# STUDIES ON RESISTANCE AND OVERWINTERING IN HOP POWDERY MILDEW (*SPHAEROTHECA HUMULI* (DC) BURR)

by

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## ABSTRACT

The development of S. humuli on hop leaves was analysed with eight isolates, collected from various regions, and with several cultivars/seedling hops containing either no resistance genes or one or more of three known major genes for resistance. Three mechanisms of resistance were discovered. (1) Controlled by gene $I_1$, in which epidermal cells reacted hypersensitively immediately after penetration; haustoria were degenerated allowing no colony development. Originally this reaction occurred under natural conditions at Wye College but selection then resulted in the appearance of a new strain to which the $I_1$ genotype was susceptible. (2) Controlled by gene B which causes a bare 'blister' to appear on an infected leaf in the glasshouse. Here, hypersensitivity was relatively delayed until after the formation of haustoria, only a proportion of which remained functional; limited hyphal growth and sparse sporulation resulted. (3) Controlled by gene $I_2$, where an immediate hypersensitivity resulted as a response to attempted penetration; no haustoria were observed and there was no hyphal development.

Among the isolates, four strains were recognised by their behaviour on the host genotypes. One of them behaved like the strain prevalent at Wye College before 1968 and to which the $I_1$ gene was effective.

Experiments showed that S. humuli can overwinter as cleistocarps in hop debris and as mycelium in dormant rootstock buds. Among cleistocarps overwintered in a hop garden, a few had dehisced naturally, and others were induced to release ascospores in the laboratory, only after April each year. Ascospores germinated in the laboratory but failed to infect leaves. However, leaf infections in the field were shown to have originated from ascospores.

Buds bearing external mycelium in autumn and overwintered naturally produced either infected or healthy shoots in spring, or died. Infected shoots originated from mycelium established between the bud scales.

Of over 40 species of hop garden weeds inoculated with S. humuli none were infected.

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# INTRODUCTION

*Humulus lupulus* L., the cultivated hop, and *H. japonicus* Sieb & Zucc., the Japanese hop, are members of the family *Cannabinaceae* which contains one other genus, *Cannabis* represented solely by *C. sativa* L., hemp.

*H. lupulus* is widely distributed and is grown commercially in the U.S.A., Canada, Argentina, South Africa, Japan, Australia, New Zealand and in most European countries. Following their introduction to England, probably from Flanders in 1524 (Simmonds, 1877), hops were grown commercially in many counties, but today commercial cultivation is confined almost entirely to Kent, Sussex and Hampshire in the south and to Herefordshire and Worcestershire in the West Midlands, though wild hops of this species are to be found in hedgerows throughout England.

The hop plant consists of a perennial, woody rootstock bearing numerous dormant buds which develop into annual climbing shoots in spring. During early May eight selected shoots of each plant are trained usually on to four strings supported on an overhead wirework system 14-18 ft. (4.3-5.5 m) high. All the remaining shoots are removed. After the stems ('bines') have reached the top wire in late June, lateral shoots grow from the leaf axils to bear subsequently most of the crop. By tradition, the lower laterals and foliage within about 4 ft. (1.2 m) of the ground are removed ('stripped') by mid-July to allow air to circulate more freely within the hop garden.

The hop is dioecious. The female flowers ('burr') which later develop into cones, the harvested crop, arise in clusters in the axils of lateral shoots. They first appear from mid-July, the precise time varying according to the season and the variety grown. Each inflorescence consists of a short central axis ('strig') bearing alternate pairs of

stipular bracts, each subtending a pair of bracteoles which enclose the female flowers. The bracts and bracteoles possess at their bases, lupulin glands containing resins and the essential oils. The former are the precursors of the bitter principles of beer and the latter impart the characteristic aroma. In English hop growing occasional male plants are grown amongst the female ones. These provide pollen which fertilise the female flowers which then mature during August in a seeded condition. Elsewhere hops are grown seedless, without male plants. In early September the cones become ripe and are harvested by removing entire bines to a machine.

Where clean cultivation of a hop garden is practised, the plants are earthed up in July and the bases of the current seasons' bines, buried within the soil ridge, thicken just prior to the onset of the dormancy period (Williams, 1960), forming a perennial stem extension to the rootstock. The thick, fleshy buds on these swollen stem bases and on the rootstock remain dormant until March-April when the annual growth cycle resumes. The soil ridges are cleared away in February/March and the swollen stem bases are pruned from the crowns, a process known as 'dressing'. Strap cuts make excellent vegetative sources of new plants both in commerce and for experimental use in pots.

Because of the desire by hop growers to reduce labour wherever possible clean cultivation has largely been replaced in the last ten years by the use of herbicides for weed control. As a result, thickened shoot bases no longer develop and the withered remains of the current year's growth are merely snipped off at a convenient time after harvest. Although most dormant buds are beneath ground level, some are present on the crown tissue at, or just above, soil level.

Fungal diseases have been major factors limiting hop production for many years. The lethal progressive wilt and less destructive fluctuating wilt (Verticillium spp.) are most serious. Downy mildew (Pseudoperonospora humuli) arises every year in most hop gardens and can cause severe damage to growth and result in loss of yield in years favourable to the disease. Powdery mildew of hops, caused by Sphaerotheca humuli (DC.) Burr., has been known in Britain for over 150 years (Whitehead, 1887) and in the last decade has so increased in severity to become of great concern to growers. The disease exists in two forms: (a) as discrete pustules of the asexual stage which often coalesce into larger colonies, this form is often called by growers 'white mould' and occurs on the leaves, young basal and lateral shoots, inflorescences and on the developing cones. (b) as the sexual stage (cleistocarps) which develops mainly on cones but also on leaves after vegetative hop growth has ceased; as a result foxy-red patches arise on affected tissues, hence the term 'red mould' by growers and in most popular accounts.

The asexual stage of powdery mildew first appears on hops in spring as two distinct symptoms. Most commonly discrete, white, sporing pustules appear on expanding leaves of basal shoots near the ground in late April (Plate 1) or on leaves higher up the trained bines in mid-May. General secondary leaf infection, as sporing pustules, is normally found shortly afterwards. Alternatively, and less frequently, a small proportion of the shoots arising from rootstock buds appear smothered in a blanket mycelium of S. humuli which spores actively (Plate 2). These also act as sources of secondary infections which soon follow. The incidence of these primary shoot infections, which have become known as 'primary mould', has increased in recent years and is almost always encountered in non-cultivated gardens.

Plate 1 Discrete sporing colonies (arrowed) on young expanding leaves of cv. Northern Brewer in a non-cultivated hop garden in the spring. Note the cone debris on the soil surface. Most of these cones contained mature cleistocarps.

Plate 2 'Primary mould' infection of shoots (arrowed) on a plant of cv. Northern Brewer in a non-cultivated hop garden. The heavily infected shoots are completely smothered with actively sporing mycelium and very often their growth is retarded. Some shoots are infected only at their bases and were apparently growing away from the infection.


1


2

As the season proceeds powdery mildew infection progresses gradually up the growing plant on both surfaces of the leaves, colonies coalesce to form large white powdery patches (Plate 3) and eventually the infected areas become necrotic. Leaf infection is thought not to cause a reduction in the growth of the plant but is potentially serious because of the risk of having high inoculum when the inflorescences are formed. The 'burr' is particularly susceptible to infection (Plate 4) and young cones may become partially or completely shrivelled (Plates 5 & 6), a common feature of an early attack. If the flowers and young cones escape infection, maturing cones may later be attacked (Plate 5) resulting in little distortion but a brown discolouration accompanied by an undesirable early 'ripening'. This effect was first observed by Hammond in 1900. Coley-Smith (1964) showed that it was associated with the presence of the sexual stage of fungus which commonly develops also on both cones and leaves infected with 'white mould' (Plates 6 & 7). Early infection of the cones or late infection causing premature ripening may lead to a considerable loss in yield and a lowering of the quality of the crop.

The most significant gaps in our knowledge of the life cycle of S. humuli lie in the methods of overwintering and initiation of first infections in spring. Until recently the 'primary mould' symptom had never been recorded and the discrete spots which characterise the appearance of the disease were considered to arise from ascospores released from dehisced cleistocarps (Salmon, 1907; Blodgett, 1913, 1915). The incidence of 'primary mould' has increased in the last decade. Such symptoms, suggestive of mycelium hibernating in dormant buds, are almost always found in non-cultivated gardens where some rootstock buds are exposed to conidial infection after their formation the previous year. Section II of this thesis considers methods of overwintering of S. humuli.

Plate 3 A leaf of susceptible hop cv. Northern Brewer very heavily infected with powdery mildew as actively sporing colonies which have coalasced.

Plate 4 The female flowers or 'burr' of cv. Northern Brewer infected with S. humuli (I) in July. Healthy flowers (H) are illustrated for comparisons.


3


I
H
4

Plate 5 Cones of cv. Northern Brewer at harvest showing various grades of cone infection. The cones infected early in their formation are distorted and malformed, while those infected late have only a few infected bracts and bracteoles and show little distortion. Some of the cones possess cleistocarps (arrowed).


5

Plate 6 Cones of cv. Northern Brewer in early August infected heavily and bearing many cleistocarps which impart a black discolouration.

Plate 7 Abaxial surface of a leaf of cv. Northern Brewer heavily infected and bearing many cleistocarps.



6



7

Until recently all hop cultivars grown in this country were susceptible in various degrees to powdery mildew. A number of cultivars most favoured by brewers or growers at the present time are also the most susceptible, in particular Northern Brewer and OT 48 (Brambling Cross). Control therefore relies heavily on a regular and routine programme of fungicide sprays and dusts. However, whereas regular applications of dinocap/sulphur once gave successful control, this is often now no longer possible. As a result, considerable losses in crop have been experienced, especially in 1971. The increase in severity of powdery mildew has been attributed to three main factors: (a) the gradual changeover from clean cultivation to non-cultivation with herbicides; this has allowed some of the buds that produce new shoots in the following spring to be exposed continuously to airborne inoculum, and also has encouraged infected cone debris to remain on the soil surface, (b) the increased use of dithiocarbamate fungicides rather than copper materials for downy mildew control which causes softer foliage, more susceptible to S. humuli, and probably also loss of direct fungicidal action by copper (Royle & Griffin, 1973), (c) increased cultivation of the highly susceptible cultivars Northern Brewer and OT 48.

The recent experiments with new systemic fungicides e.g. pyrazophos (Royle & Liyanage, 1973) has improved the prospects of achieving better chemical control of hop powdery mildew. However, the alternative approach to control lies in the breeding of resistant cultivars and during the last seven years Neve has successfully combined resistance to powdery mildew and other diseases with other desirable attributes, such as high resin content and early maturity, through a process of hybridization and selection. This has resulted in the introduction of new cultivars such as Wye Target, resistant to Verticillium wilt and to powdery mildew, Wye

Challenger, resistant to both powdery and downy mildews and Wye Northdown, resistant to downy mildew.

A certain amount is known about the genetic systems controlling powdery mildew resistance in hops. The extent of powdery mildew infection developing on a particular cultivar depends on the host compatibility and aggressiveness of the pathogen. Therefore, the known genetic systems operating in different cultivars can be exploited to best advantage in planning a hybridisation programme only if the pathological basis of resistance is understood. Such a study must include a consideration not only of the mechanisms of host responses to the infection by the fungus but also of pathogenic variability of different isolates of the fungus. These aspects are reported in Section I of the thesis.

## NOMENCLATURE

For over thirty years after the publication of Salmon's monograph on the Erysiphaceae in 1900, workers, for the most part, accepted his taxonomic system which he based on the American Code of Botanical Nomenclature. In 1933 Blumer published his Die Erysiphaceen mitteleuropas, which then served as the standard work on the Erysiphaceae. Its treatment of the species concept as well as its system of nomenclature has been generally accepted by subsequent authors. Unfortunately Blumer's nomenclature was not in accordance with the International Code of Botanical Nomenclature laid down in 1961. This was based on Fries' Systema mycologicum, which appeared in 1821.

This confusion in the taxonomy of the Erysiphaceae has resulted in several synonyms being applied to the hop powdery mildew pathogen. Thus it is opportune to review briefly the history of its nomenclature and to decide on the correct specific epithet.

Sphaerotheca castagnei Lév., was considered to be an aggregate species comprising two forms, on different host plants, in which there were constant differences in the size of cells forming the outer cleistocarp wall (Burrill & Earle, 1877). They divided S. castagnei into two species, S. humuli DC. and S. castagnei, based on the size of the cells of the outer cleistocarp wall, the nature of the appendages and the size of ascospores. Salmon (1900) made measurements of cleistocarp wall cells and lengths of ascospores of S. humuli and found them to be less than 15 µm and 20 µm respectively. The appendages were straight and slender, three or more times as long as the diameter of the cleistocarp and usually dark brown in colour when mature. Similar measurements made on S. castagnei showed cells of the cleistocarp wall to be 20-30 µm and the mean ascospore length about 15 µm. Appendages were long, stout and also flexuous; they were usually light brown in colour.

He found that, on the whole and with a few rare exceptions, these differences were constant and strongly marked. However, Salmon considered that other separative characters proposed by Burrill were more variable and therefore not reliable for separation of the species. Bouwens (1927) however, considered that the dimension of cleistocarp wall cells should not be used as a diagnostic character in the separation of these two fungi. Salmon then separated Léveillé's S. castagnei into S. humuli (DC.) Burr., and S. humuli var. fuliginea Schlecht., giving precedence to the oldest specific name from Erysiphe humuli (De Candolle, 1815) and deriving the varietal name from Alphitomorpha fuliginea (Schlechtendal, 1819). Therefore, according to Salmon's classification the correct name for the causal agent of strawberry and hop mildews is S. humuli (DC.) Burr.

Kuntze (1892) considered that the name Sphaerotheca for the present genus must be renamed Albigo. He then transferred all the species of Sphaerotheca mentioned in Saccardo's Sylloge to Albigo. For unspecified reasons Magnus (1899) stated that Albigo has no claim and instead suggested that Sphaerotheca macularis (Ehrh.), should be employed in place of S. humuli. Neither Kuntze nor Magnus, however, traced the origin of the name Albigo. Ehrhart (1792) used the term Albigo to cover all powdery mildew fungi, but no evidence was found to consider it as a genus. Thus, there were no reasons for substituting Albigo for Léveillé's Sphaerotheca. The name S. macularis (Ehrh.) for hop mildew which Magnus had supposed should supercede S. humuli (DC.) Burr., also has no claim for recognition. The strawberry mildew fungus was investigated by Jaczewski (1927) and later by Blumer (1933) who suggested that the name should be attributed to Jaczewski (S. macularis (Wallr.) Jaczewski).

Following a decision taken at the meeting of the International Botanical Congress in 1950, Cook (1952) proposed the name S. macularis (Wall. ex Fries)

having overlooked Jaczewski's proposal but recognising Fries' (1829) validation of Wallroth's epithet macularis. Measurements of cleistocarps, asci and ascospores made by Peries (1961b) agreed with that of Jaczewski and he therefore considered that according to the International Code, the strawberry mildew fungus should be S. macularis (Wall. ex Fries) Jaczewski.

Coley-Smith (1964) preferred to use the name S. macularis (Wall. ex Fries) Jaczewski, instead of retaining the name S. humuli (DC.) Burr., for the hop powdery mildew fungus. Measurements of cleistocarps, asci and ascospores made during the present investigation generally agreed with those given by Blodgett (1913) for S. humuli on hops. It was also observed that the appendages were long and slender and not flexuous as Peries (1961b) reported for S. macularis. In addition inoculation of strawberry leaf disks with S. humuli showed no development beyond germ tube production and a hypersensitive reaction of the epidermal cells of strawberry leaves was observed. A similar reaction was observed by Peries (1961b) when hop leaves were inoculated with S. macularis from strawberry and a hypersensitive reaction developed in response to infection. Therefore, the powdery mildew fungi attacking hop and strawberry should be distinguished as the separate species S. humuli (DC.) Burr. and S. macularis (Wall. ex Fries) Jaczewski, respectively.

