlation with total dry weight. A steeper decline of both specific leaf area and leaf area ratio occurred during the ninth week in infected plants and the tenth week in controls (Fig. 13) corresponding to the decline in leaf area (Fig. 11). The declining leaf area ratios suggest a diminishing amount of materials allocated for the development of the assimilatory surface in relation to the total amount available in the plant.

FIG 12

The relationship between mean total plant dry weight plotted on a log scale and the growth characteristics - leaf weight ratio, specific leaf area and leaf area ratio - of heavily E. cichoracearum infected and non-infected groundsel plants during the growth period between the 7th and 11th weeks.

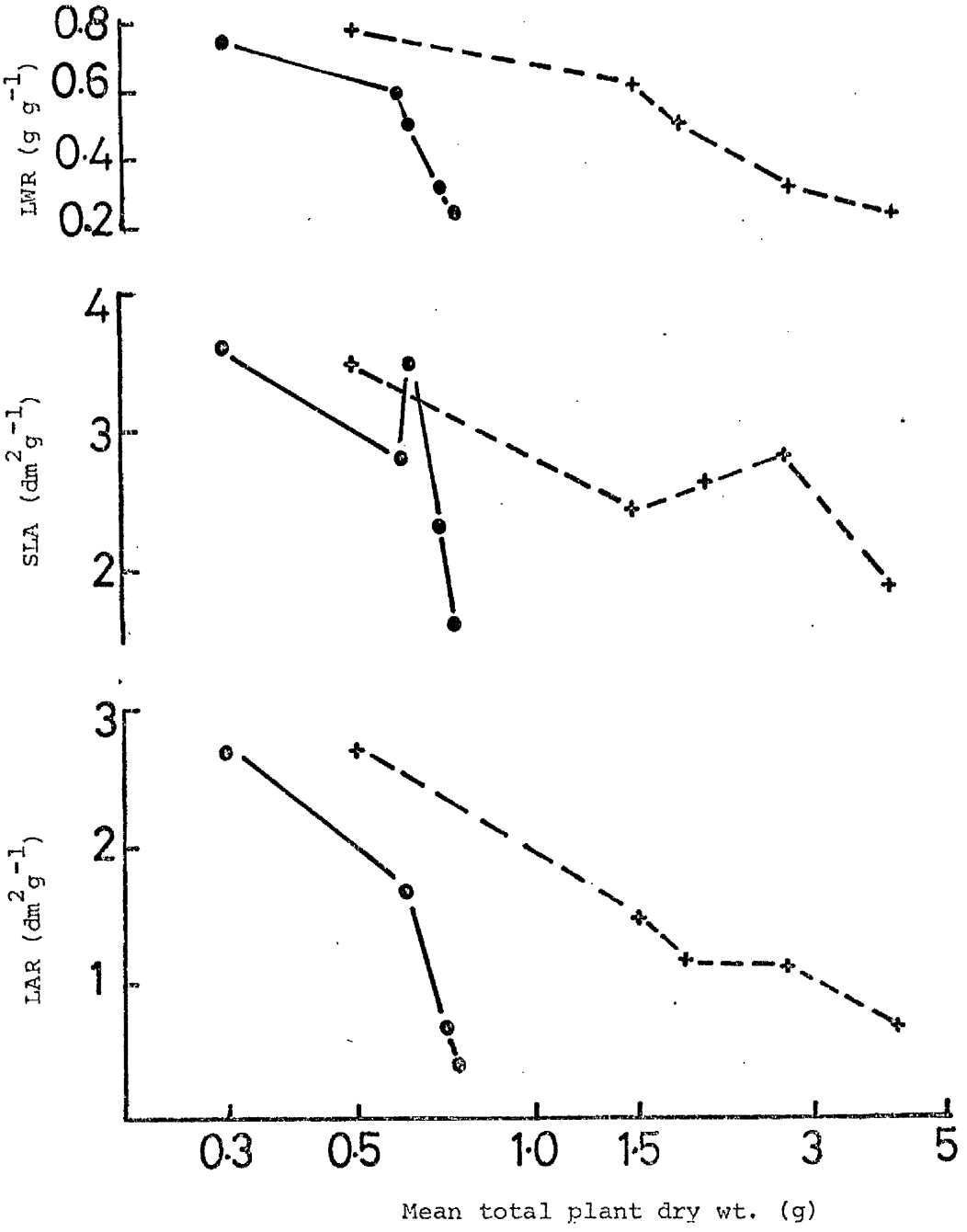
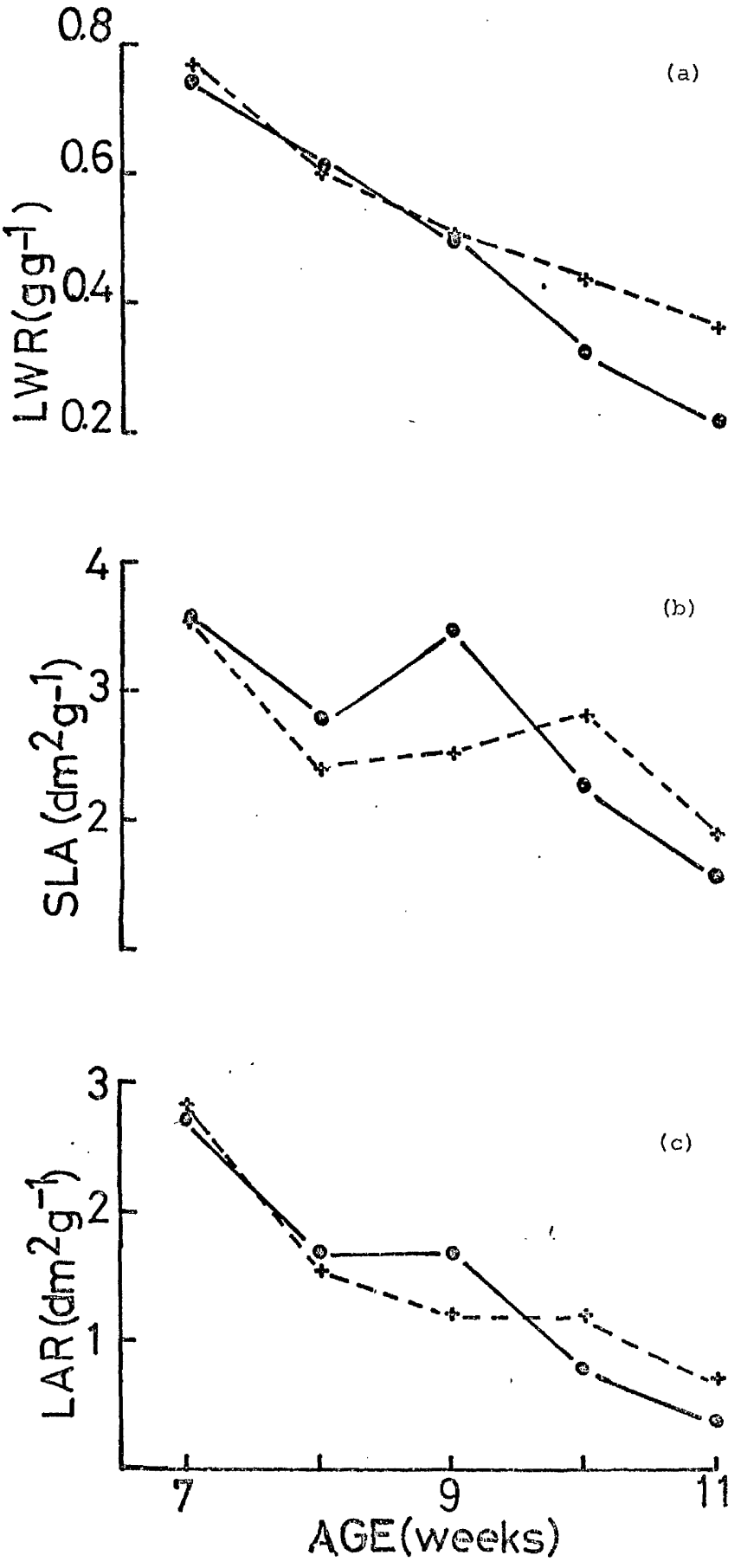


FIG. 13

Effects of heavy infection of E. cichoracearum on groundsel plants, showing comparisons of infected and non-infected in the progressions of (a) leaf weight ratio, (b) specific leaf area, and (c) leaf area ratio.