## SECTION - I

# REVIEW OF LITERATURE

## History of resistance to powdery mildew in hops.

Resistance in the hop to infection by S. humuli was first noticed by Salmon in 1914 (Salmon, 1917). By exposing hop seedlings to heavy inoculum from infected plants in a glasshouse and by inoculating conidia on to detached leaves in humidity chambers he showed that certain seedlings of hops appeared to be immune. These included some wild hop seedlings from Italy which remained persistently immune under glasshouse conditions. However, these same seedlings grown outdoors showed a slight infection of lateral shoots in the autumn of 1916, though they showed no trace of mildew during the previous summer despite being surrounded by heavily infected plants in the hop garden. Under glasshouse conditions several other seedlings of the same age and parentage became severely infected with mildew. A similar type of immunity as that shown by the wild Italian hops was also exhibited under glasshouse conditions by a female variety with yellow leaves, known as 'golden hop'.

Salmon (1919) later divided various seedlings into four groups on the basis of their responses to infection: (i) Certain wild hop seedlings were persistently immune in the glasshouse for three consecutive years 1914-18 under very heavy infection pressure. (ii) Other seedlings which were immune in 1918 in the glasshouse, remained so after being grown for four years in a hop garden. (iii) Another group of seedlings, persistently immune in the glasshouse in 1914, were susceptible when grown outdoors during 1916-18; in some cases they became severely infected. (iv) In the glasshouse, the leaves of some seedlings reacted to infection by forming humps or blisters. On the surface of the blisters a weak, faintly white growth of mycelium was produced, eventually dying away to leave minute brown patches of apparently dead epidermal cells. This was designated semi-immunity.

Salmon (1920) also observed another form of semi-immunity under glasshouse conditions exhibited by a seedling derived from the variety neo-mexicanus of H. lupulus. Here a barely visible growth of mycelium developed which eventually perished leaving green or yellow, semi-translucent patches. There was no evidence of blisters . He observed the same phenomenon, although less marked, in the variety neo-mexicanus itself under glasshouse conditions.

At the time of making this classification Salmon had not exposed group (i) seedlings to infection in a hop garden. No subsequent reference to their performance under these conditions has been found in the literature. It is likely, therefore, that these seedlings were similar in reaction to those in either group (ii) or (iii).

None of these seedlings were developed as commercial varieties by Salmon, but he retained selections of them which were subsequently used in a recent programme of breeding for powdery mildew resistance by Neve who has encountered reactions similar to those described by Salmon and has been able to establish the genetic basis of those reactions.

In his first report, Neve (1965) stated that two small families from crosses between downy mildew-resistant females and powdery mildew-resistant males were either highly resistant or highly susceptible with no intermediate types appearing. Reporting on a further series of crosses he later commented (1966) that the results suggest two types of resistance are involved: (a) a near-immunity reaction in which the plants remain completely free from infection except under abnormal environmental conditions; this appears to be controlled by a single pair of genes with that for near-immunity being dominant, and (b) a field resistance with various levels of susceptibility, probably polygenically controlled.

In a report on glasshouse testing of seedling material Neve (1968) then differentiated between three reactions thus: (a) infected seedlings gave rise to raised blisters on the surfaces of which a thin mycelium supported very sparse sporulation. This reaction was attributed to a dominant major gene closely linked with a gene controlling the production of selinene in the essential oil. The plants showing the blister reaction under glass apparently remained completely free from infection in the field. (b) a complete resistance exhibited by seedlings with or without selinene; the former were assumed to carry also the gene responsible for the blister reaction. (c) a partial resistance probably controlled polygenically.

In 1968 the population of S. humuli prevalent in the Hop Department glasshouses no longer induced the complete resistance mechanism in those seedlings which had reacted in this manner the year before. However, the blister reaction remained unaltered. Previously immune plants then exhibited either a blister reaction, as typified by the seedling 11/66/28, or became susceptible as in the case of seedling 14/66/50.

After the study described in this thesis was well underway, tests carried out in 1971 established the existence of another dominant major gene for powdery mildew resistance which confers an immune reaction, apparently based on hypersensitivity (Neve, 1972). This gene is the basis of resistance in a new hop variety, Wye Target, released in 1972 and therefore not part of the early studies reported herein. Like the blister gene, this gene was also now known to have been present in breeding material at Wye College for a long period since it was traced through three generations to male OB 79 which was raised from a wild species of neo-mexicanus by Salmon in 1936.

In addition to these major genes, the field or partial resistance recognised earlier has been attributed to a polygenic system of control.

Recently Liyanage, Neve & Royle (1973) have assigned names to the three major genes for resistance which have been recognised so far.

1. A dominant gene conferring hypersensitivity to infection by some fungal populations while it broke down to others. This is designated Immunity 1 ($I_1$).

2. A dominant gene conferring resistance to all isolates to which it is exposed. This is termed Immunity 2 ($I_2$).

3. A major gene which shows up as a blister in the glasshouse and is therefore referred to as Blister (B).

Physiologic races of powdery mildews.

Many species of powdery mildew fungi comprise a number of specialized forms which are morphologically identical but have different infective powers. Race differentiation in the Erysiphaceae was first established in the genus Erysiphe by Neger (1902). Soon afterwards Marchal (1902, 1903), published his works on host-specialization in Erysiphe graminis. The forms distinguished by Marchal existed in a number of physiologic races each restricted to a group of host varieties (Salmon, 1903 , 1904a,b; Reed, 1905). The occurrence of physiologic races of E. graminis f. sp. tritici attacking wheat, was first reported by Waterhouse (1930) in Australia and Mains (1933) in the United States. Similarly, Mains & Dietz (1930) provided evidence for the presence of physiologic races in barley. In Germany field surveys were initiated by Schlichtling (1938) who differentiated six physiologic races in wheat. Later, using differential host varieties Leijerstam (1965) identified thirty eight races affecting wheat in North-Western Europe (cited by Wolfe, 1967).

In the genus Sphaerotheca there are no reports of the existence of physiologic races. Peries (1961b), carried out an experiment by inoculating six strawberry varieties with two isolates of Sphaerotheca macularis to determine whether physiologic races exist. However, he

found no significant differences in either the patterns of symptom production or their virulence towards the different host varieties used.

The apparent change from immunity to susceptibility of persistently immune hop seedlings when transferred from glasshouse to hop garden and conversely of the change from susceptibility to immunity when the plants were brought back from the hop garden to the glasshouse led Salmon (1919) to suspect that this behaviour was possibly due to the evolution of a strain of S. humuli different in the glasshouse to that outdoors. Later, Salmon (1927) compared the infectivity of conidia produced on the plants that showed a breakdown in their immunity with that of conidia taken from a susceptible variety, by inoculating susceptible and persistently immune hop seedlings in the glasshouse. However, he observed no infection on the immune plants with conidia from both sources, and thus no evidence was found for the existence of physiologic races of S. humuli on hops.

Mechanisms of resistance in powdery mildew diseases.

(i) Pre-penetration phase. A mechanical barrier such as thick cuticle has been suggested as a cause of resistance to Erysiphe graminis, Podosphaera leucotricha and Sphaerotheca pannosa (Corner, 1935). Similarly, Graf-Marin (1934) and Homma (1938) have also attributed cuticle thickness as the reason for the inability of cereal mildew fungi to penetrate the host tissue. Hashioka (1938) considered that a cuticular layer of 0.5 µm was thick enough to prevent penetration of S. fuliginea. Evidence for cuticular resistance of roses to S. pannosa has been given by Rogers (1959) and of strawberry to S. macularis by Peries (1961b) and Jhooty & McKeen (1965). More recently Cimanowski & Millikan (1970) suggested that in resistant apple varieties the cuticle may provide a mechanical barrier which inhibits penetration of P. leucotricha. Older apple leaves have greater development of the cuticle than younger ones and this may explain increasing resistance with age (Schieferstein & Loomis, 1956). Other workers like Lowig (1933) and Germar (1934) have attributed resistance to

epidermal wall thickness. The figures for the combined thickness of the cuticle and epidermal walls of apple leaves as given by Csorba (1933), appear to explain the resistance of certain apple varieties to P. leucotricha. Stauch (1927) and Smith & Blair (1950) have, however, found no correlation between morphological features of plants and their resistance to powdery mildew. Also, the results obtained by Weinhold & English (1964) indicate that the thickenss of the cuticle is not an important factor in resistance of peach leaves of S. pannosa.

It has been shown that germination of conidia of S. pannosa on the surfaces of peach and rose leaves is greater than on inert surfaces such as glass (Longrée, 1939; Rogers, 1954; Weinhold, 1961). These workers were uncertain as to whether the stimulatory effect of the host leaf was due to a microclimatic effect at the leaf surface or to some chemical stimulus of the leaf. Germination of mildew conidia was apparently stimulated by the leaf surface, but the effect was non-specific and could be exerted by non-host leaves. The formation of mature appressoria during the primary infection process of E. graminis on wheat and barley appears to depend upon properties of the host surface. Yang & Ellingboe, 1972 gave evidence suggesting that the wax layer of the cuticle surface is a major factor for the maturation of appressoria. Roberts, Martin & Peries (1961) found no differences in the chemical composition of surface waxes of healthy leaves of mildew resistant and susceptible roses. Extracted surface waxes of healthy rose leaves stimulated germination of mildew spores on glass slides, but absorbed wax fractions and cutin acids were inhibitory. They also found that films (30 $\mu g/cm^2$) of cutin acids from rose leaves on glass slides almost completely suppressed germination of conidia on strawberry powdery mildew, S. macularis. However, the suppressive action of the cutin acid was lost when they were diluted with three times their weight of surface waxes from healthy leaves. Inhibition by cutin acids was not specific

because those from apple leaves reduced germination of conidia of strawberry mildew and rose mildew.

(ii) Post-penetration phase. Several workers have studied haustorial morphology in an effort to understand the physical relationship of the host and the fungus. Smith (1900) described the essential features of haustoria in detailed light microscope studies of a number of different powdery mildews. Haustoria are usually surrounded by sac-like sheaths separating them from the host protoplasm. The membraneous boundary between the sheaths and host protoplasm was considered by several authors (Smith, 1900; Smith, 1938). The most elaborate account on mature haustoria and on the host parasite interface of E. graminis on barley has been reported by Bracker in 1968. He demonstrated that the central body of the nucleate haustorium and branches projecting from the central body were embedded in a common sheath matrix bounded by a sheath membrane that is continuous with the host plasmalemma.

Investigating the forms of resistance in wheat to E. graminis f. sp. tritici, Corner (1935) found that though penetration of the epidermal cell occurred, the haustoria formed were small and distorted. The degeneration of haustoria due to incompatibility between host and pathogen has been also demonstrated by Lupton (1956), and Weinhold & English (1964).

Several workers including Woodward (1927), Mackie (1928) and Corner (1935) have reported no interaction between the host cell nucleus and mildew haustoria. However, Smith (1938) showed that the host nucleus moved towards the haustoria and interpreted this as a defence mechanism of the host. A similar movement of nuclei in the host cell and adjacent cells was reported by Schnathorst (1959), who also observed that the chloroplasts of a healthy epidermal cell of lettuce adjacent to infected cells often clustered about the nuclei, moving with them to a position in the cell closest to the infected cell. Sempio (1950) observed a similar

reaction in barley infected with powdery mildew except the clustering and movement of chloroplasts, which were not present in the epidermal cells of barley. Schnathorst (1959) postulated that the migration of the host nuclei and chloroplasts may increase resistance of the host because they might bring about an increase of photosynthetic activity and subsequently higher osmotic values in the region of the haustorium.

Another form of host resistance exhibited to powdery mildew infection is that based on hypersensitivity which was first recognised for the infection of Puccinia graminis (Stakman, 1915). By this reaction a level of disease is determined which is proportional to the rapidity with which most cells are killed on invasion by the pathogen. Thus, resistance actually depends on the extreme susceptibility of the host cells to infection; the more compatible the host cell with the pathogen, the slower the necrosis of the cell. Cell necrosis at any stage of infection can be regarded as a form of hypersensitivity. Thus, the observations of Mackie (1928), Sempio (1939), Dundas (1936), Smith (1938), Cherewick (1944), Smith & Blair (1950), White & Baker (1954) and Hirata (1959), that the resistance of certain plants to powdery mildews is correlated with host cell necrosis, can be considered as a form of hypersensitivity. Usually in these cases the necrotic effect was confined to a single cell but occasionally to groups of adjacent cells. The contents of penetrated resistant cells became disorganised and later these cells collapsed. In host cells of resistant varieties, the plasmalemma and nuclear membrane were rapidly destroyed (Stavely, Pillai & Hanson, 1969) resulting in a hypersensitive reaction. It was suggested by Thatcher (1939) that the induction of hypersensitivity by obligate parasites prevents their further development by starvation. White & Baker (1954) claimed that resistance in barley to infection by E. graminis hordei was a function of the rapidity and degree of mesophyll collapse, rather than being the result of death of epidermal cells or aborted development of haustoria

Physiological factors may also be involved in host resistance to powdery mildew fungi. The role of the osmotic value of host cells in regulating the growth of a number of powdery mildews has been closely linked with carbohydrate metabolism, because low carbohydrate (low osmotic value) favours growth of the pathogen, and increasing carbohydrate (high osmotic value) promotes resistance (Trelease & Trelease, 1929; Sempio, 1950; Allen, 1954; Delp, 1954). Goheen & Schnathorst (1963) and Weinhold & English (1964) have found an association between high osmotic values and resistance to mildew in grape and peach respectively. Moreover, high osmotic values of the cell sap are associated with resistance of apple to powdery mildew (Cimanowski & Millikan, 1970).

Wheat leaves floated in various sugar solutions were more susceptible to mildew than those floated on water. (Trelease & Trelease, 1929). Such observations led these workers to believe that infection by powdery mildews was restricted to tissues with higher sugar content. Yarwood (1934 a,b, 1957) came to the same conclusion from the results of similar experiments in which higher carbohydrate content of the host tissue grown in the light than in the dark was correlated with better establishment and development of powdery mildew. Yarwood (1936 , 1957), Dundas (1936, 1940, 1941), Allen (1936) and Schnathorst (1959) have used detached leaves floating on sugar solutions as a routine measure for the culture of powdery mildews. Horsfall & Dimond (1957) have classified powdery mildews as high sugar pathogens. On the other hand, Grainger (1947) has found no correlation between carbohydrate content of the host tissues and their infection by mildews. Evidence obtained by many other workers, e.g. Reed (1914), Trelease & Trelease (1928) and Yarwood (1938), suggests, moreover, that carbohydrate content of the host tissue has no relationship with mildew infection, but none of these authors carried out tissue analyses to confirm their conclusions.

The amino compounds in rose leaves of susceptible and resistant varieties and in young and old leaves which have become resistant with age are correlated with susceptibility of these tissues to mildew (Bartlett, 1963).

External factors predisposing host plants to infection.

Several factors may affect the plant's response to powdery mildew infection and therefore contribute to its level of resistance.

There are a number of reports of the influence on the affects of environment on host susceptibility to mildew (Salmon, 1921; Hardison, 1944; Last, 1953; Schnathorst 1959). Several workers including Tapke (1951, 1953) and Salmon (1920, 1921) have shown that plants grown under glasshouse conditions produce soft 'sappy' tissue which are susceptible to infection, whereas plants grown in the field produce 'tough' leaves which are resistant. The changes in the environmental conditions not only change the nature of the host susceptibility but also alter the infectivity of the parasite (Benada, 1970).

Susceptibility to powdery mildews is known to be influenced by the nutritional status of the plant. In general, it is considered that nitrogen increases and potassium decreases susceptibility of plants to mildew infection (Salmon, 1925; Trelease & Trelease, 1928; Arland, 1931; Last, 1953; Cole, 1964, 1966). Cole (1966) suggested that the fungus grows poorly on potassium-deficient tobacco leaves, despite ample supplies of carbohydrate and nitrogen, because potassium deficiency restricts protein synthesis or related processes and decreases the availability of essential nutrients. Inconsistent effects of phosphorus have been reported. Milikan (1939) reported that phosphorus increased susceptibility to powdery mildew of wheat while Schulz (1939) found that it decreased susceptibility to E. graminis. Hirata (1971) showed that calcium increases the susceptibility of primary barley leaves to powdery mildew

caused by Erysiphe graminis f. sp. hordei. Hogland & Snyder (1939) and several others reported that plants were free from mildew, when they are grown in complete nutrient solutions, containing extra elements like boron, copper and zinc. The other micro-nutrients which increased resistance of various plants to their respective powdery mildews were lithium (Wortley, 1936 and Kent, 1941), silicon (Lowig, 1937 and Wagner, 1940) and cadmium (Sempio, 1937, 1950 and Tomlinson & Webb, 1960).

## MATERIALS AND METHODS

### (a) Selection of plant material.

A number of hop cultivars and seedlings either susceptible or representative of the different resistant genotypes were chosen for the experiments. Their reactions in the glasshouse and field to infection by S. humuli are shown in Table 1.

Of the resistant selections, 11/66/28 was known to possess both the $I_1$ and B genes while the genetic constitution of 4/63/25 (later introduced into commerce as Wye Challenger) was only partially known at the start of this investigation. The seedlings of family 4/63 were not screened for powdery mildew resistance in the glasshouse before 1968, though they were known to be resistant in the field. Subsequently, however, under heavy inoculum pressure in the glasshouse, 4/63/25 was found to be slightly infected. This seedling was thought to possess only the blister gene (Neve, personal communication), and was a potential commercial cultivar. For these two reasons Wye Challenger was included in the present investigation The resistance of 14/66/50, due to the $I_1$ gene, broke down after 1968 and this seedling has been susceptible ever since. Of the seedlings in the family 11/65, 11/65/92 was chosen as it was being propagated in large numbers in 1972 prior to its eventual introduction into commerce as a new cultivar, Wye Target. This possessed the $I_2$ gene. Apart from the cultivars with known genetic background, those which were used widely in commercial hop growing e.g. Fuggle and OT 48 were also included in some of the experiments as was also the seedling 35/68/58 which was judged in 1970 to show a partial polygenic resistance.

Table 1. Reactions of chosen hop cultivars and seedlings in glasshouse and field to infection by the prevalent population of S. humuli

| cv./seedling | Glasshouse reaction | | Field reaction | | |
|---|---|---|---|---|---|
| | leaf | | leaf | | cone |
| | before 1968 | since 1968 | before 1968 | since 1968 | |
| Northern Brewer | S | S | S | S | S |
| Fuggle | | | | | |
| OT 48 | | | | | |
| 35/68/58 | | | | | |
| 11/66/28 | I | B | B | I | I |
| 4/63/25 (Wye Challenger) | ? | B | B | s | I |
| 14/66/50 | I | S | ? | S | S |
| 11/65/92 (Wye Target) | n.a. | I | n.a. | I | I |

Key: S = susceptible, s = slightly susceptible, B = blister, I = immune
n.a. = not available, ? = unknown.

(b) Collection and maintenance of isolates.

Isolates of S. humuli were collected from infected hops growing at eight sites throughout the country. Four isolates were obtained from cultivated hops from the Weald of Kent, two from the West Midlands, one from wild hops in Rutland and one from the Hop Research Department glasshouses as shown in Table 2.

Detached infected leaves and cones were brought to the laboratory in sealed polythene bags, incubated for 24h in humid chambers and then the fungus was transferred by shaking the conidia from a single pustule on to the leaves.

S. humuli is an obligate parasite and therefore requires maintenance on living hop tissues. In addition each isolate has to be kept in isolation. To achieve this, hop plants cv. Northern Brewer were raised from strap cuts in plastic pots (13 cm diameter) inoculated and then enclosed in perspex cylinders (15 cm diameter, 30 cm high) the open tops of which were covered with 3-4 layers of butter muslin. The pots were stood on inverted Petri dish lids placed on clean quartz sand in trays (18 cm diameter) (Plate 8). Excess water in the tray ensured an airtight seal at the base of each cylinder. An inoculated plant enclosed in this fashion was placed first in the growth room for one week, to allow the pathogen to establish, and then in a growth cabinet at 11-12°C and with a 16h light regime (3-4000lx). Under these conditions the plants grew slowly, allowing intervals of 4-6 weeks between the transfer inoculations. Special care was always taken to prevent any cross-contamination between isolates by carrying out the transfers in separate rooms. Also, between each transfer, clothes were dusted to remove any possible conidia.

This method of isolation has been very successful in retaining the integrity of isolates for over three years.

Table 2. Description of the isolates used for experiments

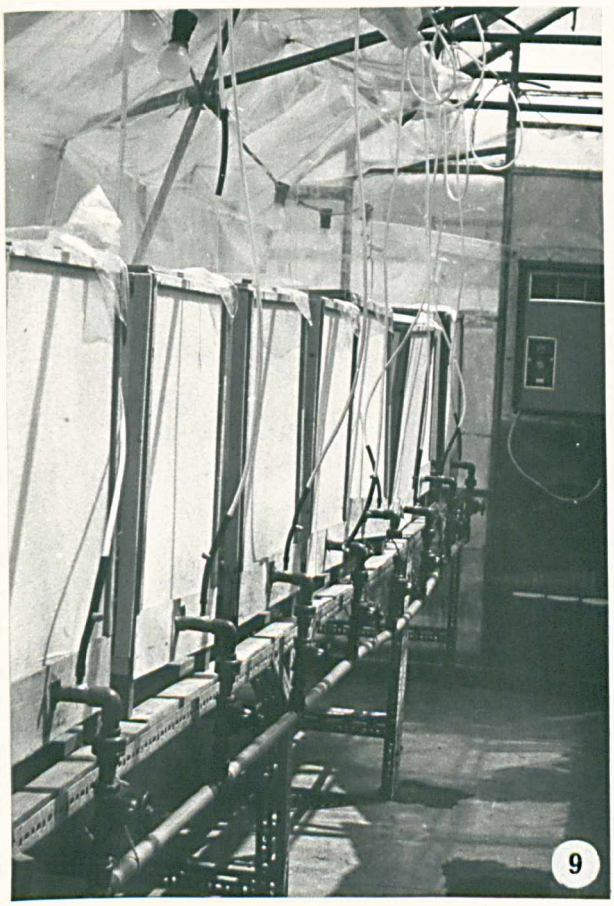
| Isolate | Source | Date of collection |
|---|---|---|
| East Farleigh | leaves and cones, cv. Northern Brewer, Gallant's Farm, East Farleigh, Kent. | 17 Sept 1969 |
| East Malling | leaves of unknown cultivar from glasshouses. East Malling Research Station, Maidstone, Kent. | 30 Sept 1970 |
| Glasshouse | leaves of various cultivars from glasshouses. Hop Research Department, Wye College, Ashford, Kent. | 1 Sept 1970 |
| Marden | leaves and cones, cv. Northern Brewer, Thorn, Marden, Kent. | 28 Aug 1970 |
| Mordiford | leaves and cones, cv. Northern Brewer, Larport Farm, Mordiford, Hereford. | 9 Sept 1970 |
| Tenbury Wells | cones, cv. Northern Brewer, Kyrewood Farm, Worcestershire. | 17 Aug 1970 |
| Rutland | leaves, wild hedgerow hops Langham, Rutland. | 23 Sept 1970 |
| Staplehurst | leaves and cones, cv. OT 48 Hawkenbury Farm, Staplehurst. | 28 Aug 1970 |

Plate 8 Method of maintaining S. humuli in isolation. Infected plants of cv. Northern Brewer, raised from strap cuts, are enclosed in a perspex cylinder covered on top with 3-4 layers of butter muslin. Wet quartz sand in the tray ensures an airtight seal.

Plate 9 Arrangement of wooden-framed isolation boxes in the air-cooled glasshouse. These are provided with a capillary watering system to allow plants to be grown in isolation undisturbed.




8



9

(c) Production and maintenance of plant material.

Experimental plant material was grown vegetatively from either perennial strap cuts or two-node softwood cuttings. All plants were grown in J.I.3 compost. Strap cuts were obtained in October/November from the rootstocks of plants growing in the Department's Withersdane hop garden. They were kept in polythene bags in a refrigerator at 0-2°C for at least six weeks, conditions found by Williams, Roberts & Coley-Smith (1961), to be required for vernalisation of perennial hop material. The strap cuts were then planted in compost in plastic pots (8 cm diameter) and placed in a glasshouse with the light supplemented with 500 W mercury vapour and 60 W tungsten lamps during the winter months to provide an 18h photoperiod, thereby keeping the plants in a vegetative state. An air-conditioning plant in the glasshouse enabled the temperature to be maintained usually at 18± 3°C throughout the year, but during occasional hot days in summer the temperature increased to about 27°C. White-washing the roof and south-facing wall of the glasshouse greatly assisted the maintenance of this temperature range.

Two-node softwood cuttings were taken from lateral shoots of field plants in May-July. They were rooted in 1:2 peat:sand mixture in jiffy pots placed on the bench of a mist-propagation unit (Williams & Sykes, 1959). After 10-14 days rooted cuttings were planted in plastic pots 13 cm diameter with compost, and grown in the air-cooled glasshouse. To retain the vigour of these plants over a long period they were later repotted in larger pots (22 cm diameter). Usually only two shoots per plant were trained up canes 30 cm high. Because of the rapid growth rate of the shoots they were regularly re-trained by wrapping the shoot system round the base of the plant. Occasional infestation of aphids were checked by spraying with carbamate or organophosphorus insecticides,

white flies were controlled with lindane fumigant. Red spider mites were kept under control by spraying with dinobuton.

(d) Bulking of inoculum for experiments.

The experiments required large quantities of conidial inoculum, hence various methods to produce such quantities were explored. Two methods were finally used: (a) Most commonly two strap cuts, cv. Northern Brewer, obtained from the field in late autumn and vernalised for about six weeks were planted in a single 13 cm pot. The pots were enclosed in muslin-covered cylinders and kept in the air-cooled glasshouse. When the shoots were about 14-15 cm high they were inoculated with the desired isolate which established quickly and sporulated heavily under these conditions.

(b) When strap cuts were in short supply, the main shoots of potted plants were cut back to leave about 5-6 nodes and old leaves were removed. Each plant was then enclosed in a muslin-covered perspex cylinder where axillary buds eventually developed into shoots producing a bushy plant. The leaves of the new lateral shoots were ideal substrates for quick establishment and sporulation of the pathogen.

(e) Method of inoculation.

In order to obtain conidia of high and uniform germinability, only those conidia produced within 24h of an inoculation were used in experiments. This was achieved by shaking the inoculum-bearing plants 24h before an experiment in order to remove older conidia.

To obtain a uniform spore deposit on receptor leaf disks and whole plants, the settling tower technique (McCallan & Wilcoxon, 1940) was used.

Cardboard settling towers (64 x 38 x 26 cm) were placed over the plant material to be inoculated and donor plants were shaken gently to release conidia. These were then allowed to settle under draught-free conditions.

A different tower located in separate rooms of the Department was used for each isolate.

Usually the first, second or third pairs from the terminal bud of expanded leaves were removed from healthy plants of the required cultivars. Unless stated otherwise, disks 13 mm diameter, were punched from the leaves using a cork borer and placed adaxial surface uppermost, on squares of 'Airoporena' soaked in deionised water in compartments of polystyrene boxes (10 x 10 x 2cm).

The whole-plant inoculation experiments were carried out in isolation in wooden frame cabinets (0.5 x 0.6 x 0.7m). These had hardboard bases and were covered on the top and two sides with two layers of butter muslin plus two layers of sticky muslin. The back was covered with clear polythene and a vertically-sliding door in the front consisted of a sheet of perspex 4mm thick. Each cabinet was supplied with a capillary watering system, sufficient to supply 12 pots which was attached to a main water-conducting pipe running along the front of the bench. All gaps in the boxes were sealed with either polythene or foam rubber draught excluder strip, thus ensuring a completely sealed environment within each cabinet (Plate 9).

(f) Preparation of leaf material for microscope examination.

(i) Whole mounts for light microscopy.

These were prepared by the method of Preece (1965). Leaf disks were decolourized in methanol in the dark overnight. After warming in a mixture of rectified spirit and glacial acetic acid (1:1) they were stained in phenol:acetic:aniline blue (15:4:1) for 2-3 minutes, then rinsed in 95% alcohol, dehydrated in two changes of absolute alcohol, cleaned in Euparal essence and finally mounted in Euparal.

(ii) Whole mounts for scanning electron microscopy.

Observations with the scanning electron microscope (s.e.m.) were made on leaf disks of various cultivars inoculated with S. humuli.

Preparation of material for the s.e.m. followed the procedure described by Royle & Thomas (1971). Disks, 3mm diameter, were punched from the larger disks and fixed for 1h in 2.5% glutaraldehyde in 0.2M sodium phosphate buffer at pH 7.0. Penetration of the fixative was assisted by intermittent evacuation in a desiccator. The disks were then rinsed twice in deionised water and slowly dehydrated in an ethanol:water series graded at 10% intervals. Several changes of absolute alcohol were finally made before gradually impregnating the disks with iso-amyl acetate using a series of eight amyl acetate:absolute alcohol graded solutions and ending with several changes of pure amyl acetate. To minimise distortion of the material during dehydration and infiltration with amyl acetate the specimens were left covered by a small quantity of the previous solution at each change. The disks were then dried by the critical point method (Anderson, 1951) using the apparatus of Boyde & Wood (1969). This involved replacement of amyl acetate with liquid $CO_2$ which evaporated when heated to slightly above its critical temperature (31°C) in the apparatus. Each disk was finally mounted on a galvanized metal stub and coated uniformly with gold-palladium alloy in a vacuum coating unit in order to make the surface electrically conductive and to avoid shadowing effects. The specimens were examined under the s.e.m. (Cambridge Stereoscan Mk II) at 45° to the beam and with a beam voltage of 20 kV. This method allowed the material to be examined at high accelerating voltages for long periods without distortion.

Fresh material without metal coating was sometimes examined directly in the s.e.m. by first attaching on to a stub with 'Durofix' and then using a low accelerating voltage (10 kV). The drawback in this method was that specimen damage occurred when exposed for longer period.

(iii) Sections for light microscopy.

Leaf material was fixed and stored in formalin:acetic:50%ethyl alcohol (5:5:90). It was then dehydrated and embedded in paraffin wax according to the procedure of Johansen (1940). The wax-embedded material was sectioned on a Cambridge rocking microtome at 10µm, and the sections were stained with phenol-acetic-aniline blue.

(iv) Sections for transmission electron microscopy.

Transmission electron microscopy was carried out on two cvs. only, Northern Brewer and Wye Target. Leaf disks (13mm) were placed, adaxial surface uppermost, in compartment boxes, and were then inoculated with the Glasshouse isolate. The disks were then incubated for 7 days in the growth room when squares (1-2mm) of leaf lamina were cut with a sharp razor blade under a chilled fixative of 2.5% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7.0. These leaf pieces were placed in a desiccator and subjected to intermittent evacuation of air to assist fixation and then left for 24h at room temperature in small vials.

The leaf pieces were washed through four changes of 30 min each with 0.1M sodium cacodylate buffer to remove all traces of glutaraldehyde as osmium tetroxide reacts with glutaraldehyde to produce a fine electron dense precipitate (Gay, personal communication). The buffer solution was replaced by freshly prepared 1% osmium tetroxide in 0.1M sodium cacodylate buffer which was left for 2h at room temperature. This was followed by dehydration with a series of ethanol solutions graded in 10% steps, keeping the material immersed for 10 min at each stage. After the 90% ethanol change leaf pieces were immersed in rectified spirit for 10 min and then in four changes of absolute ethanol at 15 min intervals.

Prior to introducing the Epoxy:resin mixture, epoxy propane (propylene oxide) was used as a link reagent. The concentration of epoxy propane was increased through 2:1, 1:1, 1:2, 0:1, 0:1, of absolute alcohol:epoxy propane with leaf disks kept 10 min at each stage. At this stage introduction of epon resin mixture with the catalyst commenced with mixing 2 parts of epoxy propane:1 part of resin and accelerator in which the leaf material was left overnight at room temperature. The following day the specimens were transferred to small, clean vials containing small quantities of fresh resin. A further four changes of pure resin were carried out leaving the tissue 2h in each change. Then the specimens were transferred to labelled foil cases or 'Taab' beem capsules and the tissue allowed to soak overnight at room temperature in the resin mixture and finally placed at 60°C for 48h to polymerise. After about 4-5h the leaf pieces were lifted off the bottom of the foil cases using a pointed needle.