●——● infected

+-----+ control


0.8
0.6
0.4
0.2
LWR(gg-1)
(a)
4
3
2
SLA (dm2g-1)
(b)
3
2
1
LAR(dm2g-1)
(c)
7
9
11
AGE(weeks)

Unit leaf rate (Fig. 14, Table 9) : A decline in the capacity of the photosynthetic area to produce plant biomass as determined by unit leaf rate occurred during the growth period between the seventh and eighth weeks in both infected and control plants. This decline may have been due to the initiation and development of flowers which occurred at this time since such a process usually requires mobilisation of materials to the sites where flowers are being initiated.

Both infected and controls, however, showed a steady rise in ULR during the next three growth periods. This subsequent increase is probably associated with the development of fruits and seeds which were in progress during this period.

Discussion

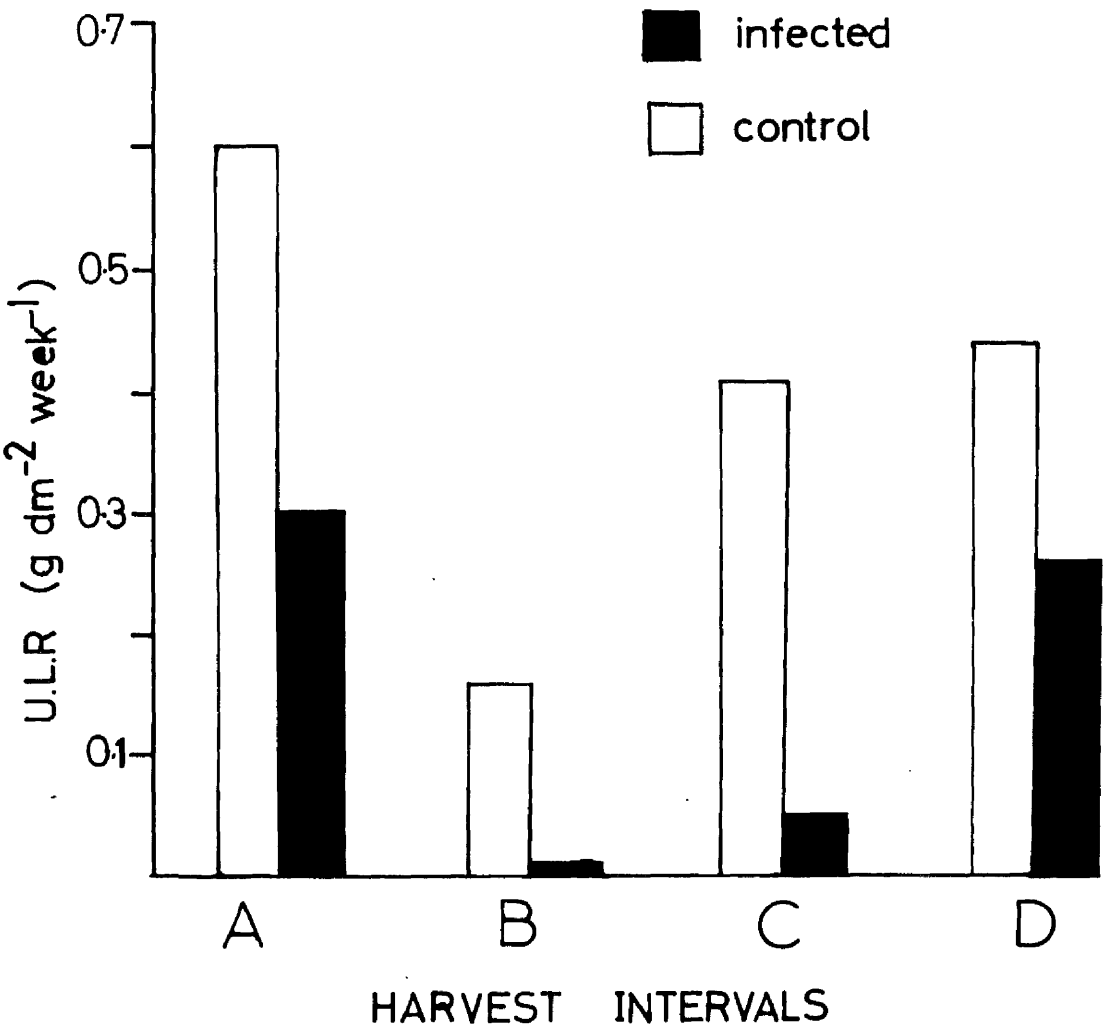
Low levels of infection of up to 10% had little effect on the growth and development of the host. The only significant effect of the disease at these levels was manifest as an increased number of senescent leaves at each harvest. It was this feature, except for the presence of mildew colonies, which clearly distinguished infected plants from uninfected ones.

Normally, senescence commences on the lower leaves and progresses to the upper leaves and mildew infection which occurs first on the lower leaves appears to increase the rate at which the leaves senesced. Allen (1942) reported that the chlorophyll content of wheat leaves lightly infected with mildew decreased. Leaf senescence is characterised by loss of chlorophyll and the chlorophyll content of normal leaves in many plants is a function of the age of the plant. Hence, the presence of the parasite on the lower leaves may have accentuated the natural sequence of events leading to leaf senescence.

It is striking that although a significant number of leaves were lost through senescence, the number of green, apparently functional leaves

FIG. 14

Effect of heavy infection of E. cichoracearum on groundsel plants, showing comparisons infected and non-infected plants in the progression of unit leaf rate.


0·7
0·5
0·3
0·1
U.L.R (g dm^{-2} week^{-1})
infected
control
A
B
C
D
HARVEST INTERVALS

and their total area was not significantly affected at low disease intensity. The mean total number of leaves produced on infected plants was about the same as in the controls during the greater part of the growth period but increased by about three leaves per plant during the last week of growth (Table 6). Thus, mild infection stimulated the production of more leaves and their expansion to compensate for those lost through senescence.

Severe E. cichoracearum infection at 75% intensity, however, significantly retarded the growth and development of the host plant. It resulted in the production of a much smaller leaf area and to a reduced rate of production of dry matter per unit leaf area and this was probably the main factor responsible for the observed decrease in number of flowers, the height of the plant and seed weight.

Although the plants were inoculated at three weeks old and mildew was apparent by the fourth week, the major effects on growth did not become apparent until about 7 weeks of age. The capability of such heavily infected plants to produce viable seeds in spite of the enormous reductions in the development of the various organs indicates that groundsel has a level of tolerance to E. cichoracearum infection.

In contrast to the findings of Millerd and Scott (1956) that mildew increased the fresh weight per unit leaf area and of Last (1962) who found that mildew had little effect on fresh weights of barley, mildew in the present investigation at the high levels of infection greatly decreased fresh weights, at all harvests.

Last (1962) found a relatively greater reduction of root development than of leaves in E. graminis infected barley plants. Similarly, Doodson et al. (1964) found an unexpectedly large retardation in root development in Puccinia infected wheat plants. However, heavy mildew infection of groundsel did not result in any greater reduction in root development than occurred in other organs.

Drastic retardation of root development in mildew infected cereals has been ascribed to a distortion of the translocation system resulting in the accumulation of normal and "fungal carbohydrates" in the infected leaves. Recent researches with radioactive materials have produced results in support of this. For example Fric (1975) found that export of labelled carbon assimilate from mildew infected wheat leaves was significantly retarded. Yellow rust produced similar effects on wheat. In addition to reducing export from infected leaves, diversion to and accumulation of translocates in infected leaves has been reported (Daly et al. 1962; see also Crowdy and Manners, 1971 and Smith et al. 1969 b)

Minarcic and Pauleck (1975) attributed the reduction in root development of powdery mildew infected barley to inhibition of mitotic cell division of the apical root meristems. This inhibition could have been due to shortage of essential carbohydrate or to the presence of a fungal toxin.

It may be that the disproportionately large retardation of root development by powdery mildew or rust infection in cereals is a feature of host-parasite combinations in cultivated crops.

It is clear from Table 10 that the dry weight ratios of each organ in infected plants at all harvests were similar to those of control plants. The growth characteristics, leaf weight ratio, specific leaf area, leaf area ratio and unit leaf rate exhibited similar ontogenetic changes in infected as in control plants, although the rates of change were different. Thus in contrast to the work with cereals, in groundsel dry matter distribution in relation to the development of the various organs was more or less identical in both infected and control plants. Thus, there is no evidence to indicate that the translocation of the proportion of photo-synthates to the roots is being affected. The difference appears to lie only in the pool of dry matter available for

distribution.

Harrison and Isaac (1969) suggested that a more rapid decline in specific leaf area of Verticillium infected potatoes was indicative of a correspondingly more rapid maturation of the leaf system of infected plants than of the controls. A similar, more rapid decline of specific leaf area of mildew infected groundsel as compared with that of non-infected plants was found in the present investigation. It may be that, in this case also, mildew infected plants attained maturity earlier and in consequence died two to three weeks earlier than the controls.

In conclusion, it is clear that although infection significantly affected the development of the assimilatory apparatus and thus reduced the extent of dry matter production, the pattern of dry matter distribution and the overall growth patterns were not affected. The infected plants produced seeds in the same period of growth as the control plants, though in less quantity and of lighter weight but with high viability.

The level of infection obtained in this study was far higher than that which normally occurs in nature and the production of viable seeds under such severe attack indicates that groundsel has a high level of tolerance to mildew. Thus, the survival of the species appears assured under almost any level of mildew infection.

CHAPTER 6

THE EFFECT OF INFECTION ON THE DISTRIBUTION OF NITROGENOUS MATERIALS IN THE HOST PLANT.

Introduction

A remarkable feature of mildew infection revealed by the results of the growth analytical studies was the enhancement of leaf senescence. Woolhouse (1967) in his review of leaf senescence classified the causes into genetic and stochastic processes. The genetic processes are believed to involve a group of genes which are repressed during development and normal functioning but which become active at a predetermined time in the life of the organ and lead to morphogenetic changes which disrupt normal function and eventually lead to death. The stochastic processes are those events in the environment including physical agents such as diseases, frost and drought and chemical agents such as nutrient deficiencies and chemical toxins.

It has been amply documented that, in general, the major factor of leaf senescence is loss of proteins due to hydrolysis to their constituent amino acids and amides and the translocation of these to the regions of growth and development (Williams, 1955; Zimmerman, 1960; Bevers, 1968; Luckwill, 1968 and Spencer and Titus, 1972). Thus the size of the alcohol soluble fraction which includes the amino acids and amides, normally increases during senescence (Wood et al. 1943). Williams (1955) reported that mineral elements also migrated from the leaves of deciduous plants prior to leaf fall. Nitrogen, phosphorus, potassium magnesium and iron may migrate in this way, sometimes to the extent of 90% of the total. Since nitrogen metabolism is clearly an important factor in leaf senescence it seemed appropriate to investigate the levels of nitrogenous materials in the different organs of infected plants to compare with uninfected

plants of the same age. Earlier work had also given clear indication that infection did not affect the distribution of translocates within the plant, only the total amounts available for translocation. Thus, studies of the distribution of nitrogenous materials between the various organs of the plant might provide further clarification of the effects of infection on the translocation system of the host.

The subject of the effect of parasitism on the nitrogen metabolism of the host plant has been reviewed, e.g. Goodman et al., (1967). Parasites may also induce the morphological symptoms of senescence and changes in nitrogen metabolism which are similar to those reported in senescing tissue.

Shaw and Colotelo (1961) carried out a detailed study of the nitrogen economy of wheat leaf discs infected by stem rust. They found that as the fungus developed on a susceptible host, total protein and soluble nitrogen and also the ratio of soluble to insoluble nitrogen increased compared with those of healthy tissues. The concentration of free amino acids in infected leaves, 9 days after inoculation, rose steadily and was more than fourfold that in uninfected leaves. In the same period, the total concentration of protein amino acids per gram fresh weight rose twofold.

In contrast, increases in nitrogenous fractions in resistant leaves was slight and transitory occurring very soon after infection, the concentration declining drastically with time. Farkas and Kiraly (1964) also found that the ammonia content of wheat leaves increased only in leaves infected by a race of stem rust which resulted in a susceptible reaction. Total resistance involving a hypersensitive reaction exhibited no increases in ammonia content while partial resistance yielded intermediate amounts.

Similarly, Sadler and Scot (1974) found that powdery mildew infection of barley leaves increased the content of inorganic nitrogen largely in

the form of ammonium nitrogen but caused little change in nitrate nitrogen levels. Evolution of ammonia gas was also increased by infection. It was suggested that the ammonium dependent glutamine and asparagine biosynthesis and probably also an increased protein synthesis might be responsible for the stimulation of respiration which was another characteristic of the powdery mildew infected tissues.

Goodman et al. (1967) in their review concluded that increases in nitrogenous substances are characteristic of diseased tissues. They ascribed such increases in biotrophic relationships either to a stimulated protein synthesis by the host and the parasite in and around the infection centre or due to diversion of such substances from uninfected to infected parts. It is also possible that the increases in soluble nitrogen result from the breakdown of host protein by processes which are similar to those involved in senescence.

In the present study, total nitrogen concentrations in mildew infected and non-infected groundsel plants at different stages of growth were investigated. The relative proportions of 70% alcohol soluble and insoluble fractions were also determined. The soluble fraction is usually taken to represent the low molecular weight substances mainly amino acids and amides whilst the insoluble fractions represent the high molecular weight peptides and proteins.

Materials and Methods

Plant materials

The plants used for the growth analytical studies described in the previous chapter were used for the determination of total nitrogen in leaves, stems and roots. After determining their dry weight, the plant parts were ground to a fine powder with a pestle and mortar and stored in McCartney bottles.

A further set of infected and uninfected plants were raised under

the same conditions as the previous set for the determination of alcohol soluble and insoluble fractions. The maximum mildew intensity on the second set was approximately 90% at the last harvest.

Nitrogen analysis

Reagents

Digestion catalyst:	8 g potassium sulphate
	+ 1 g mercuric oxide
	+ 1 g copper sulphate
Mixed indicator:	6 ml methyl red (0.16% in 95% alcohol)
	12 ml Bromocresol green (0.04% in water)
	6 ml 95% alcohol
Digestion medium:	100 ml Sulphuric acid + 3 gm Salicylic acid

The Kjeldahl method

About 0.2 g of powdered sample was digested in a micro-Kjeldahl digestion flask with about 5 ml of the digestion medium and 0.5 g of catalyst in a fume cupboard. Digestion was continued until the solution assumed a clear green colour. After cooling, the digest was diluted with about 10 ml of distilled water. It was allowed to cool before making up to 100 ml in a volumetric flask. 10 ml of this solution was transferred quantitatively into a markham still and ammonia was distilled off with excess of sodium hydroxide (10 ml of 20% NaOH). The distillate was received in about 5 ml of 2% Boric acid plus 4 drops of the mixed indicator and titrated with 0.01 N Hydrochloric acid. The amount of Nitrogen present was calculated using the following formula:

$$1 \text{ ml } 0.01 \text{ N HCl} \equiv 0.14 \text{ mg N}$$

The results are expressed as milligram Nitrogen per gram dry weight of material.

Soluble and Insoluble Nitrogen determination

Plants were inoculated five weeks after germination and the first samples were harvested one week later. At each harvest six uninfected and an equal number of infected plants were taken for analysis.

Because the dry weights of shoot and root of individual plants at the first harvest were small, analyses were done on the combined dry material from two plants giving three measurements only for uninfected and infected plants but for subsequent harvests the shoot and root of individual plants were analysed separately.

The soluble and insoluble nitrogenous fractions were separated as shown in the flow diagram (Fig. 15).