The embedded pieces of leaf tissue were removed from foil cases and cut with a fine saw after securing in a vice. . These were then mounted with 'Araldite' and hardener on the body of a sawed 'Taab' beem capsule, and allowed to harden at 60°C for 24h. These blocks were used to cut transverse sections and those embedded on the 'Taab' beem capsules were used to obtain median sections. Prior to cutting sections the blocks were trimmed with a razor blade and finer trimming was done on the LKB ultramicrotome. Sections were then cut on the ultramicrotome using a new glass knife. Sections in the silver-grey range, and about 60 nm thick (Peachy, 1958), were picked up on 100 µm 'Taab' copper grids.

There is often insufficient contrast in the image to resolve the fine structure, therefore sections had to be stained. The two most commonly used stains are uranyl acetate and lead citrate. In both cases the effect is to give greatly increased electron density to nucleic acids, lipoproteins,

some lipids and some carbohydrates (Gay, personal communication). Grids were first placed in 2% aqueous uranyl acetate for 30 min at 60°C. The grids were then removed with a fine pair of forceps and washed thoroughly in two changes of glass distilled water. They were then removed again and the excess water was drained off by touching the edge of each grid with filter paper. They were then immersed singly for 3 min in drops of lead citrate stain (Reynolds, 1963) on a layer of dental wax in a covered Petri dish. Before adding the lead stain, a few pellets of sodium hydroxide were placed inside the Petri dish to remove $CO_2$ and prevent any precipitation of lead carbonate. Grids were then washed thoroughly in several changes of glass distilled water and dried before storing in grid boxes.

The material was examined and electron micrographs taken with either an EM6B or AE3 108 type Electron microscope.

# RESULTS

## 1. Characterization of symptoms exhibited by the recognised genotypes to infection by S. humuli in glasshouse and field.

Observations and measurements of the principal features of the disease caused by the population of S. humuli prevalent in the Wye College glasshouses and hop garden were made between 1970-73. In the glasshouse, natural development of the disease occurred during June-July each year on leaves of seedlings being screened for resistance. Pot plants of eight cultivars and seedlings representative of the various genotypes were infected by placing them among the test seedlings. Records of the disease in the field were made on both leaves and cones of the same cultivars and seedlings, unsprayed and planted randomly in single row plots in an area of the Nursery garden. There were eleven replicates of each plot. Records were made in August 1972 of the number of leaves infected on each plant, at three heights. At this time a severe epidemic had built up on susceptible plants. Cone infection was assessed on 300 cones randomly selected at harvest in September 1972. A leaf was considered to be infected only if it showed visible, white, sporulating colonies while a cone was infected if white mould was apparent or cleistocarps were seen on the bracts and bracteoles.

### (a) On susceptible genotypes possessing no major resistance gene or the $I_1$ gene alone.

In both the glasshouse and field colonisation of young expanding leaves of susceptible hosts resulted first in a raised blister (Plate 10), which later supported heavy sporulation obscuring the blisters (Plates 11 & 12). On older leaves, however, infection rarely caused the blister effect, colonies forming on the original, flat leaf surfaces. Different

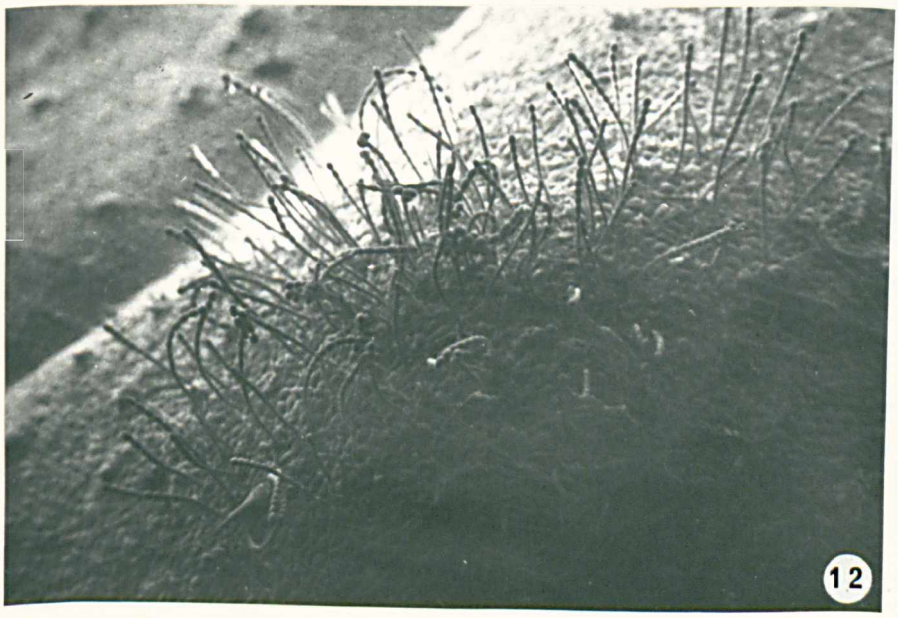
Plate 10 Raised blisters (arrowed) on a third expanded leaf of cv. Northern Brewer, on which the fungus is just beginning to sporulate.

Plate 11 Discrete sporing pustules on a fourth expanded leaf of cv. Northern Brewer. The blisters are obscured by the dense sporulation.

Plate 12 A raised pustule on a fourth expanded leaf of cv. Northern Brewer on which sporulation is occurring (Scanning electron micrograph, x 70).


10


11


12

numbers of leaves infected in the field showed there to be various degrees of susceptibility between the susceptible cultivars, Northern Brewer being the most susceptible and Fuggle, 14/66/50, OT 48 and 35/68/58 showing decreasing susceptibility (Table 3). As a result of the epidemic build-up of the disease, cones of susceptible host plants either failed to develop or became severely distorted, and bore many cleistocarps at harvest (Plate 13). In terms of cone infection, Northern Brewer was again the most susceptible followed by OT 48, 14/66/50, 35/68/58 and Fuggle.

(b) On genotypes possessing the B + $I_1$ genes or the B gene alone.

In this reaction infection of young leaves of plants in the glasshouse resulted in blisters similar to those developing initially on young leaves of susceptible hosts. However, these never supported profuse fungal growth and sporulation, only weak sporulation (Plate 14). On older leaves also infection caused quite prominent blisters, the tissues of which often became necrotic (Plate 15). In the glasshouse both Wye Challenger and 11/66/28 reacted in this way while in the field they differed. Blisters were not formed on 11/66/28 in the field while only few blisters were observed on Wye Challenger. More often brown necrotic patches with no evidence of tissue overgrowth were produced on Wye Challenger in response to infection in the hop garden. Similar symptoms were produced by 11/66/28 under field conditions. The formation of blisters on hosts possessing the B gene seems therefore to be influenced by environmental conditions.

Cones of 11/66/28 and Wye Challenger remained free of infection with no evidence of blisters on the bracts and bracteoles (Plate 13).

Table 3. Leaf and cone infection on eight cultivars and seedlings in the field in 1972

| cv./seedling | % leaves with sporing colonies | % cones infected | % cones with cleistocarps |
|---|---|---|---|
| Northern Brewer | 67.6 | 99.2 | 99.0 |
| Fuggle | 52.4 | 79.1 | 76.2 |
| OT 48 | 25.4 | 96.5 | 87.6 |
| 35/68/58 | 15.1 | 85.3 | 69.5 |
| 11/66/28 | 0 | 0 | 0 |
| Wye Challenger $R_1$ | 3.6 | 0 | 0 |
| 14/66/50 | 42.7 | 91.1 | 89.6 |
| Wye Target $R_2$ | 0 | 0 | 0 |
| S.E. | ±3.9 | ±4.6 | ±5.5 |
| L.S.D.(5%) | 7.7 | 9.1 | 10.9 |

Plate 13 Cones (300) of cvs. Wye Challenger and Northern Brewer obtained from unsprayed plants in the Nursery garden, September, 1972. Cones of Northern Brewer are severely infected with cleistocarps while those of Wye Challenger are completely free from infection. The brown discolouration is caused by wind.


W.Challenger
N.Brewer
13

Plate 14 Prominant bare blisters (arrowed) on a third expanded leaf of cv. Wye Challenger, with very sparse sporulation.

Plate 15 Infection of a fifth expanded leaf of cv. Wye Challenger showing slight blistering and necrosis (arrowed).

Plate 16 Infection of a fourth expanded leaf of cv. Wye Target showing chlorotic specks (arrowed) with no evidence of mycelium or sporulation.


14


15


16

(c) On genotypes with the $I_2$ gene.

Infection of leaves of Wye Target in both glasshouse and field showed only yellow chlorotic flecks which sometimes became necrotic as is typical of a hypersensitive reaction (Plate 16). There was apparently no mycelial development or sporulation.

2. Characterization of symptoms exhibited by the recognised genotypes to infection by separate isolates of S. humuli in the glasshouse.

Experiments were now conducted in isolation to observe the symptoms of infection with each isolate of S. humuli on leaves of potted plants in a glasshouse.

Plants of four cultivars and seedlings, one susceptible and three carrying known genes for resistance were inoculated separately with eight isolates in 1971. Each treatment was replicated three times. Following inoculation the plants were placed in the isolation boxes kept in the air-cooled glasshouse. Records of leaf infection were obtained twice, two and four weeks after inoculation. The results (Table 4) are the means of these two recordings. A leaf was recorded as infected only if it showed visible, white sporing lesions.

Under these conditions raised blisters were produced but were less prominent than those formed on plants kept in the open glasshouse. On Northern Brewer they gave rise to sporulating colonies. On leaves of Wye Challenger and 11/66/28, however, few bare blisters were formed, most leaves showing a necrotic reaction. The number of blisters produced were many fewer than would have been expected with similar inoculations in the open glasshouse, suggesting again that environmental conditions govern the precise expression of the B gene to infection.

Table 4. Infection of leaves of whole plants of four cultivars and seedlings inoculated with eight isolates in 1971*

% leaves infected

| Isolate \ cv./seedling: | Northern Brewer | 11/66/28 | Wye Challenger | 14/66/50 | Mean |
|---|---|---|---|---|---|
| East Farleigh | 48.7( 772)** | 0 (0) | 0 (0) | 0 (0) | 12.2(193) |
| East Malling | 78.5(1090) | 0 (0) | 18.6(315) | 24.9(521) | 30.5(482) |
| Glasshouse | 70.7( 999) | 27.8(554) | 48.7(771) | 66.0(949) | 53.3(818) |
| Marden | 64.9( 941) | 0 (0) | 0 (0) | 0 (0) | 16.2(235) |
| Mordiford | 73.0(1025) | 0 (0) | 38.7(659) | 0 (0) | 27.9(421) |
| Rutland | 79.5(1105) | 0 (0) | 28.5(560) | 63.5(923) | 42.9(647) |
| Staplehurst | 75.8(1067) | 0 (0) | 0 (0) | 0 (0) | 19.0(267) |
| Tenbury Wells | 67.8( 971) | 14.4(387) | 44.3(728) | 65.9(947) | 48.1(758) |
| Mean | 69.9( 996) | 5.3(118) | 22.4(379) | 27.5(418) | |

*Each figure is a mean of three replicates

**Data (in parentheses) are arcsin transformed x $10^3$

(i) For isolate differences; S.E.$\pm$(22), L.S.D.(5%)=(62)

(ii) For cultivar differences; S.E.$\pm$(16), L.S.D.(5%)=(44)

(iii) For isolate/cultivar interactions; S.E.$\pm$(44), L.S.D.(5%)=(124)

On the basis of the qualitatively differential reactions of selected cultivars and seedlings (Table 4), the isolates could be grouped into four categories according to whether or not they caused any degree of leaf infection: (i) Glasshouse and Tenbury Wells infected all four cultivars and seedlings. (ii) East Malling and Rutland infected all except 11/66/28. (iii) Mordiford alone infected only Northern Brewer and Wye Challenger. (iv) Marden, Staplehurst and East Farleigh infected only Northern Brewer.

The experiment was repeated in 1972 to include Wye Target and using isolates representing only groups i, ii and iv. The results (Table 5) show Wye Target to be immune to all isolates tested. The general pattern of leaf infection of the other cultivars and seedlings was similar to that in the previous experiment. However, the amounts of leaf infection differed between the two experiments. For example, in 1971, significant differences were observed between Rutland and Glasshouse isolates on Northern Brewer the former being more infective, while in 1972, similar amounts of leaf infection occurred.

3. Analysis of resistance mechanisms.

During colonisation of the hop leaf by S. humuli several phases in the development of the pathogen which may be involved in expressions of resistance can conveniently be distinguished as follows: (i) germination of conidia (ii) production of appressoria (iii) penetration of the cuticle and epidermal cell walls (iv) formation of haustoria (v) formation of primary, secondary and tertiary hyphal branches (vi) formation of conidiophore initials and (vii) sporulation.

The penetration and post-penetration events (iii) and (iv) will be considered in later experiments. Meanwhile, of the remaining phases describing characterization of pathogen growth and development on the

Table 5. Infection of leaves of whole plants of five cultivars and seedlings inoculated with four isolates in 1972*

% infected leaves

| Isolate \ cv./seedling: | Northern Brewer | 11/66/28 | Wye Challenger | 14/66/50 | Wye Target | Mean |
|---|---|---|---|---|---|---|
| East Farleigh | 44.0(725)** | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 8,8(145) |
| Glasshouse | 69.7(990) | 40.2(686) | 44.0(725) | 62.4(912) | 0 (0) | 43.3(663) |
| Rutland | 67.1(960) | 0 (0) | 30.1(667) | 39.4(674) | 0 (0) | 24.3(460) |
| Tenbury Wells | 66.3(952) | 13.3(363) | 45.1(729) | 62.3(911) | 0 (0) | 37.4(591) |
| Mean | 58.0(907) | 13.4(262) | 29.8(530) | 41.0(624) | 0 (0) | |

*Each figure is a mean of four replicates

**Data (in parentheses) are arcsin transformed x $10^3$

(i) For isolate differences; S.E.$\pm$(19), L.S.D.(5%)=(56)

(ii) For cultivar differences; S.E.$\pm$(22), L.S.D.(5%)=(62)

(iii) For isolate/cultivar interactions; S.E.$\pm$(44), L.S.D.(5%)=(124)

plant surface (i) and (v) require definition for the purpose of this study, thus: Germination of conidia. The criterion for germination was that proposed by Manners & Hossain (1963). A conidium was regarded as germinated when its germ tube was as long as it was broad. Germination was recorded for the first twenty five conidia encountered on each leaf disk. Total length of hyphae per germinated conidium (hyphal length). This comprises the combined lengths of the germ tube(s) and primary, secondary and tertiary hyphal branches. In the early stages of fungal growth only the germ tube(s) were therefore measured, whereas later all the hyphae within the colony were measured. Measurements were made only up to 48h after inoculation, since thereafter colony growth often became too dense for accurate and rapid measurements. An ocular micrometer was used for this purpose.

(a) Growth and development of two isolates on recognised genotypes.

Four genotypes with known genes for resistance to powdery mildew plus the susceptible cv. Northern Brewer were inoculated with the Glasshouse and East Farleigh isolates. These isolates were selected on the basis of results of the previous experiments; they not only represented the most and least active of the eight isolates tested on whole plants but they also allowed each genotype to express its resistance.