Results

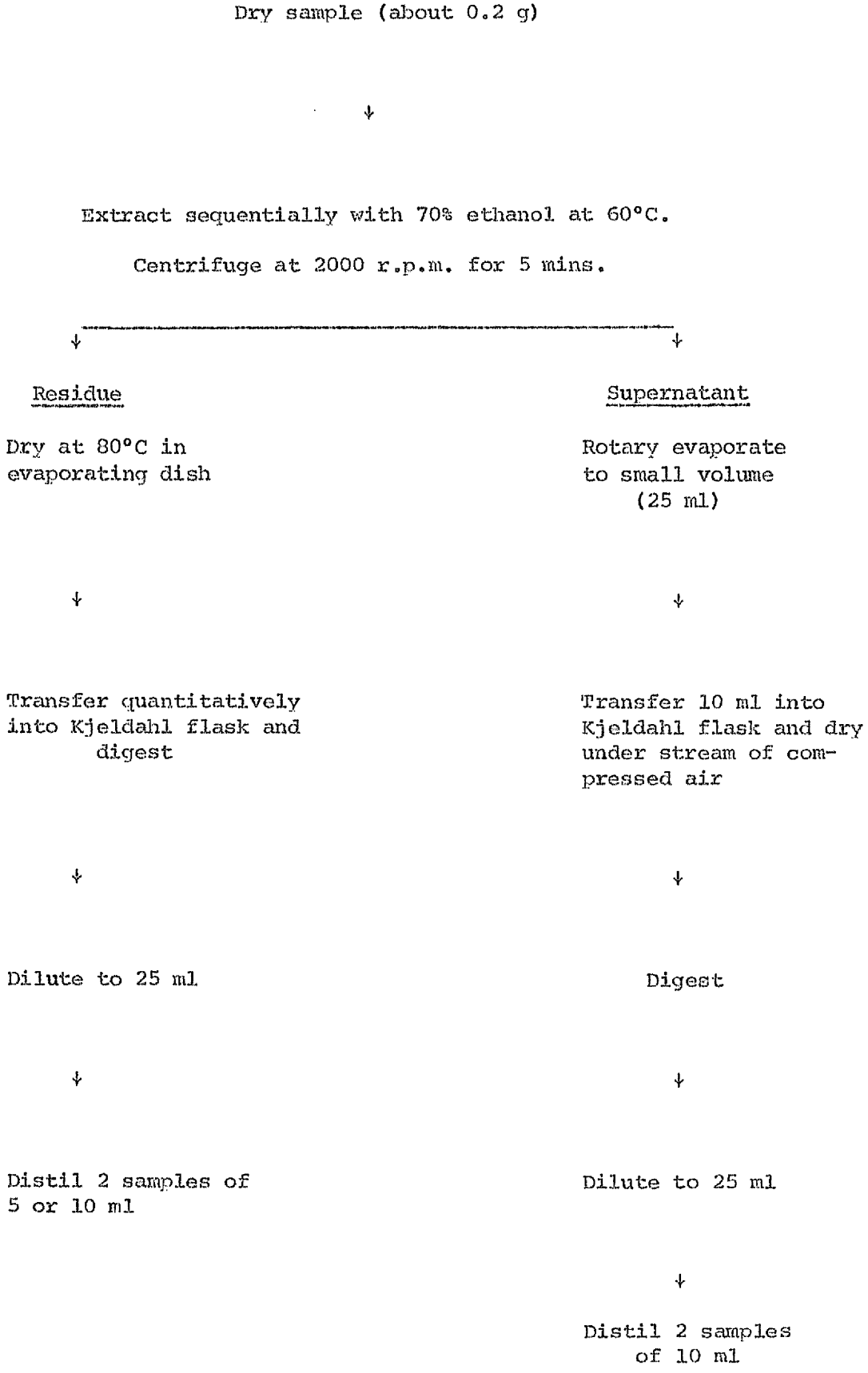
Total Nitrogen

The results are given in Table 11. The roots of infected plants contained more nitrogen per unit dry weight than those of the controls at all harvests. Analysis of the data revealed that these differences were highly significant ($p = 0.02$-0.001).

When the nitrogen concentration of the various plants was summed up to give the concentration in the entire plant, infected plants at all harvests contained more nitrogen on a unit weight basis than the controls. The reason for this is due largely to the higher concentration in the roots and also to greater concentrations in the stem in the later harvests.

Nitrogen concentrations in the leaves did not appear to change very much over the period of investigation but a decline with age in the stem of controls occurred resulting in a significant difference ($p = 0.02$) between infected and controls at the fifth harvest.

Fig. 15. Flow diagram showing extraction and analysis of soluble and insoluble nitrogenous fractions.



Soluble and Insoluble Nitrogenous fractions

The mean mildew intensities at the various harvests are recorded in Table 12. The results are given in Appendix Tables 5A to D and the critical comparisons of the means are recorded in Tables 12 and 13.

Significantly higher concentrations of soluble and insoluble nitrogen and thus of total nitrogen were found in the shoots than in roots of both infected and control plants ($p = 0.01$). Statistical analysis also showed a significant decline in total Nitrogen concentrations with age ($p = 0.05$) in both infected and control plants.

Statistical analyses did not show any significant differences in the concentration of soluble or insoluble forms of Nitrogen in the shoots and roots between infected and control plants. However, the concentration of soluble Nitrogen was consistently higher in both shoots and roots of infected than of control plants. On the other hand, the ratios of insoluble/soluble fractions at each harvest were always higher in the controls than in the infected plants (Table 13). The concentration of total nitrogen (i.e. soluble + insoluble forms) was also always higher in the infected plants than in the controls except at the first harvest (Table 13).

Discussion

Greater concentrations of nitrogenous materials were found in mildew infected plants and the excess appeared to be present largely in the soluble, non-protein forms. The results of this investigation thus provide further evidence in support of the findings of Shaw and Colotelo (1961) with rust-infected wheat and Sadler and Scott (1974) with mildew-infected barley that infection caused an accumulation of nitrogenous materials in terms of unit weight of Nitrogen per unit fresh weight of host plant.

The excess nitrogen in the living tissues of infected plants might

have originated from protein hydrolysis in the senescing leaves which then was transported into the living tissues particularly the roots and remained there in a soluble form. This could be an explanation for the significant difference in nitrogen content of roots between infected and control plants. Another possible explanation is that the roots of infected plants were more efficient at absorbing nitrogen from the soil than those of control plants and that a larger proportion of this was retained in the roots. Thus infection appears to increase the efficiency of nitrogen absorption from the soil. Infection may also have interfered with the translocation of nitrogenous materials within the host. The latter, if it is the case, would be at variance with the pattern of the overall distribution of dry matter since this gave no indication of a change in the pattern of distribution.

Only two experiments, each covering a different growth period of the host plant, were carried out to study the distribution of nitrogenous materials in the host. The results of both experiments together do not provide sufficient evidence to allow definite conclusions to be drawn on the effects of infection on the translocation system of the host. This aspect of the work therefore requires further investigation.

Table 11. Concentrations of Total Nitrogen in E. cichoracearum infected (i) and non-infected (c) groundsel at different stages of growth. (All values are means of five determinations.)

Harvests/Age		Total plant dry weight (g)	mg N/g dry weight			
			Leaf	Stem	Root	Whole plant
2	(i)	0.5992	54.55	-	-	-
(8 weeks)	(c)	1.4509	49.67*	-	-	-
3	(i)	0.6116	56.27	47.21	33.76	137.24
(9 weeks)	(c)	1.7880	50.76	47.56	27.89***	126.21
4	(i)	0.6467	54.62	49.33	44.33	148.28
(10 weeks)	(c)	2.7291	55.38	46.00	35.83***	126.21
5	(i)	0.7419	50.58	39.27	38.44	128.29
(11 weeks)	(c)	4.0771	51.69	32.10**	31.34**	115.13

* significantly different at p = 0.05

** " " " p = 0.02

*** " " " p = 0.001

Table 12. Concentration of soluble and insoluble N-fractions in shoot and roots of infected and control plants and the corresponding mean dry weights at different harvests.

Harvest Occasions/Age		Dry weight (g)		Mean disease intensity (%)	N-concentrations (mg/g)			
					Shoot		Root	
		Shoot	Root		Soluble	Insoluble	Soluble	Insoluble
1 (32 days)	(i)	0.2586	0.0789	50	6.68	30.55	5.15	15.30
	(c)	0.2678	0.0761		6.21	33.26	4.82	16.68
2 (35 days)	(i)	0.3000	0.0794	75	4.90	29.23	3.78	13.95
	(c)	0.3585	0.0954		4.37	28.32	3.61	13.67
3 (38 days)	(i)	0.3922	0.1246	75	5.70	25.57	3.74	11.82
	(c)	0.4182	0.1449		4.97	24.69	2.89	12.23
4 (41 days)	(i)	0.3854	0.1207	90	4.76	24.36	2.76	12.05
	(c)	0.4692	0.1503		4.17	23.36	2.76	10.95

Table 13. Concentrations of soluble and insoluble forms of Nitrogenous materials in whole infected and control plants during four harvests.

Harvests (Age)	Treatment	N. concentration (mg/g)			
		Insoluble	Soluble	A/B	A + B
1	Infected	45.85	11.83	3.88	57.68
(32 days)	Control	49.92	11.02	4.53	60.94
2	Infected	43.18	8.68	4.97	51.86
(35 days)	Control	41.99	7.98	5.26	49.97
3	Infected	37.39	9.44	3.96	46.90
(38 days)	Control	36.92	7.86	4.70	44.78
4	Infected	36.41	7.52	4.84	43.93
(41 days)	Control	34.31	6.93	4.95	41.24

It is clear from this work that low levels of infection of up to 10% mildew intensity did not affect the growth and development of groundsel to any extent. The rate of senescence of the older leaves was the only significant effect of infection. The total number of green leaves per plant was not affected indicating that some degree of compensatory growth occurred to replace those lost through senescence.

On the other hand, heavy mildew infection (about 75% mildew intensity or more) severely retarded plant growth although the distribution pattern of dry matter did not appear to be affected, only the total amount available for distribution was reduced. Thus the overall growth patterns were similar between infected and control plants. The more rapid decline of the growth characteristic, specific leaf area of infected plants suggests a correspondingly faster rate of maturity. This was substantiated by the death of the infected plants at least two weeks earlier than the controls. The infected plants produced seeds which were lighter in weight than those from control plants. However, about 72% of them were viable.

The mildew colonies probably physically obstructed the plant's photosynthetic surface area and in this way limited the plant's capability of dry matter production. Even though this obstruction ultimately led to a drastic reduction of plant biomass, the pattern of distribution of dry matter amongst the various organs was not affected.

Mildew infection was consistently associated with slightly higher but not significantly higher concentrations of nitrogenous materials per unit weight of plant material than in controls. A large proportion of this was present in a soluble, non-protein form. It is not possible to say whether the larger concentrations result from the redistribution of hydrolised materials from the senescing tissues or from a greater uptake

from the environment by the roots.

Of the two experiments on the distribution of nitrogenous material between the various parts of the plant, only one gave any indication that infection alters the pattern of distribution of N-materials and thus interferes with the translocatory system. Thus, out of four experiments, two on the distribution of dry matter and the other two on the distribution of nitrogenous materials, only one gave any indication that infection might distort the translocatory patterns. This one experiment covered the growth period between the eighth and eleventh weeks when the plants had reached an advanced stage of growth and infected plants had lost about four times as many leaves through senescence than the controls. Thus, at this stage of growth, the aerial portion of the plant which is constituted largely of leaves was in a state of reorganisation of its stock of materials. It may thus be that the excess nitrogenous materials found in the roots of infected plants originated from the senescing leaves and was translocated to the roots since the aerial portion of the plant was not as active in function. Hence, as no direct effects was detected on those organs of the plant other than those bearing mildew colonies, the involvement of a toxin affecting the distribution of translocates can be ruled out. There is thus a degree of compatibility between the host and parasite in relation to translocation which indicates an appreciable level of tolerance of the host to the activities of the parasite.

APPENDICES

APPENDIX TABLES 1A AND 1B

Length of germ tubes of *E. cichoracearum* conidia after 24 and 48 hours of incubation at different temperatures.

Appendix Table 1A. Length of germ tube after 48 hrs (μ).

6°	11.7	11.7	11.7	11.7	11.7	11.7	8.8	11.7	11.7	8.8	11.7	M = 11.17	S.E. = 0.125
12°	23.4	26.3	23.4	23.4	23.4	23.4	20.5	23.4	17.6	17.6	20.5		
	23.4	20.5	20.5	23.4	20.5	23.4	23.4	20.5	23.4			M = 22.10	S.E. = 0.02
18°	23.4	23.4	23.4	23.4	23.4	35.1	29.3	26.3	23.4	29.3	23.4		
	29.3	32.2	26.3	23.4	23.4	23.4	23.4	32.2				M = 24.87	S.E. = 0.59
21°	23.4	23.4	23.4	20.5	26.3	23.4	17.6	17.6	23.4	26.3	23.4		
	23.4	23.4	20.5	35.1	35.1	23.4	29.3	23.4	23.4			M = 24.29	S.E. = 1.03
25°	23.4	29.3	29.3	29.3	23.4	35.1	23.4	29.3	41.0	29.3			
	23.4	29.3	29.3	23.4	35.1	23.4	29.3	23.4	29.3	23.4		M = 28.11	S.E. = 1.19
28°	23.4	29.3	26.3	23.4	29.3	23.4	23.4	26.3	29.3	23.4			
	29.3	26.3	17.6	26.3	17.6	29.3	17.6	32.2	23.4	26.3		M = 25.17	S.E. = 0.87
34°	No germination.												

Appendix Table 1B. Length of germ tube (μ) after 24 hrs.

Temperature												
6°	8.8	7.0	8.8	11.7	11.7	5.9						M = 8.97 S.E. = 1.00
12°	11.7	23.4	23.4	20.5	23.4	14.6	23.4	23.4	14.6	23.4	23.4	
	17.6	23.4	17.6	17.6	23.4	20.5	11.7	23.4	26.3			M = 20.34 S.E. = 0.9655
18°	23.4	29.3	17.6	17.6	17.6	24.6	17.6	29.3	23.4	17.6	17.6	
	29.3	23.4	17.6	23.4	29.3	35.1	17.6	29.3	23.4			M = 23.2 S.E. = 1.528
21°	23.4	17.6	23.4	20.3	17.6	23.4	11.7	23.4	29.3	23.4		
	17.6	23.4	23.4	29.3	29.3	26.3	23.4	29.3	23.4	29.3		M = 23.86 S.E.= 0.826
25°	23.4	23.4	23.4	23.4	20.5	23.4	23.4	23.4	29.3	26.3		
	17.6	29.3	17.6	29.3	23.4	29.3	23.4	23.4	17.6	23.4		M = 23.71 S.E. = 0.67
28°	23.4	23.4	23.4	17.6	20.5	17.6	17.6	5.9	17.6	23.4	23.4	
	23.4	20.5	17.6	17.6	23.4	17.6	17.6	20.5	20.5			M = 19.63 S.E. = 0.85

34° C No germination; conidia had shrivelled.

APPENDIX TABLES 2A AND 2B

Data on some primary values of growth of Benomyl-treated and non-treated groundsel plants.

Appendix Table 2A

		Fresh weight (g)				Dry weight (g)			
		Benlate-treated		Non-treated		Benlate-treated		Non-treated	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
1st Harvest	1	0.020	0.0320	0.018	0.0172	0.014	0.0138	0.021	0.0122
(7 weeks)	2	0.030	0.0149	0.020	0.0268	0.022	0.0180	0.020	0.0178
	3	0.016	0.0232	0.020	0.0171	0.009	0.0108	0.014	0.0101
	4	0.016	0.0137	0.025	0.0191	0.017	0.0115	0.013	0.0120
	5	0.027	0.0270	0.017	0.0173	0.013	0.0100	0.017	0.0110
	6	0.017	0.0180	0.018	0.0253	0.022	0.0078	0.008	0.0106
	7	0.026	0.0207	0.019	0.0185	0.012	0.0135	0.020	0.0136
	8	0.019	0.0164	0.016	0.0150	0.017	0.0067	0.010	0.0106
	Mean	0.021	0.0207	0.019	0.0195	0.016	0.0115	0.015	0.0122
	S.E.	0.002	0.001	0.001	0.002	0.002	0.0013	0.0017	0.001
2nd Harvest	1	0.038	0.0621	0.036	0.0642	0.029	0.0264	0.032	0.037
(8 weeks)	2	0.065	0.0400	0.036	0.0517	0.022	0.0368	0.029	0.0293
	3	0.031	0.0327	0.064	0.0442	0.020	0.0496	0.018	0.028
	4	0.047	0.0395	0.059	0.0495	0.021	0.0311	0.019	0.0312
	5	0.026	0.0754	0.042	0.0350	0.031	0.0255	0.017	0.0257
	6	0.049	0.0553	0.043	0.0714	0.026	0.0395	0.021	0.0444
	7	0.066	0.0377	0.042	0.0666	0.018	0.0382	0.018	0.0187
	8	0.032	0.0546	0.061	0.0535	0.036	0.0254	0.021	0.0384
	Mean	0.044	0.0497	0.048	0.0545	0.025	0.0341	0.022	0.0316
	S.E.	0.0054	0.0052	0.0041	0.0043	0.0022	0.003	0.002	0.0029