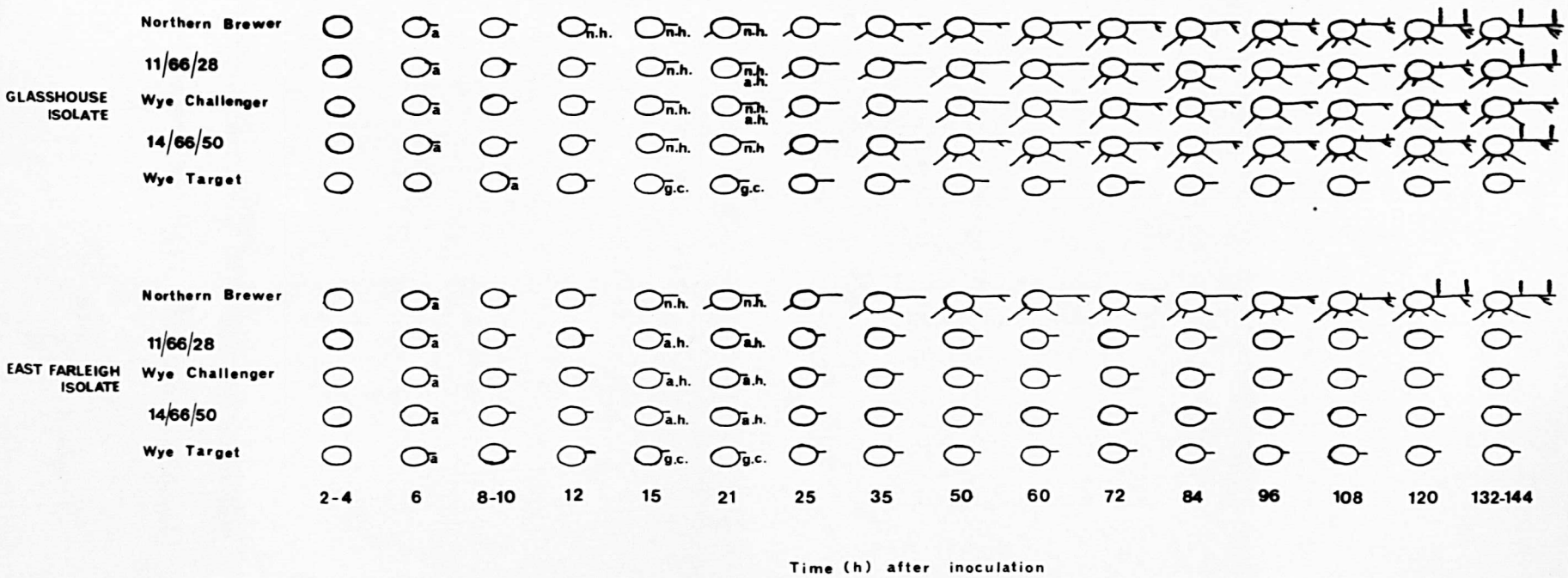
Disks were cut from leaves and placed, adaxial surface uppermost, in compartment boxes. They were then inoculated with 24h-old inoculum and placed in the growthroom. Four inoculated disks of each cultivar/seedling were removed every 2h up to 12h, then at 15, 21, 25, 35, 50, 60 and 72h and then at 12h intervals up to 144h after inoculation. These were prepared as stained whole mounts for microscopic examination of the surface development of the fungus. Other leaf disks, for sectioning, were removed 2, 4, 6, 8, 10, 12, 15 and 21h after inoculation, fixed and permanent preparations made.

The phase-by-phase development of each isolate in the various genotypes is illustrated diagramatically in Fig. 1. Plates 17-32, illustrate some phases in pathogen development.

Germination of conidia of both isolates was first recorded on all cultivars except Wye Target 6h after inoculation (Figs. 2 & 3). On Wye Target germination of conidia of the Glasshouse isolate was observed 8h after inoculation. Within both isolates, on all genotypes, conidial germination increased rapidly during the first 15h after inoculation, thereafter there was very little increase. Some differences in the extent of germination were noted at 6-8h but these later became less evident. More conidia of the East Farleigh than of the Glasshouse isolate had germinated 6-15h after inoculation, though the final proportions, 35-50h after inoculation, were similar. In all isolate/host combinations consistently over 80% conidia finally germinated. There were no consistent differences on susceptible or resistant genotypes. Conidial germination therefore appeared not to be a component in the expression of resistance.

Appressoria were produced singly, generally by only one germ tube of conidia of both isolates, 6-8h after inoculation of all the genotypes. Appressoria were produced anywhere on the leaf surface rather than specifically over the junctions of epidermal cells as Stanbridge, Gay & Wood (1971) showed for E. graminis f. sp. hordei. On all host genotypes germ tubes of the Glasshouse isolate produced more appressoria than those of the East Farleigh isolate. With both isolates, the number of appressoria formed on resistant hosts were generally fewer than on susceptible hosts. Since they were usually produced between a germ tube and the leaf surface appressoria were not always clearly visible in whole mounts. Their formation and frequency were not therefore good criteria for measurement.

Fig. 1 Schematized stages of development of Glasshouse and East Farleigh isolates on five cultivars/seedlings which include all three known major genes for resistance and a susceptible host. Abbreviations indicate first appearance of a - appressoria, n.h. - normal haustoria, a.h. - abnormal haustoria and g.c. - granular epidermal cells.


GLASSHOUSE ISOLATE
Northern Brewer
11/66/28
Wye Challenger
14/66/50
Wye Target
EAST FARLEIGH ISOLATE
Northern Brewer
11/66/28
Wye Challenger
14/66/50
Wye Target
2-4
6
8-10
12
15
21
25
35
50
60
72
84
96
108
120
132-144
Time (h) after inoculation

Plates 17-32 Scanning electron micrographs of the development of the Glasshouse isolate on leaves of various cultivars. All are of material prepared by critical point drying unless otherwise indicated.

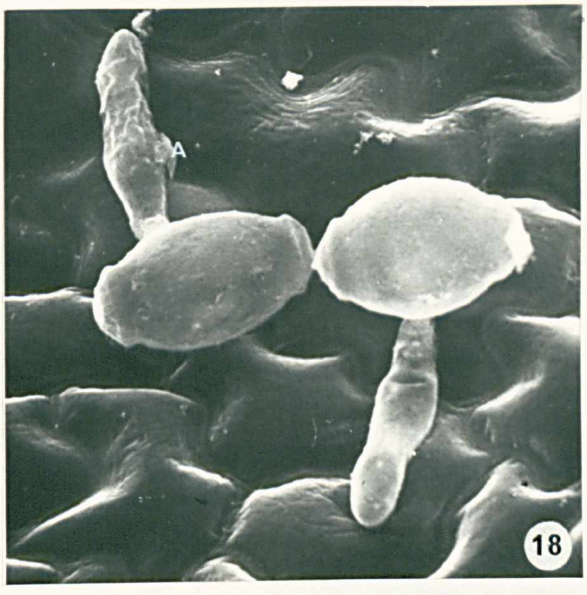
Plate 17 A conidium with a single germ tube 24h after inoculation of cv. Northern Brewer. (x 2,140).

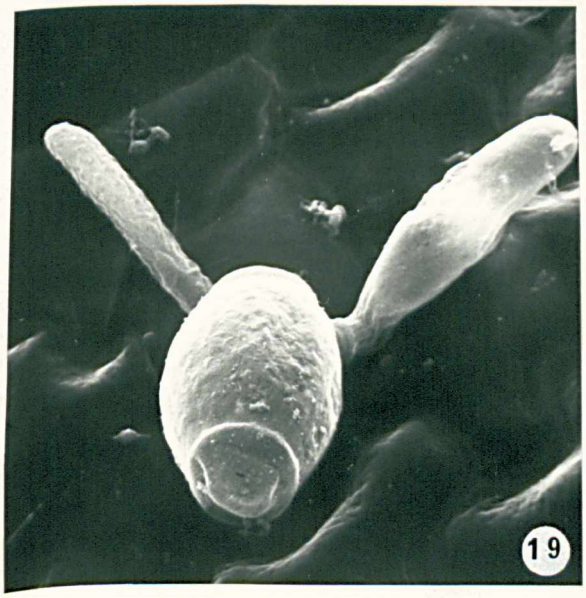
Plate 18 Two conidia with single germ tubes 24h after inoculation of cv. Northern Brewer. On one germ tube an appressorium (A) can be seen (x 1,140).

Plate 19 A conidium with two germ tubes 24h after inoculation of cv. Northern Brewer. (x 1,575).

Plate 20 Two conidia, one with three germ tubes 48h after inoculation of cv. Northern Brewer (x 490).


17


A
18


19

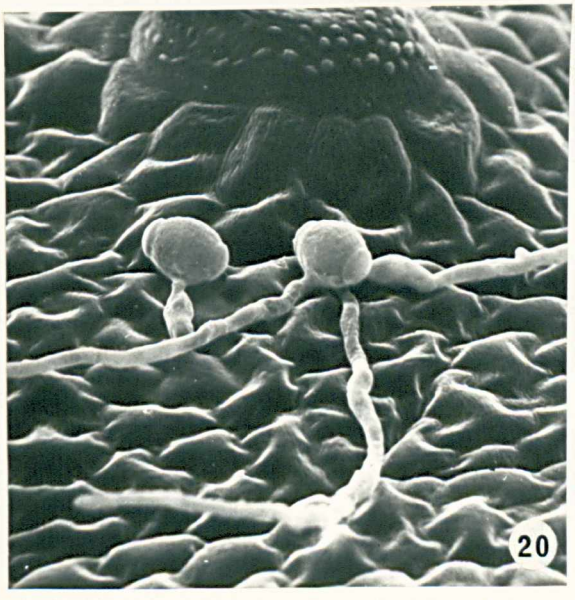
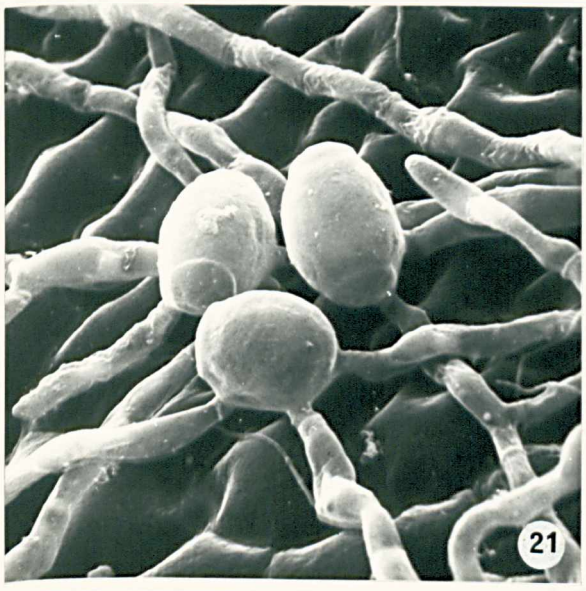

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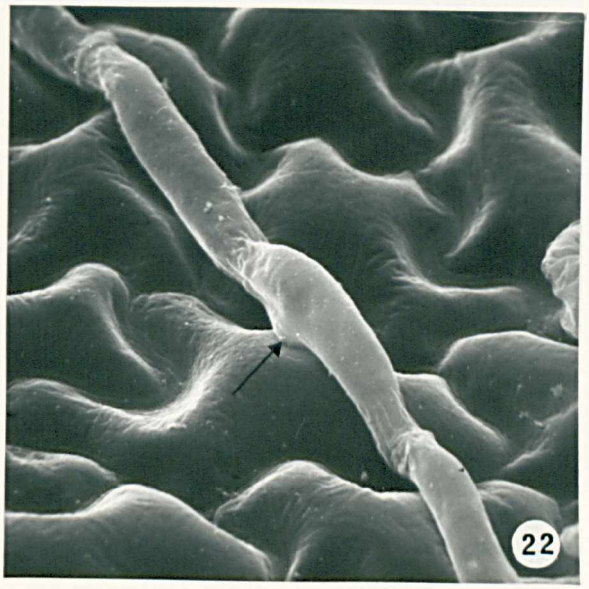
Plate 21 Part of a colony initiated by three conidia on cv. Northern Brewer, 96h after inoculation. (x 930).

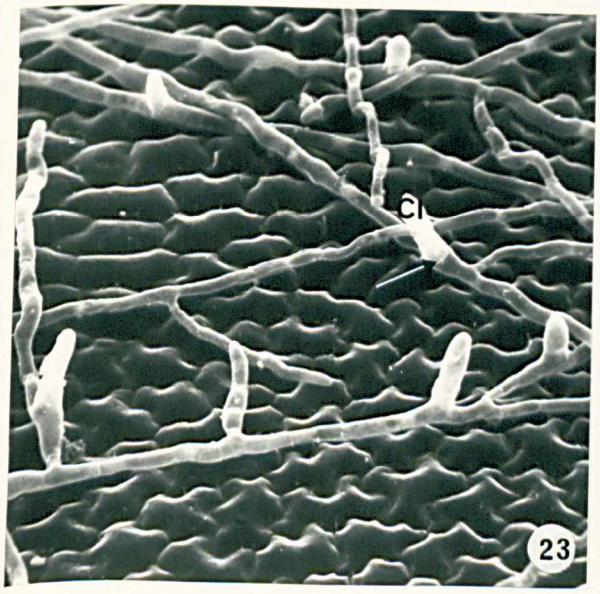
Plate 22 A hypha on cv. Northern Brewer, 96h after inoculation with a point of attachment and presumably penetration of the host (arrowed). Note the cross walls on the hypha. (x 1,575).

Plate 23 Part of a colony on cv. Northern Brewer 120h after inoculation, showing conidiophore initials (CI). Note the distinct septa at the bases of the conidiophore (arrowed). (x 420).

Plate 24 Part of a colony showing early stages of conidial chain development (arrowed) on cv. Northern Brewer 144h after inoculation ( x 105).


21


22


Cl
23

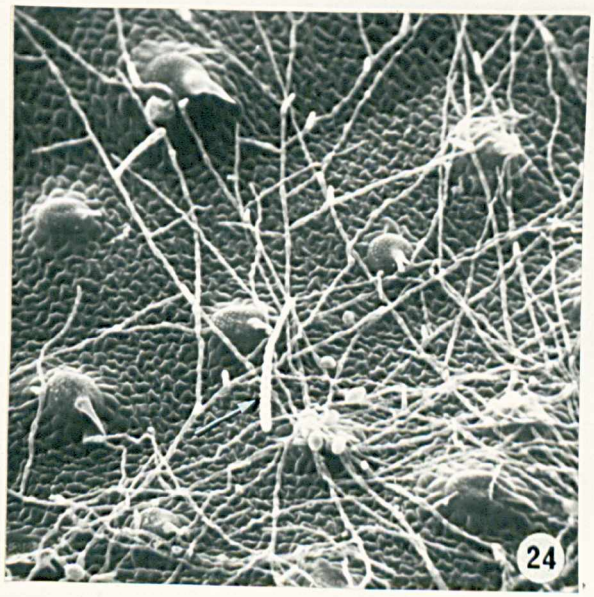
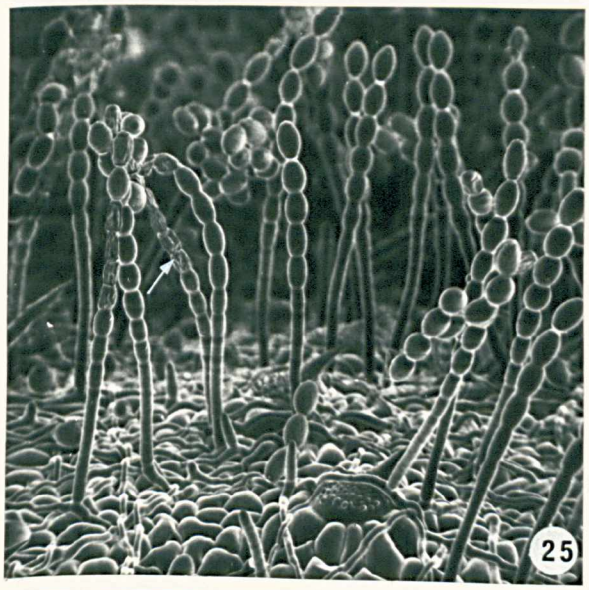

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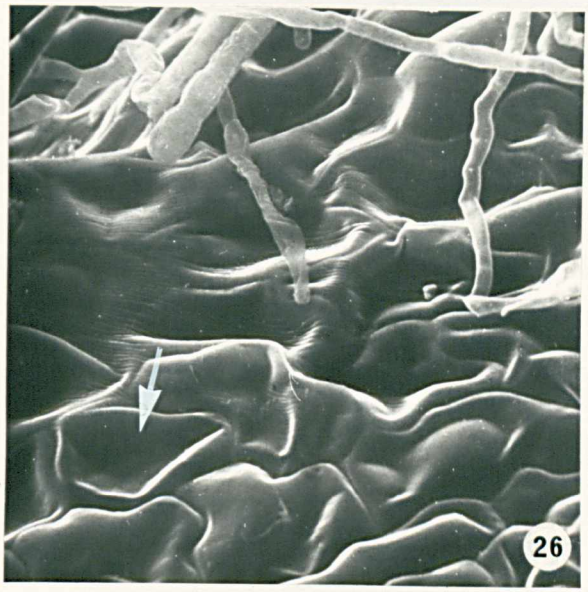
Plate 25 Conidial chains, 7 days after inoculation. Note the electron beam damage (arrowed) of this uncoated, fresh specimen. (x 160).