Appendix Table 2B(i) 1st Harvest (10 weeks)

Benlate treated

Replicates	Fresh weight (g)			Leaf area (sq.dm)	Dry weight (g)		
	Stem	Root	Leaves		Stem	Root	Leaves
1	-	-	-	-	-	-	-
2	1.2017	2.2575	5.5030	1.635	0.0792	0.1018	0.3352
3	1.3459	2.0488	6.8208	1.723	0.0782	0.0885	0.4101
4	0.8102	1.2836	4.8759	1.385	0.0495	0.0729	0.2928
5	1.0290	1.7625	4.9512	1.564	0.0589	0.0789	0.3316
Mean	1.0967	1.8381	5.5378	1.577	0.0665	0.0856	0.3425
S.E.	0.115	0.21	0.45	0.069	0.007	0.006	0.022
Control							
1	1.5645	1.8200	5.4714	1.728	0.0808	0.0904	0.3433
2	1.3482	1.9057	5.9910	1.762	0.0818	0.0887	0.3757
3	-	-	-	-	-	-	-
4	1.3181	2.1758	5.7661	1.533	0.0810	0.1135	0.3557
5	0.6304	1.2738	3.7370	1.061	0.0393	0.0577	0.2129
Mean	1.2203	1.7939	5.2414	1.521	0.0708	0.0876	0.3219
S.E.	0.192	0.189	0.512	0.161	0.01	0.01	0.036

Appendix Table 2B(ii) 2nd Harvest (12 weeks)

Benlate treated

Replicates	Fresh weight (g)			Leaf area (sq.dm)				No. of Lateral Shoots
	Stem	Root	Leaves		Stem	Root	Leaves	
1	2.6738	4.5050	12.499	2.485	0.1923	0.2231	0.8344	7
2	1.7297	2.8666	8.6351	2.448	0.1151	0.1360	0.5477	8
3	2.3038	3.7968	11.0954	3.112	0.1551	0.2003	0.7495	10
4	1.6694	3.1602	9.3896	2.581	0.1306	0.1748	0.6036	9
Mean	2.0942	3.5822	10.4056	2.657	0.1483	0.1836	0.6826	8.5
S.E.	0.24	0.363	0.864	0.151	0.014	0.017	0.065	0.645

Control

1	2.1529	3.0755	9.7100	2.741	0.1573	0.1585	0.6229	7
2	2.3651	4.0201	12.0471	2.734	0.1655	0.1972	0.7444	10
3	1.8337	3.3134	8.6005	2.438	0.1306	0.1467	0.5306	7
4	2.4118	3.8036	11.2052	2.961	0.1104	0.1792	0.6950	6
Mean	2.1909	3.5532	10.3907	2.719	0.1410	0.1704	0.6483	7.5
S.E.	0.131	0.217	0.77	0.103	0.01	0.01	0.046	0.87

APPENDIX TABLES 3A to 3D

Data on some primary values of growth of 5-10% *E. cichoracearum* infected and non-infected groundsel plants taken at weekly intervals during the growth period between the eighth and eleventh weeks.

Appendix Table 3A. 1st Harvest (8 weeks)

	Replicates	Stem Height (mm)	Fresh weight (g)			Leaf area (sq.dm)	Dry weight (g)			No. of senescent leaves	Total No. of leaves	Total plant dry wt. (g)
			Stem	Leaf	Root		Stem	Leaf	Root			
INFECTED	1	22	0.1292	0.5430	0.0876	0.111	0.0071	0.0392	0.0071	0	9	0.0534
	2	25	0.0651	0.2862	0.0456	0.221	0.0031	0.0218	0.0037	1	7	0.0286
	3	18	0.0638	0.2575	0.0538	0.108	0.0048	0.0296	0.0049	0	7	0.0383
	4	25	0.0800	0.3836	0.0501	0.198	0.0056	0.0045	0.0206	0	9	0.0307
	5	27	0.1470	0.5609	0.1172	0.132	0.0083	0.0468	0.0106	1	9	0.0657
	Mean	23.40	0.0971	0.4063	0.0709	0.154	0.0058	0.0284	0.0094	0.4	8	0.0434
	S.E.	1.568	0.017	0.063	0.014	0.022	0.0010	0.0073	0.0032	-	1.02	0.0070
CONTROL	1	22	0.0741	0.3329	0.00479	0.152	0.0047	0.0279	0.0048	0	10	0.0374
	2	20	0.0614	0.3055	0.0453	0.153	0.0034	0.0237	0.0038	0	8	0.0309
	3	25	0.0705	0.3503	0.0540	0.142	0.0040	0.0280	0.0055	0	9	0.0375
	4	23	0.0934	0.3960	0.0708	0.141	0.0046	0.0321	0.0066	0	9	0.0433
	5	18	0.0533	0.2985	0.0340	0.176	0.0036	0.0218	0.0031	0	9	0.0285
	Mean	21.6	0.0706	0.3367	0.0504	0.149	0.0041	0.0267	0.0048	0	9	0.0356
	S.E.	1.208	0.0066	0.0172	0.0061	0.008	0.0001	0.0018	0.0005	-	0.3	0.0023

Appendix Table 3B. 2nd Harvest (9 weeks)

	Replicates	Stem Height (mm)	Fresh weight (g)			Leaf area (sq.dm)	Dry weight (g)			No. of senescent leaves	Total No. of leaves	Total plant dry wt. (g)
			Stem	Leaf	Root		Stem	Leaf	Root			
INFECTED	1	46	0.3767	0.8438	0.4460	0.609	0.0304	0.1465	0.0339	3	11	0.2108
	2	42	0.2965	0.4873	0.3643	0.503	0.0245	0.1110	0.0290	2	12	0.1645
	3	34	0.2325	1.2370	0.1039	0.440	0.0200	0.1022	0.0157	3	9	0.1379
	4	38	0.2444	1.3587	0.3487	0.494	0.0202	0.1058	0.0291	2	10	0.1551
	5	-	-	-	-	-	-	-	-	-	-	-
	Mean	40.0	0.2876	0.9817	0.3158	0.512	0.0238	0.1164	0.0270	3	11	0.1671
	S.E.	2.58	0.0993	0.1980	0.0736	0.032	0.0024	0.0101	0.0037		1.12	0.0155
CONTROL	1	45	0.4668	2.1954	0.6159	0.803	0.0265	0.1646	0.0400	0	13	0.2311
	2	39	0.2755	1.5283	0.3106	0.586	0.0170	0.1119	0.0262	0	14	0.1551
	3	32	0.1700	0.8762	0.1536	0.335	0.0105	0.0642	0.0097	0	10	0.0844
	4	43	0.4200	1.9380	0.3040	0.684	0.0298	0.1392	0.0268	0	11	0.1958
	5	36	0.2395	1.3021	0.2664	0.450	0.0159	0.1080	0.0219	0	13	0.1458
	Mean	39	0.3144	1.5680	0.3301	0.572	0.0200	0.1176	0.0250	0	12	0.1625
	S.E.	2.34	0.0558	0.2325	0.0768	0.0821	0.0035	0.0168	0.0048		0.735	0.0246

Appendix Table 3C. 3rd Harvest (10 weeks)

	Replicates	Stem Height (mm)	Fresh weight (g)			Leaf area (sq.dm)	Dry weight (g)			No. of senescent leaves	Tota of l
			Stem	Leaf	Root		Stem	Leaf	Root		
INFECTED	1	66	1.0292	3.4304	1.3381	1.147	0.0896	0.1706	0.0984	4	13
	2	58	0.5179	2.1560	0.3330	0.699	0.0385	0.1983	0.0327	3	12
	3	34	0.3180	1.8481	0.3370	0.629	0.0301	0.3209	0.0392	2	12
	4	85	1.6840	4.7599	1.1607	1.393	0.1455	0.4308	0.0982	6	15
	5	50	0.5968	3.0328	1.3628	0.921	0.0457	0.2309	0.0875	3	13
	Mean	58.6	0.8292	3.0455	0.9064	0.9598	0.0699	0.2703	0.0712	4	13
	S.E.	8.45	0.2341	0.5152	0.2357	0.1382	0.0215	0.0474	0.0146	0.67	0.
CONTROL	1	60	0.6809	2.8150	0.9212	0.683	0.0492	0.2194	0.0677	4	13
	2	68	0.7632	2.3429	0.6787	0.788	0.1609	0.2238	0.0494	2	12
	3	62	0.9077	3.6000	1.3218	1.114	0.0673	0.2976	0.1004	1	18
	4	84	1.4777	5.1463	1.8967	1.405	0.1036	0.4114	0.1188	1	18
	5	62	0.8384	4.0182	1.1969	1.145	0.0581	0.2976	0.0861	2	15
	Mean	67.2	0.9336	3.5845	1.2031	1.027	0.0878	0.2900	0.0845	2	15
	S.E.	4.409	0.1411	0.4879	0.2061	0.130	0.0205	0.0347	0.0121	0.55	1.