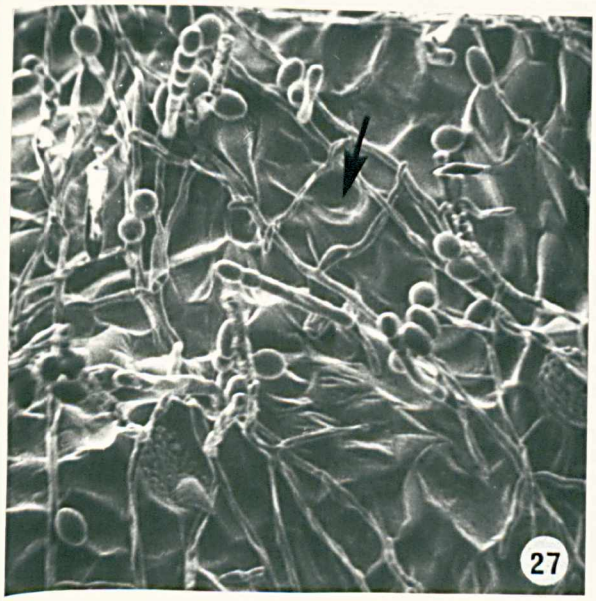
Plate 26 Surface of the edge of a blister on cv. Wye Challenger showing sparse mycelium and collapsed epidermal cells (arrowed). (x 410).

Plate 27 Surface of a blister on cv. Wye Challenger showing collapsed epidermal cells (arrowed) and the sparse sporulation of the fungus. (x 190).

Plate 28 Surface of a blister of cv. Wye Challenger showing some collapsed epidermal cells (arrowed) with little or no mycelium. Note the unaffected epidermal cells adjoining the collapsed ones. (x 440).


25


26


27

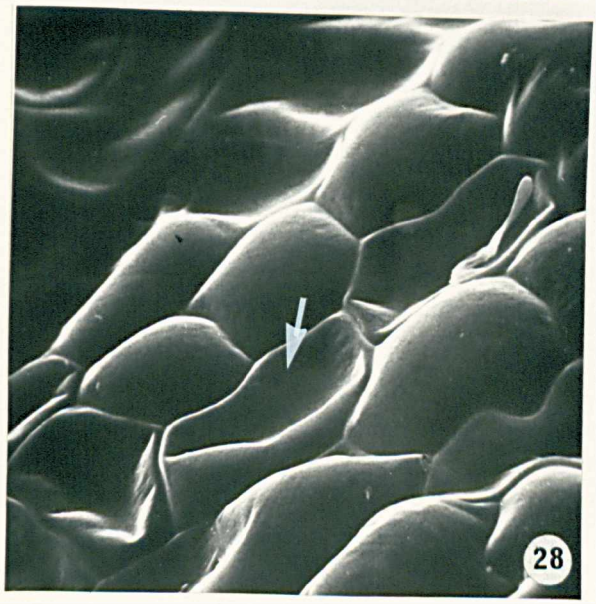
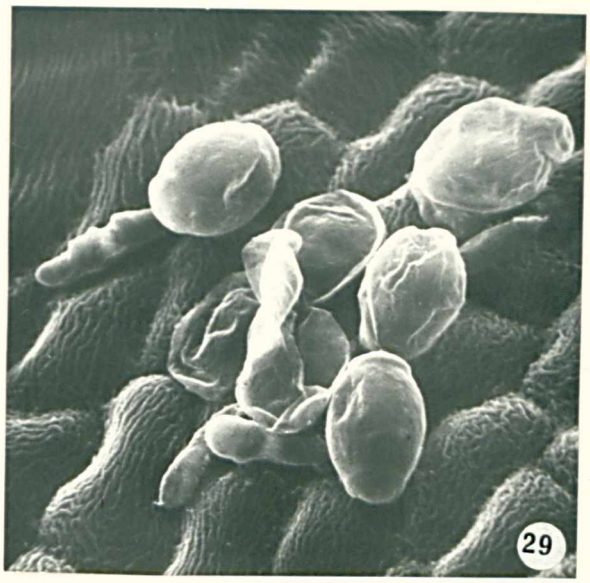

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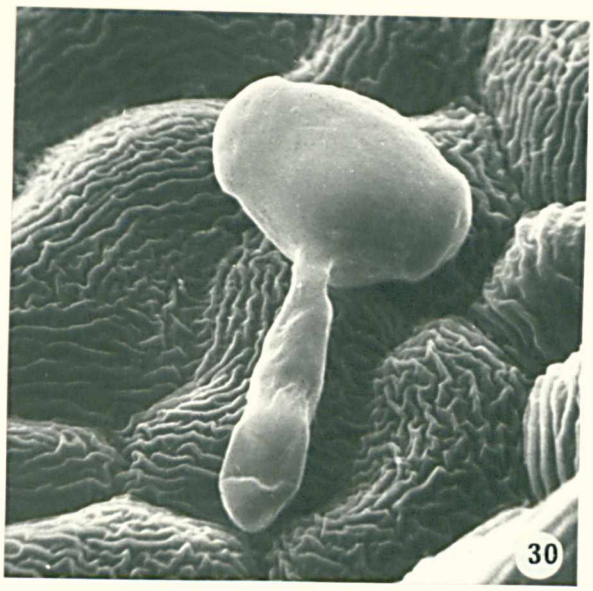
Plate 29 A group of conidia on cv. Wye Target, 96h after inoculation showing poor germ tube growth and some collapse of conidia and germ tubes. (x 790).

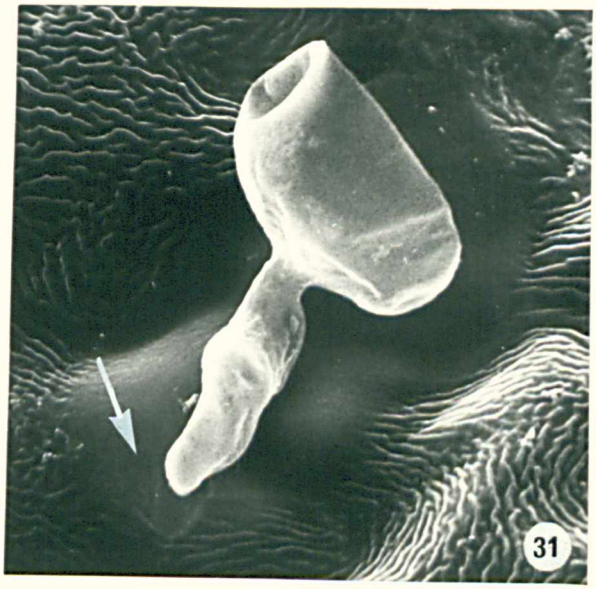
Plate 30 A conidium on cv. Wye Target, 96h after inoculation, showing a single germ tube. Note the cuticular ridges on this leaf surface. (x 1,560).

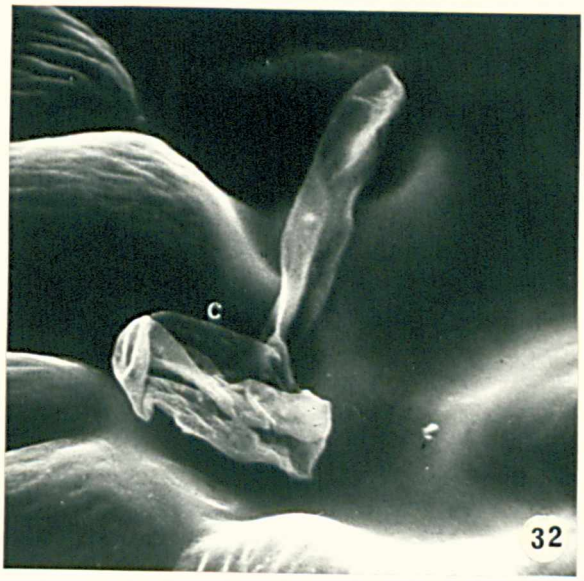
Plate 31 A conidium on cv. Wye Target, 144h after inoculation, showing a clear cuticular area (arrowed) which may be an effect of the pathogen. (x 1,880).

Plate 32 A conidium (c) on cv. Wye Target, 144h after inoculation, showing complete collapse of both conidium and germ tube. (x 1,800).


29


30


31


C
32

Figs. 2 & 3 Conidial germination with time of the Glasshouse and East Farleigh isolates on five cultivars/seedlings which include all three known major genes for resistance and a susceptible host.

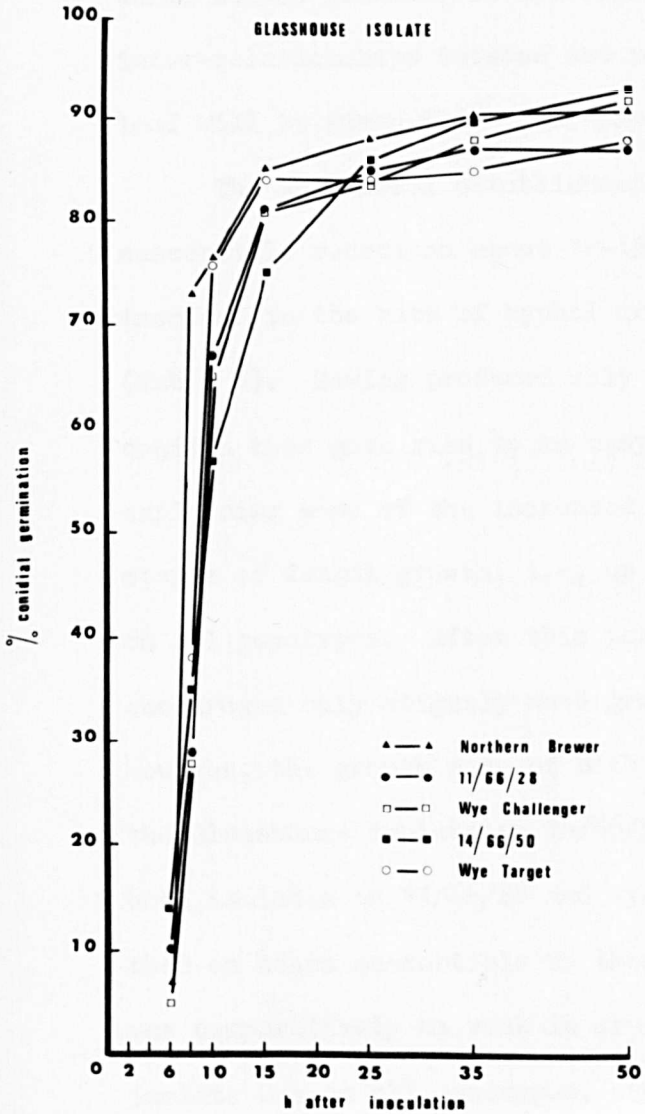

GLASSHOUSE ISOLATE
% conidial germination
h after inoculation
Northern Brewer
11/66/28
Wye Challenger
14/66/50
Wye Target

Fig. 2

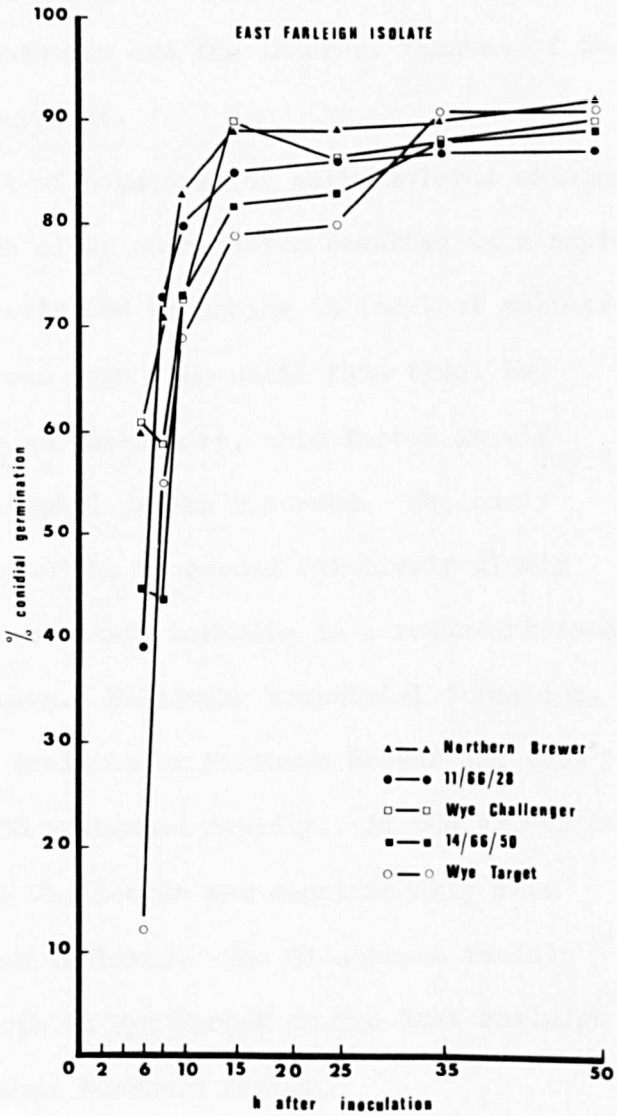

EAST FARLEIGH ISOLATE
% conidial germination
h after inoculation
Northern Brewer
11/66/28
Wye Challenger
14/66/50
Wye Target

Fig. 3

On leaves of susceptible hosts each appressorium produced a thin infection hypha which penetrated the cuticle and epidermal cell wall (Plate 33), giving rise to a haustorium inside the epidermal cell lumen. Penetration of resistant hosts occurred similarly but either abnormal haustoria or no haustoria at all formed in the epidermal cells which became granular in appearance. A more detailed account of the inter-relationships between the pathogen and the internal tissues of the leaf will be given in a later experiment.

The successful establishment of haustoria of both isolates causing susceptible reactions about 12-15h after inoculation resulted in a rapid increase in the rate of hyphal growth and branching on the leaf surface (Table 6). Having produced only one germ tube until this time, the conidia then gave rise to as many as three more, this factor itself explaining some of the increased hyphal growth recorded. The early stages of fungal growth, i.e. up to 15h, proceeded relatively slowly on all genotypes. After this period hosts behaving in a resistant fashion encouraged only slightly more growth. Following haustorial formation, however, the growth rate of both isolates on Northern Brewer and that of the Glasshouse isolate on 14/66/50 increased rapidly. At 50h growth of both isolates on 11/66/28 and Wye Challenger was significantly less than on hosts susceptible to these isolates. The Glasshouse isolate was comparatively as weak in growth on Wye Target as the East Farleigh isolate was on all genotypes, except Northern Brewer.

In Fig. 4 the estimated incremental growth of the two isolates per hour on the various hosts is given to demonstrate the declining rate of hyphal growth with most combinations during the first 15h. When haustorial establishment failed, as in the Glasshouse isolate/Wye Target and East Farleigh/11/66/28, Wye Challenger, 14/66/50 and Wye Target

Plates 33-43 Light micrographs of transverse sections of leaves variously infected with powdery mildew.
Unless otherwise indicated all are 7 days after inoculation with the Glasshouse isolate.

Plate 33 Upper surface of cv. Northern Brewer infected with the Glasshouse isolate. A conidium (C) with a germ tube (GT) has produced an appressorium (A). The infection peg (IP) has penetrated the cuticle and wall of the upper epidermal cell and formed a single haustorium (H). (x 1,800).