Appendix Table 3D. 4th Harvest (|| weeks)

	Replicates	Stem Height (mm)	Fresh weight (g)			Leaf area (sq.dm)	Dry weight (g)			No. of senescent leaves	Total No. of leaves	Total plant dry wt. (g)
			Stem	Leaf	Root		Stem	Leaf	Root			
INFECTED	1	152	2.1743	4.0870	2.5455	1.489	0.1404	0.3407	0.1248	8	15	0.6059
	2	89	1.3144	5.2791	2.8297	1.854	0.0802	0.3627	0.1489	5	16	0.5918
	3	107	1.3792	4.3416	2.0177	1.508	0.0847	0.3072	0.1047	7	15	0.4966
	4	83	1.0571	3.6674	1.6388	1.219	0.0705	0.2612	0.0802	9	13	0.4119
	5	-	-	-	-	-	-	-	-	-	-	-
	Mean	107.75	1.4813	4.3438	2.2580	1.518	0.0940	0.3180	0.1147	7	15	0.5266
	S.E.	15.61	0.2411	0.3412	0.2660	0.1281	0.0156	0.0218	0.0145	0.85	0.63	0.0451
CONTROL	1	92	1.4700	5.1514	1.9686	1.681	0.0907	0.3488	0.1015	3	15	0.5410
	2	117	1.7990	6.0762	2.3448	1.790	0.1148	0.4172	0.1477	1	15	0.6797
	3	95	1.4596	5.5844	2.4209	1.625	0.1142	0.3779	0.1406	2	16	0.6327
	4	114	1.6032	5.0620	1.7760	1.507	0.1013	0.3382	0.1011	2	16	0.5406
	5	115	1.7790	6.0009	2.9016	1.753	0.1175	0.4063	0.1388	2	15	0.6626
	Mean	106.6	1.6222	5.5750	2.2824	1.671	0.1077	0.3777	0.1260	2	15	0.6114
	S.E.	5.3907	0.0725	0.209	0.1950	0.0516	0.0051	0.0153	0.0099		0.25	0.0293

APPENDIX TABLES 4A TO 4E

Data on some primary values of growth of 75% or more *E. cichoracearum* infected and non-infected groundsel plants taken at weekly intervals during the growth period between the seventh and eleventh weeks.

Appendix Table 4A. 1st Harvest (7 weeks)

	Replicates	Height (mm)	Fresh weight (g)			Dry weight (g)			Leaf area (sq.dm.)	No. of Leaves
			Stem	Root	Leaves	Stem	Root	Leaves		
INFECTED	1	28	0.3370	0.7964	2.6467	0.0234	0.0409	0.1682	0.771	27
	2	-	-	-	-	-	-	-	-	-
	3	26	0.4516	0.9586	3.9047	0.0250	0.0637	0.2671	0.997	34
	4	25	0.3678	0.7384	3.4275	0.0275	0.0492	0.2701	0.728	29
	5	26	0.3396	0.6353	2.7026	0.0215	0.0431	0.1904	0.716	25
	Mean	24.2	0.3275	0.7270	2.8017	0.0244	0.0539	0.2240	0.803	29
	S.E.	0.629	0.108	0.179	0.909	0.001	0.012	0.001	0.066	2
CONTROL	1	30	0.6300	-	6.4613	0.0534	0.0890	0.4489	1.580	37
	2	35	0.8621	-	5.8515	0.0474	0.0613	0.3728	1.189	36
	3	35	0.7542	-	5.8073	0.0652	0.1107	0.4171	1.228	35
	4	28	0.5942	-	5.5663	0.0446	0.0800	0.3432	1.271	36
	5	35	0.6965	-	5.6360	0.0495	0.0756	0.3664	1.284	36
	Mean	32.6	0.7074	-	5.8645	0.0521	0.0634	0.3697	1.310	36
	S.E.	1.503	0.048	-	0.158	0.003	0.028	0.065	0.071	0.316

Appendix Table 4B. 2nd Harvest (8 weeks)

	Replicates	Height (mm)	Fresh weight (g)			Dry weight (g)			Leaf area (sq.dm)	No. of Flowers	No. of Senescent Leaves
			Stem	Root	Leaves	Stem	Root	Leaves			
INFECTED	1	58	1.3819	0.9619	4.0065	0.1205	0.0919	0.3356	0.956	18	17
	2	48	1.3933	0.9286	4.2808	0.1351	0.0873	0.3927	-	12	14
	3	53	1.4721	1.0903	3.7292	0.1289	0.0792	0.3176	0.875	18	8
	4	64	2.5674	1.8118	6.6193	-	-	-	-	27	9
	5	65	2.4618	1.2991	5.9042	0.2105	0.0895	0.4077	1.202	20	10
	Mean	57.6	1.8553	1.2184	4.9080	0.1489	0.0870	0.3634	1.011	19	12
	S.E.	3.234	0.270	0.162	0.571	0.021	0.003	0.021	0.098	2.41	0.71
CONTROL	1	74	2.4823	3.4708	12.4162	0.2989	0.3066	1.0756	-	48	3
	2	69	4.8744	2.2622	11.2350	0.4893	0.1654	0.7338	1.955	49	2
	3	83	3.7875	2.1835	10.6393	0.3626	0.2172	0.8702	-	44	2
	4	74	3.7096	2.6371	11.8840	0.3692	0.2430	0.9504	2.255	27	4
	5	72	3.0938	2.0635	11.2067	0.2567	0.1709	0.7336	2.178	38	1
	Mean	74	3.5896	2.5235	11.4763	0.3554	0.2227	0.8728	2.129	41	2.4
	S.E.	4.506	0.398	0.255	0.306	0.039	0.025	0.065	0.094	4.52	0.51

Appendix Table 4C. 3rd Harvest (9 weeks)

	Replicates	Height (mm)	Fresh weight (g)			Dry weight (g)			Leaf area (sq.dm)	No. of flowers	No. of Senescent Leaves
			Stem	Root	Leaves	Stem	Root	Leaves			
INFECTED	1	90	2.8384	1.2873	3.2728	0.2687	0.1100	0.2973	1.071	33	24
	2	82	2.3568	1.1836	3.2866	0.2237	0.0900	0.3150	-	30	21
	3	84	2.3495	0.8883	3.0115	-	-	-	1.002	25	23
	4	84	2.0145	1.0481	3.0192	0.2105	0.0930	0.2842	-	22	25
	5	80	1.9691	1.1363	3.1481	0.1907	0.0953	0.2871	1.051	18	22
	Mean	84	2.3057	1.1088	3.1488	0.2234	0.0973	0.2959	1.041	26	23
	S.E.	1.673	0.156	0.067	0.041	0.017	0.002	0.007	0.028	1.449	0.707
CONTROL	1	105	7.5072	4.0810	15.6765	0.6743	0.2781	0.9927	2.750	74	4
	2	100	6.5492	3.1530	11.2359	0.7379	0.2512	0.8416	-	79	7
	3	105	8.1846	3.0718	12.6085	0.8512	0.2381	0.7955	-	67	7
	4	95	5.2422	2.5022	9.3257	0.5522	0.1927	0.7575	1.908	-	8
	5	95	5.9034	3.4914	11.2426	0.5845	0.2996	0.8922	1.978	-	6
	Mean	100.0	6.6774	3.2599	12.0179	0.6801	0.2520	0.8559	2.212	73	6
	S.E.	2.236	0.531	0.259	1.053	0.054	0.018	0.041	0.269	3.48	1.30

Appendix Table 4D. 4th Harvest (10 weeks)

	Replicates	Height (mm)	Fresh weight (g)			Dry weight (g)			Leaf area (sq.dm)	No. of flowers	No. of senescent leaves
			Stem	Root	Leaves	Stem	Root	Leaves			
INFECTED	1	94	2.7213	0.9770	2.0992	0.2904	0.0802	0.2487	0.577	36	25
	2	114	3.8892	1.3960	2.3491	0.4413	0.1118	0.2726	0.477	49	22
	3	107	3.270	1.2973	1.1316	0.3371	0.1030	0.1425	-	41	27
	4	100	2.3544	0.6006	1.3214	0.2493	0.0559	0.1987	-	38	27
	5	98	3.7028	1.1148	1.5571	0.3907	0.1224	0.1882	0.399	32	30
	Mean	102.6	3.1877	1.0772	1.6917	0.3418	0.0947	0.2102	0.4843	39	26
	S.E.	3.544	0.289	0.167	0.231	0.034	0.019	0.023	0.052	2.85	2.09
CONTROL	1	138	11.3986	3.7598	16.4880	1.1206	0.2592	1.0900	-	110	5
	2	140	11.4162	3.8346	15.2588	1.1001	0.3126	1.0513	-	114	6
	3	128	13.2417	3.3859	18.0838	1.2827	0.3193	1.1896	3.374	136	9
	4	156	17.5268	4.0643	20.5786	1.4986	0.3807	1.4184	3.907	154	8
	5	128	11.5142	2.5192	17.7398	1.0779	0.2517	1.2925	2.788	120	9
	Mean	138	13.0195	3.5128	17.6298	1.2160	0.3047	1.2084	3.356	127	7
	S.E.	5.14	1.179	0.271	0.890	0.079	0.023	0.067	0.178	7.290	0.81

Appendix Table 4E. 5th Harvest (11 weeks)

	Replicates	Height (mm)	Fresh weight (g)			Dry weight (g)			Leaf area (sq.dm)	No. of side branches
			Stem	Root	Leaves	Stem	Root	Leaves		
INFECTED	1	111	3.3320	1.1845	0.8848	0.3936	0.0878	0.1361	0.20	9
	2	144	6.8400	1.6569	2.1508	0.7271	0.1221	0.2512	0.36	11
	3	142	4.9743	1.1662	2.0274	0.6008	0.1098	0.2559	0.37	12
	4	112	2.8710	0.5180	0.9634	0.3281	0.0449	0.1188	0.22	9
	5	117	3.2069	0.9298	0.8384	0.3504	0.0723	0.1103	0.25	8
	Mean	125.2	4.2448	1.0910	1.3730	0.4800	0.0874	0.1745	0.28	10
	S.E.	7.344	0.744	0.186	0.294	0.078	0.014	0.033	0.036	0.74
CONTROL	1	190	24.6644	4.7740	25.5038	2.4829	0.3445	1.7015	-	21
	2	176	21.9883	5.0733	21.4280	2.0013	0.3569	1.6372	3.25	20
	3	160	23.0577	4.4958	-	2.0910	0.2830	1.3016	-	20
	4	149	24.5306	4.6067	20.5280	2.2884	0.3269	1.5234	2.78	21
	5	200	21.0205	5.5888	17.3242	2.2609	0.4175	1.3682	2.68	17
	Mean	175	23.0523	4.9002	21.1962	2.2249	0.3458	1.5064	2.903	20
	S.E.	9.359	0.709	0.239	1.684	0.084	0.022	0.076	0.178	0.73

Appendix Table 5A. Concentrations of alcohol soluble and insoluble nitrogenous fractions in roots and shoots of mildew infected and non-infected groundsel plants.

	Replicates	S H O O T				R O O T			
		Infected		Control		Infected		Control	
		Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
1st Harvest (32 days old)	1	7.14	31.19	6.21	34.86	5.49	14.82	4.96	15.82
	2	6.73	29.10	5.91	32.72	5.60	15.53	4.85	17.50
	3	6.18	31.37	6.51	32.21	4.36	15.56	4.65	16.67
	Mean	6.68	30.55	6.21	33.26	5.15	15.30	4.82	16.66
2nd Harvest (35 days old)	1	4.70	30.13	4.20	27.21	3.85	13.54	-	-
	2	4.65	30.90	5.08	29.38	4.17	14.17	-	-
	3	4.56	29.46	4.85	28.92	3.83	16.63	3.50	13.30
	4	4.80	29.97	4.94	30.64	3.84	12.28	3.72	13.77
	5	5.63	28.17	3.37	26.92	3.28	13.56	3.61	13.81
	6	5.05	26.73	3.78	26.82	3.69	13.49	-	13.78
	Mean	4.90	29.23	4.37	28.32	3.78	13.95	3.61	13.67
	S.E.	0.15	0.59	0.29	0.60	1.69	0.57	0.06	0.18

Appendix Table 5B.

	Replicates	SHOOT				ROOT			
		Infected		Control		Infected		Control	
		Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
3rd Harvest (38 days old)	1	5.60	29.62	5.51	25.35	4.35	12.75	3.03	12.13
	2	5.58	30.36	5.45	24.16	4.17	14.17	2.87	11.49
	3	5.78	22.93	5.20	25.35	3.82	10.53	3.21	12.20
	4	5.55	23.51	4.92	29.01	2.87	10.33	2.79	12.00
	5	5.88	22.72	4.27	22.33	3.43	11.67	1.06	12.42
	6	5.79	23.68	4.44	21.94	3.79	11.44	4.38	13.13
	Mean	5.70	25.57	4.97	24.69	3.74	11.82	2.89	12.23
	S.E.	0.07	1.03	0.19	1.05	0.21	0.57	0.44	0.20
4th Harvest (41 days old)	1	5.55	27.79	4.32	24.42	2.23	10.18	2.55	11.47
	2	4.33	27.72	4.26	24.76	2.46	11.17	2.96	11.14
	3	4.65	22.81	4.81	24.50	2.19	10.31	3.47	12.48
	4	5.50	21.59	4.77	23.45	2.24	11.98	3.05	11.99
	5	4.26	23.33	3.71	21.16	3.89	14.58	2.05	9.11
	6	4.24	23.12	3.16	21.89	3.55	14.09	2.47	9.53
	Mean	4.76	24.36	4.17	23.36	2.76	12.05	2.76	10.95
	S.E.	0.23	1.23	0.27	0.64	0.31	0.78	0.20	0.57

Appendix Table 5C. Dry weight data of roots and shoots of mildew infected and non-infected groundsel plants used for the determination of soluble and insoluble Nitrogenous fractions.

1st Harvest (50% mildew intensity

Replicates	Dry weight (g)			
	Control		Infected	
	Shoot	Root	Shoot	Root
1	0.5775	0.1830	0.5129	0.1812
2	0.5271	0.1422	0.5500	0.1517
3	0.5024	0.1313	0.4886	0.1402
Mean	0.2678	0.0761	0.2586	0.0789

The replicates were paired and so the means from the data were divided by two.

2nd Harvest (75% mildew intensity)

Replicates	Dry weight (g)			
	Control		Infected	
	Shoot	Root	Shoot	Root
1	0.3912	0.0767	0.2998	0.1001
2	0.3220	0.0774	0.2963	0.0860
3	0.3922	0.1359	0.2781	0.0798
4	0.3631	0.0684	0.2900	0.0617
5	0.3328	0.0936	0.3308	0.0823
6	0.3497	0.1206	0.3052	0.0863
Mean	0.3585	0.0954	0.3000	0.0794

Appendix Table 5D.

3rd Harvest (75% mildew intensity)

Replicates	Dry weight (g)			
	Control		Infected	
	Shoot	Root	Shoot	Root
1	0.4101	0.1649	0.3276	0.1191
2	0.3775	-	0.4908	0.1142
3	0.4169	-	0.3387	0.1487
4	0.3860	0.1391	0.3725	0.1428
5	0.4438	0.1308	0.4185	0.1019
6	0.4748	-	0.4052	0.1211
Mean	0.4182	0.1449	0.3922	0.1246

4th Harvest (about 90% mildew intensity)

Replicates	Dry weight (g)			
	Control		Infected	
	Shoot	Root	Shoot	Root
1	0.5605	0.1661	0.2616	0.0905
2	0.3735	0.1023	0.4681	0.1553
3	0.5252	0.1523	0.4504	0.1653
4	0.3741	0.0965	0.3806	0.1082
5	0.4809	0.1756	0.3607	0.0894
6	0.5008	0.2289	0.3912	0.1152
Mean	0.4692	0.1503	0.3854	0.1207

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