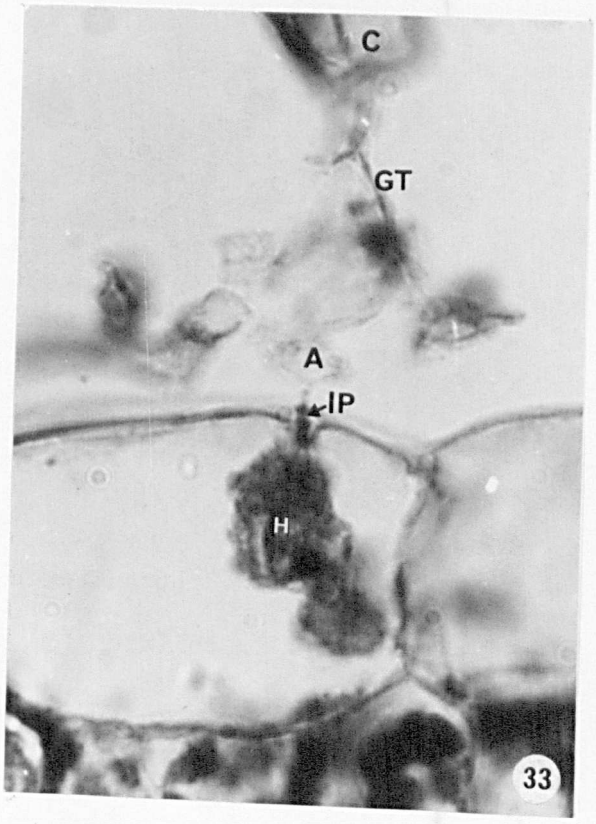
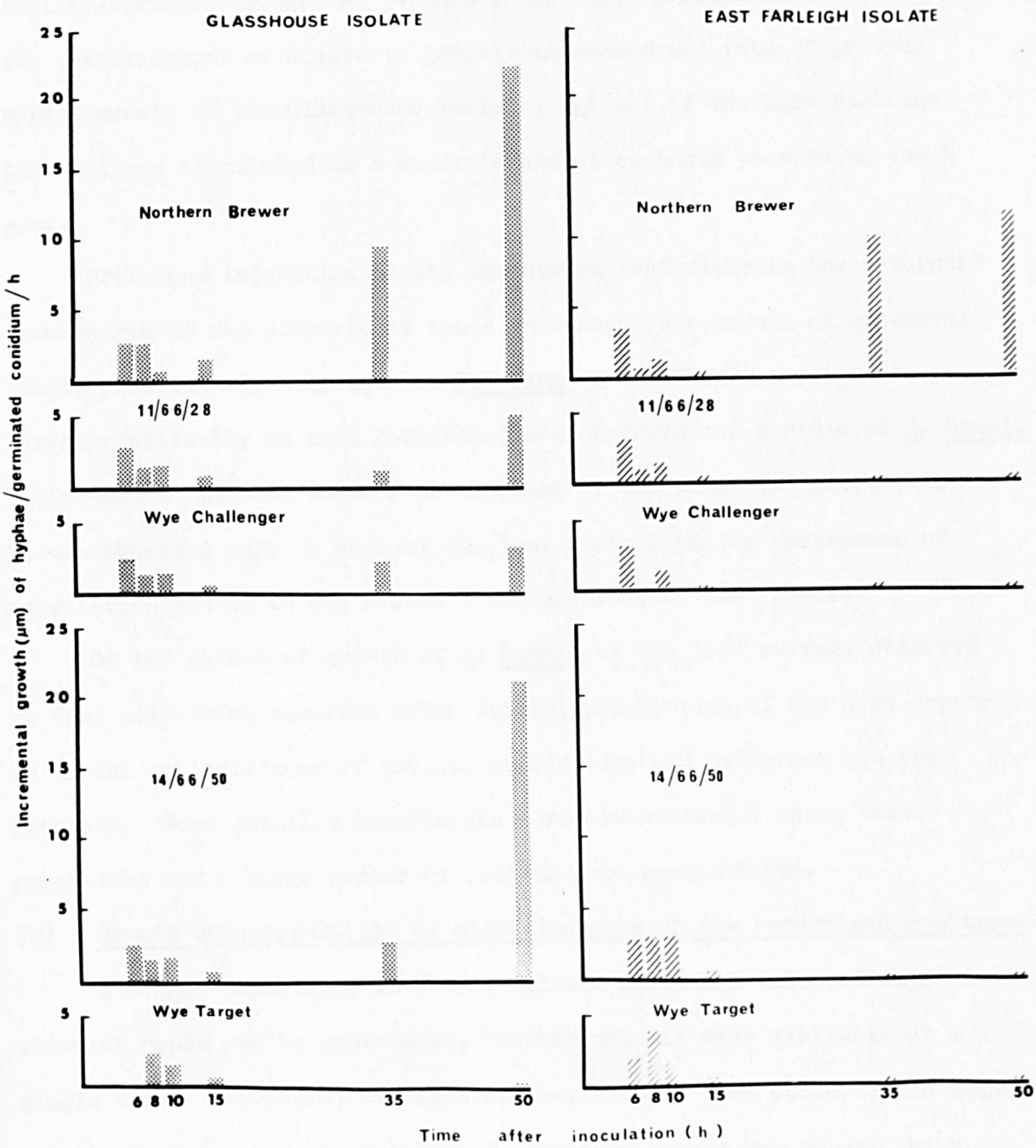

C
GT
A
IP
H
33

Table 6. Hyphal length (µm) per germinated conidium of five cultivars and seedlings inoculated with the Glasshouse and East Farleigh isolates*

| cv./seedling \ h after inoculation: | Glasshouse isolate | | | | | | East Farleigh isolate | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6 | 8 | 10 | 15 | 35 | 50 | 6 | 8 | 10 | 15 | 35 | 50 |
| Northern Brewer | 16.5 | 21.1 | 23.3 | 26.5 | 217.7 | 550.5 | 19.6 | 20.9 | 23.9 | 25.6 | 200.9 | 378.6 |
| 11/66/28 | 16.4 | 18.6 | 21.9 | 25.6 | 46.6 | 121.1 | 17.8 | 19.6 | 22.3 | 24.5 | 24.9 | 26.2 |
| Wye Challenger | 15.0 | 17.8 | 20.7 | 23.1 | 64.8 | 112.8 | 18.5 | 18.7 | 21.2 | 24.7 | 24.9 | 25.1 |
| 14/66/50 | 15.7 | 18.3 | 21.6 | 24.7 | 79.8 | 381.2 | 16.4 | 18.9 | 21.5 | 23.4 | 24.6 | 25.8 |
| Wye Target | 0 | 19.2 | 22.1 | 24.7 | 25.1 | 27.8 | 12.2 | 19.7 | 23.2 | 24.2 | 25.8 | 26.1 |
| L.S.D.(5%) | 8.4 | 2.7 | 1.4 | 2.0 | 23.3 | 51.4 | 6.6 | 1.7 | 1.8 | 1.1 | 11.1 | 43.8 |

*Each figure is a mean of four replicates

Fig. 4 Incremental hyphal growth per hour between successive measurements of the Glasshouse and East Farleigh isolates on five cultivars/seedlings, which includes all three known major genes for resistance and a susceptible host.


GLASSHOUSE ISOLATE
EAST FARLEIGH ISOLATE
Northern Brewer
Northern Brewer
11/66/28
11/66/28
Wye Challenger
Wye Challenger
14/66/50
14/66/50
Wye Target
Wye Target
Incremental growth (µm) of hyphae/germinated conidium/h
Time after inoculation (h)
25
20
15
10
5
6 8 10 15 35 50

combinations, the growth rate remained consistently low throughout the duration of the experiment. In compatible relationships, i.e. Glasshouse isolate/Northern Brewer and 14/66/50, and East Farleigh/Northern Brewer, the establishment of haustoria greatly increased the rate of growth. Hyphal growth of the Glasshouse isolate, but not of the East Farleigh isolate, was stimulated to a moderate extent on hosts possessing the B gene.

Prolonged incubation of the inoculated leaf disks in the saturated atmospheres of the compartment boxes encouraged the growth of ephemeral fungi, particularly a species of Fusarium. This fungus appeared to grow hyperparasitically on both germinated and ungerminated conidia of S. humuli and seemed to prevent further development of the pathogen (Plate 34). It was observed only on some of the leaf disks kept for assessment of sporulation and in no way affected the accuracy of the results.

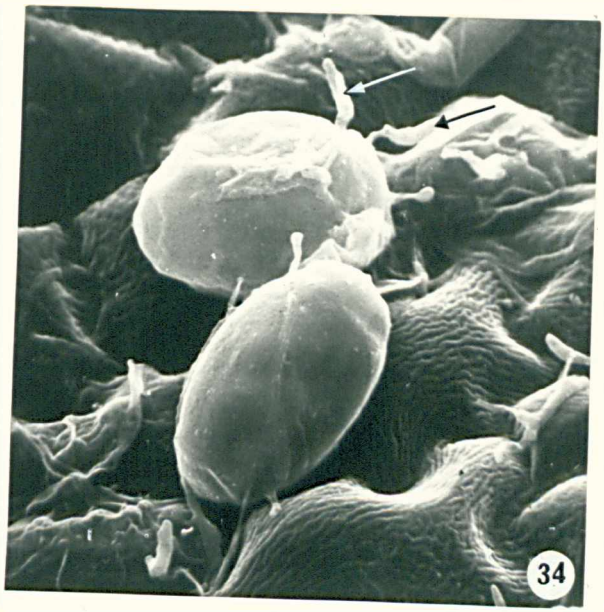
Of the phases of growth of S. humuli on the leaf surface observed so far, only those recorded after initial penetration of the host appear to be useful as indicators of isolate specificity and different genotype reaction. More detailed experiments were now conducted using these parameters and a large number of host/isolate combinations.

(b) Growth and sporulation of eight isolates on the recognised genotypes.

A single experiment involving all the cultivars and seedlings of interest could not be undertaken, because not all were available at a single time. Therefore, two separate experiments were conducted to include all the isolates and the recognised genotypes except Wye Target which became available only after this phase of the work had been completed.

Disks were punched from young, expanded leaves, inoculated with 24h old conidia of the selected isolates and incubated in compartment boxes in the growthroom. Leaf disks were fixed 48h after inoculation

Plate 34 Conidia on cv. Northern Brewer being parasitised by hyphae of a species possibly of Fusarium, with sickle-shaped conidia (arrowed). (x 1,315).


34

and then prepared for light microscopy. Total hyphal length and number of germ tubes per germinated conidium were then measured. Additional leaf disks were incubated for seven days when sporulation was assessed visually according to an arbitrary scale shown in Tables 8 and 10. To minimise errors in visual assessment, recordings on all cultivars/seedlings was performed on the same day for both experiments.

In the first experiment, four cultivars and seedlings were inoculated with three isolates, and in the second, six cultivars and seedlings were inoculated with five isolates. Seedling 35/68/58 was included in the second experiment because it was thought at the time to be polygenically resistant. In both experiments each isolate/host combination was replicated six times and twelve additional leaf disks of each treatment were used for assessment of sporulation.

In general, the extent of hyphal growth could be assigned to one of three categories (Table 7). Northern Brewer encouraged extensive hyphal growth of all isolates while in some isolate/host combinations very little growth occurred. An intermediate amount of growth was also recognised for other combinations. Varying degrees of sporulation were recorded only on those cultivars/seedlings that encouraged extensive or intermediate hyphal growth (Table 8).

The results of the second experiment are shown in Tables 9 & 10. The East Farleigh isolate was the least virulent of the five isolates on all cultivars/seedlings. Susceptible cultivars/seedlings all encouraged substantial hyphal growth and dense sporulation, while 11/66/28, Wye Challenger and 14/66/50, permitted a wide range of fungal response according to the isolate used.

The results of these two experiments show that all isolates, except Glasshouse, Tenbury Wells and Rutland, failed to develop on 14/66/50.

Table 7. Hyphal length (um) 48h after inoculation of four cultivars and seedlings with three isolates*

| cv./seedling: / Isolate | Northern Brewer | 11/66/28 | Wye Challenger | 14/66/50 | Mean |
|---|---|---|---|---|---|
| Marden | 289.9(2.3)** | 20.5(1.3) | 27.5(1.4) | 26.7(1.4) | 91.2(1.7) |
| Mordiford | 242.6(2.3) | 20.2(1.3) | 80.9(1.8) | 26.8(1.4) | 92.6(1.7) |
| Rutland | 288.3(2.4) | 31.0(1.5) | 125.8(1.9) | 67.3(1.8) | 128.1(1.9) |
| Mean | 273.6(2.3) | 23.9(1.4) | 78.1(1.7) | 40.3(1.5) | |

*Each figure is a mean of six replicates.

**Data (in parentheses) are log transformed.

(i) for isolate differences, S.E.$\pm$ (0.1), L.S.D. (5%) = (0.2)

(ii) for cultivar differences, S.E. $\pm$(0.1), L.S.D.(5%) = (0.2)

Table 8. Sporulation of three isolates seven days after inoculation of leaf disks of four hop cultivars and seedlings*

| cv./seedling: / Isolate | Northern Brewer | 11/66/28 | Wye Challenger | 14/66/50 |
|---|---|---|---|---|
| Marden | +++++ | - | - | - |
| Mordiford | +++++ | - | ++ | - |
| Rutland | +++++ | - | ++ | ++++ |

*Each figure is a mean of twelve replicates

Scale: +++++ Very large number of spores with sporulating colonies
++++ Large number of spores with sporulating colonies
+++ Fair number of spores with sporulating colonies
++ Few spores with sporulating colonies
- Non-sporulating colonies and germinated conidia with short germ tubes

Table 9. Hyphal length (µm) 48h after inoculation of six cultivars and seedlings with five isolates.*

| cv./seedling: / Isolate | Northern Brewer | Fuggle | 35/68/58 | 11/66/28 | Wye Challenger | 14/66/50 | Mean |
|---|---|---|---|---|---|---|---|
| East Farleigh | 212.0(232)** | 252.9(238) | 288.0(245) | 28.0(145) | 26.2(142) | 38.6(157) | 141.0(193) |
| East Malling | 380.1(257) | 361.0(255) | 420.7(262) | 38.8(151) | 244.3(239) | 43.6(162) | 248.1(221) |
| Glasshouse | 297.0(247) | 365.4(256) | 333.0(252) | 201.3(225) | 233.1(235) | 370.6(256) | 300.1(245) |
| Staplehurst | 354.7(255) | 300.1(246) | 355.9(255) | 31.2(149) | 34.6(154) | 66.5(180) | 190.5(207) |
| Tenbury Wells | 235.0(237) | 311.4(249) | 302.9(248) | 122.3(204) | 105.7(199) | 282.3(245) | 226.6(230) |
| Mean | 295.8(246) | 318.2(249) | 340.1(252) | 84.3(175) | 128.8(194) | 160.3(200) | |

*Each figure is a mean of six replicates

**Data (in parentheses) are log transformed x $10^2$

(i) for isolate differences; S.E.±(2), L.S.D.(5%)=(6)

(ii) for cultivar differences; S.E.±(2), L.S.D.(5%)=(6)

(iii) for isolate/cultivar interactions; S.E.±(5), L.S.D. (5%)=(15)

Table 10. Sporulation of five isolates seven days after inoculation of leaf disks of six hop cultivars and seedlings*

| cv./seedling: / Isolate | Northern Brewer | Fuggle | 35/68/58 | 11/66/28 | Wye Challenger | 14/66/50 |
|---|---|---|---|---|---|---|
| East Farleigh | ++++ | +++ | ++++ | - | - | - |
| East Malling | +++++ | ++++ | ++++ | - | ++ | - |
| Glasshouse | +++++ | ++++ | ++++ | ++ | +++ | ++++ |
| Staplehurst | +++++ | ++++ | ++++ | - | - | - |
| Tenbury Wells | +++++ | ++++ | ++++ | ++ | ++ | ++++ |

*Each figure is a mean of twelve replicates.

Scale:
+++++ Very large number of spores with sporulating colonies
++++ Large number of spores with sporulating colonies
+++ Fair number of spores with sporulating colonies
++ Few spores with sporulating colonies
- Non-sporulating colonies and germinated conidia with short germ tubes.

Table 11. Analysis of variance of hyphal length of five isolates of S. humuli on six hop cultivars and seedlings*

| Source of variation | Degrees of freedom | Sums of squares | Mean square | F value | Significance (P=0.001) |
|---|---|---|---|---|---|
| Isolates | 4 | 5.89159 | 1.47290 | 85.83 | *** |
| Cultivar | 5 | 16.93247 | 3.38649 | 197.35 | *** |
| Isolate x Cultivar | 20 | 8.06911 | 0.40346 | 23.51 | *** |
| Error | 150 | 2.57327 | 0.01716 | | |
| Total | 179 | 33.46643 | | | |

*Data transformed to logarithms.

Growth of the East Farleigh, East Malling and Marden isolates was suppressed by both 11/66/28 and 14/66/50. Similar growth suppression of East Farleigh, Staplehurst and Marden isolates but not of Glasshouse, Tenbury Wells and Rutland isolates by Wye Challenger, whose present-day resistance is based on the B gene, suggests that Wye Challenger most probably possesses, in addition to the B gene, the $I_1$ gene also contained in 14/66/50 and which broke down in 1968. Wye Challenger, like 11/66/28, now exhibits the blister reaction to infection by the prevalent fungal population in the glasshouse at Wye College. The ability of the Glasshouse, Tenbury Wells and Rutland isolates but not of the other isolates to overcome the resistance conferred by the $I_1$ gene in 14/66/50 must be due to the presence of the corresponding gene for virulence in these three isolates.

The East Malling, Mordiford and Rutland isolates were active on Wye Challenger but not on 11/66/28, although both are known to possess the B gene and 11/66/28 the $I_1$ gene. This behaviour could be due either to the modifying effects of superimposed polygenes or to the existence in 11/66/28 of a hitherto unknown major gene.

Using the hyphal growth parameter, these results allow the isolates to be grouped identically in the four categories chosen from the results of earlier whole plant experiments.

Analysis of variance of the data in the second experiment (Table 11) which included representatives of three isolate groups shows that there were highly significant differences between isolates, cultivars/seedlings and between their interactions.

When the number of germ tubes per germinated conidium was used as a criterion of fungal growth (Table 12) very similar relationships were found. Estimating the number of germ tubes is easier and quicker than measuring the total hyphal length, especially at later stages of growth, and may, therefore, be a convenient way of screening future host/isolate combinations.

Table 12. Number of germ tubes per germinated conidium 48h after inoculation of five isolates on six different cultivars and seedlings*

| cv./seedling: / Isolate | Northern Brewer | Fuggle | 35/68/58 | 11/66/28 | Wye Challenger | 14/66/50 | Mean |
|---|---|---|---|---|---|---|---|
| East Farleigh | 2.0(289)** | 2.0(288) | 2.3(355) | 1.0( 6) | 1.0( 0) | 1.1( 43) | 1.6(164) |
| East Malling | 2.7(423) | 2.6(402) | 2.8(451) | 1.1( 35) | 2.1(316) | 1.1( 38) | 2.1(278) |
| Glasshouse | 2.2(323) | 2.6(417) | 2.4(368) | 2.0(286) | 2.2(353) | 2.7(427) | 2.4(362) |
| Staplehurst | 2.4(385) | 2.4(368) | 2.6(411) | 1.1( 36) | 1.1( 35) | 1.3(102) | 1.8(223) |
| Tenbury Wells | 2.1(313) | 2.5(397) | 2.4(378) | 1.7(222) | 1.6(205) | 2.4(386) | 2.1(317) |
| Mean | 2.3(347) | 2.4(374) | 2.5(393) | 1.4(117) | 1.6(182) | 1.7(199) | |

*Each figure is a mean of six replicates

**Data (in parentheses) are log transformed x $10^3$

(i) for isolate differences; S.E.±(8), L.S.D. (5%)=(22)

(ii) for cultivar differences; S.E.±(9), L.S.D.=(5%)=(24)

(iii) for isolate/cultivar interactions; S.E.±(20), L.S.D.(5%)=(54)

(c) Experiments to determine the absence or presence of $I_1$ gene for resistance in Wye Challenger.

Since there was no cultivar or seedling which was known to possess solely the B gene without the $I_1$ gene, it was not possible to test for the presence of the $I_1$ gene in Wye Challenger by a direct comparison. Hence, the reaction of Wye Challenger to the Glasshouse and East Farleigh isolates was compared with a selection of seedlings which exhibited the blister reaction in the glasshouse and which were progenies of other seedlings known to have shown a blister rather than an immune reaction before 1967. They were therefore known to possess the B gene and were unlikely to contain the $I_1$ gene. These seedlings were 3/67/26 and 4/67/1. Seedlings derived from Wye Challenger and which are now susceptible to powdery mildew were also included in the experiment. They therefore do not possess the B gene but may have inherited $I_1$ if Wye Challenger contains this gene. These seedlings were 37/67/30, 11/67/83, and 11/67/88. In this experiment Wye Challenger and 14/66/50 were used as controls.

All plants were raised from cuttings and were grown disease-free in the air-cooled glasshouse. Disks punched from the leaves were placed in perspex compartment boxes, inoculated with 24h-old inoculum of each isolate and incubated in the growthroom. For measurement of total hyphal length, leaf disks from only 3/67/26, 4/67/1, 14/66/50 and Wye Challenger were removed 48h after inoculation. However, sporulation, seven days after inoculation, was assessed on leaf disks of all the seedlings and controls. Four leaf disks from each treatment were used for measurement of hyphal length and four others for assessment of sporulation. Additionally, a whole-plant experiment was carried out in the isolation boxes in a glasshouse using all the chosen seedlings and inoculating with the Glasshouse and East Farleigh isolates. There were four replicate plants for each treatment.

The results are presented in Tables 13-15 and show that only Wye Challenger compares closely with 14/66/50 in its effect on hyphal growth and sporulation of the East Farleigh isolate.

On all other seedlings this isolate grew and sporulated to an extent dependent on whether no gene for resistance or only the B gene was present. Clearly, therefore, Wye Challenger possesses the $I_1$ gene in addition to the B gene. Of the remaining seedlings, with hitherto unknown genotype, 3/67/26, 4/67/1 and 11/67/83 appear to contain the B gene and 37/67/30 and 11/67/88 no genes for resistance.

(d) Effect of temperature on symptom development.

The particular association of the blister symptom with glasshouse but not field condition could be due to relatively high temperatures during the incubation period. To examine this possibility inoculated plants were subjected to two temperature regimes in two ways. The opportunity also allowed additional observations to be made on the effects of temperature on disease development on a susceptible and immune genotype.

Plants of cvs. Northern Brewer, Wye Challenger and Wye Target were grown in the glasshouse until they reached a height of 30-45cm. Eight plants of each cultivar were then separated into two groups of four plants (Table 16). Group I plants were inoculated with the Glasshouse isolate and then incubated in a glasshouse for four days to allow infection to establish. Two plants were then placed in (a) a growthroom at $25\pm2^{\circ}C$ and (b) in the Hop Department growthroom at $16\pm2^{\circ}C$ for a further nine days. Group II plants were first equilibrated in the same glasshouse for three days, inoculated with 24h old inoculum of the Glasshouse isolate and then two plants each transferred to the two growthrooms (a) and (b) as earlier described. These plants were kept under these conditions for ten days.

Table 13. Hyphal length (µm) on three seedlings and a cultivar inoculated with two isolates 48h after inoculation*

| cv./Seedling | Genotype | Glasshouse isolate | | East Farleigh isolate | |
|---|---|---|---|---|---|
| | | Total hyphal length | S.E. | Total hyphal length | S.E. |
| 3/67/26 | B | 261.6 | ± 20.4 | 201.3 | ± 24.4 |
| 4/67/1 | B | 264.3 | ± 21.0 | 120.4 | ± 25.6 |
| Wye Challenger | B(+$I_1$?) | 131.3 | ± 16.4 | 25.1 | ± 1.4 |
| 14/66/50 | $I_1$ | 363.6 | ± 27.1 | 29.2 | ± 1.9 |

*Each figure is a mean of four replicates

Table 14. Sporulation of two isolates seven days after inoculation of leaf disks of seven hop seedlings and cultivars*

| cv./seedling | Genotype | Glasshouse isolate | East Farleigh isolate |
|---|---|---|---|
| 3/67/26 | B | ++ | ++ |
| 4/67/1 | B | ++ | ++ |
| 37/67/30 | ? | +++++ | ++++ |
| 11/67/83 | ? | +++ | +++ |
| 11/67/88 | ? | ++ | +++ |
| Wye Challenger | B(+$I_1$?) | ++ | - |
| 14/66/50 | $I_1$ | ++++ | - |

*Each figure is a mean of four replicates

Scale:
+++++ Very large number of spores with sporulating colonies
++++ large number of spores with sporulating colonies
+++ fair number of spores with sporulating colonies
++ few spores with sporulating colonies
- non-sporulating colonies and germinated conidia with short germ tubes

Table 15. Leaf symptoms on entire plants of seven hop cultivars and seedlings infected with Glasshouse and East Farleigh isolates fourteen days after inoculation

| cv./seedling | Glasshouse isolate | East Farleigh isolate |
|---|---|---|
| 3/67/26<br>4/67/1<br>11/67/83 | A few slight blisters with very sparse sporulation, often with necrotic areas in the centre of lesion. | A few slight blisters with very sparse sporulation, often with necrotic areas in the centre of lesion. |
| 37/67/30<br>11/67/88 | Large number of lesions, very actively sporulating. | Large number of lesions, very actively sporulating. |
| Wye Challenger | A few slight blisters with very sparse sporulation, often with necrotic areas in the centre of lesion. | Only a few necrotic lesions. |
| 14/66/50 | Large number of lesions, very actively sporulating. | A few necrotic lesions. |

Table 16. Symptom development on three cultivars subjected to different environmental conditions*

| Group | Cultivar | days after inoculation | | | |
|---|---|---|---|---|---|
| | | 5 | 7 | 10 | 14 |
| Ia | Northern Brewer | pre—sp. blisters | active sp. | very active sp. | |
| | Wye Challenger, Wye Target | | yellow flecks | | |
| Ib | Northern Brewer, Wye Challenger | | pre-sp. blisters | blisters sp. | very active sp. / sparse sp. blisters necrotic |
| | Wye Target | | yellow flecks | | |
| IIa | Northern Brewer, Wye Challenger, Wye Target | | yellow flecks | | |
| IIb | Northern Brewer, Wye Challenger | | pre-sp. blisters | many blisters, active sp. | |
| | | | | few blisters, sparse sp | blisters necrotic |
| | Wye Target | | yellow flecks | | |

*For further details, see text

Sp. = Sporulation

At the lower temperature regime blisters in Wye Challenger were formed five days after inoculation. However, if the plants were incubated in the glasshouse before transfer to this regime, blister symptoms were evident seven days after inoculation. This delay may have been due to interruption of the environmental conditions during the incubation period. The blisters formed under these conditions were less prominent than those normally encountered on similar types of plants in the open glasshouse. At the higher temperature regime, blisters failed to develop, only yellow flecks as in Wye Target. The immediate transfer of inoculated plants of any cultivar from the glasshouse to the higher temperature regime appeared to be lethal since even Northern Brewer failed to develop symptoms, but produced only yellow flecks. This contrasts with the delayed transfer to the high temperature when infection was allowed to establish first. Under these conditions Northern Brewer encouraged a very rapid appearance of actively sporulating colonies. It is therefore, not easy to understand the failure of Wye Challenger to establish and produce more definite symptoms under these conditions. Continuous high temperature and possibly low light intensity conditions may have affected the physiological condition of the plant and thereby fungal development.

(e) Effect of leaf age on hyphal growth and sporulation of the Glasshouse isolate.

Resistance of plants to powdery mildews is commonly regarded as increasing with age of leaves. To examine this relationship in hop/ S. humuli situations, leaf disks were punched from the third, fifth, eighth, eleventh and fourteenth pairs of expanded leaves from the tips of shoots of a single plant of each of four cultivars - Northern Brewer, Fuggle, Wye Target and Wye Challenger. They were placed, adaxial surface uppermost, in compartment boxes, inoculated with 24h-old conidia of the Glasshouse isolate and incubated in the growthroom. For each cultivar there were four disks

from each age of leaf. Leaf disks were removed 12, 24 and 48h after inoculation for microscopic examination of hyphal growth, and others were allowed to incubate for seven days when sporulation was evaluated. An additional two disks from the third, fifth and eighth leaf pairsof leaves from cultivar were removed seven days after inoculation, fixed in formalin-acetic-alcohol and embedded in wax for sectioning.

Table 17 shows that conidial germination whether recorded 12, 24 or 48h after inoculation markedly declined on leaves of increasing age up to the eleventh leaf pair of all cultivars. In this experiment Wye Target consistently supported less germination than other cultivars, though in earlier experiments no differences were recorded.

On all cultivars hyphal development also declined with increasing leaf age (Table 18, Fig. 5). The most substantial reduction on hyphal growth was on the susceptible cultivars, Northern Brewer and Fuggle between the third and eighth leaf. On Wye Challenger growth dropped most significantly from the third to fifth leaf. Otherwise on all cultivars there was a slight but definite decline in the recorded hyphal length with increasing leaf age. It is worth noting that differences in conidial germination had no influence on measurements of hyphal length, which were made only on germinated conidia.

As may be expected from these results so far, the extent of sporulation was also affected by leaf age (Table 19). Only Northern Brewer supported sporulation on the eighth leaf while on Fuggle it was restricted to the fifth oldest leaf and Wye Challenger to the youngest leaf used in the experiment.

Haustoria were present abundantly in leaf sections obtained from third and fifth expanded leaves of Northern Brewer and Fuggle, but with increasing leaf age only a few haustoria or none were found. Most of the

Table 17. Percentage germination of conidia of the Glasshouse isolate on different ages of leaves of four cultivars*

| Cultivar \ Time after inoculation(h): | Expanded leaf number from shoot tip | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | | | 5 | | | 8 | | | 11 | | | 14 | | |
| | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 |
| Northern Brewer | 89.0 | 89.5 | 81.0 | 71.5 | 70.0 | 69.0 | 59.5 | 62.5 | 64.0 | 50.0 | 55.0 | 57.0 | 50.0 | 54.5 | 60.5 |
| Fuggle | 91.5 | 95.0 | 90.0 | 87.0 | 77.0 | 77.5 | 54.0 | 53.5 | 60.5 | 47.5 | 47.5 | 54.0 | 46.5 | 46.0 | 55.5 |
| Wye Challenger | 88.0 | 83.5 | 84.5 | 55.0 | 67.5 | 75.0 | 45.5 | 58.0 | 56.0 | 42.5 | 65.5 | 58.0 | 41.5 | 60.0 | 62.5 |
| Wye Target | 75.0 | 73.5 | 71.0 | 63.5 | 67.5 | 62.0 | 45.5 | 52.0 | 52.5 | 41.5 | 50.5 | 50.5 | 40.0 | 47.5 | 50.5 |
| L.S.D.(5%) | 6.4 | 5.3 | 7.6 | 5.9 | 8.0 | 6.8 | 6.1 | 5.0 | 4.8 | 6.6 | 6.0 | 5.2 | 5.2 | 4.6 | 6.6 |

*Each figure is a mean of four replicates

Table 18. Total hyphal length(μm) of the Glasshouse isolate recorded on different ages of leaves of four cultivars*

| | Expanded leaf number from shoot tip | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time after inoculation (h): | 3 | | | 5 | | | 8 | | | 11 | | | 14 | | |
| Cultivar | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 |
| Northern Brewer | 25.6 | 46.8 | 328.2 | 21.5 | 23.5 | 158.0 | 20.0 | 22.5 | 23.0 | 17.4 | 20.4 | 20.9 | 16.6 | 19.2 | 20.2 |
| Fuggle | 24.5 | 41.4 | 278.1 | 24.3 | 28.0 | 83.4 | 19.0 | 22.3 | 22.6 | 18.0 | 21.2 | 22.1 | 17.3 | 20.1 | 21.3 |
| Wye Challenger | 23.9 | 24.9 | 24.8 | 19.8 | 22.4 | 22.4 | 17.5 | 21.3 | 21.5 | 16.8 | 20.7 | 20.6 | 15.9 | 19.6 | 19.6 |
| Wye Target | 25.3 | 28.8 | 121.4 | 20.0 | 21.3 | 26.3 | 19.2 | 20.6 | 22.2 | 17.2 | 19.4 | 20.2 | 17.0 | 19.2 | 20.4 |
| L.S.D.(5%) | 1.0 | 4.3 | 19.4 | 1.8 | 2.7 | 23.1 | 2.0 | 0.9 | 1.9 | 2.1 | 1.0 | 2.2 | 1.6 | 2.1 | 1.6 |

*Each figure is a mean of four replicates

Fig. 5 Hyphal growth of the Glasshouse isolate with time on leaves of five ages of four cultivars.

▼, 3rd; △, 5th; ■, 8th; □, 11th; ●, 14th expanded leaf from the shoot tip

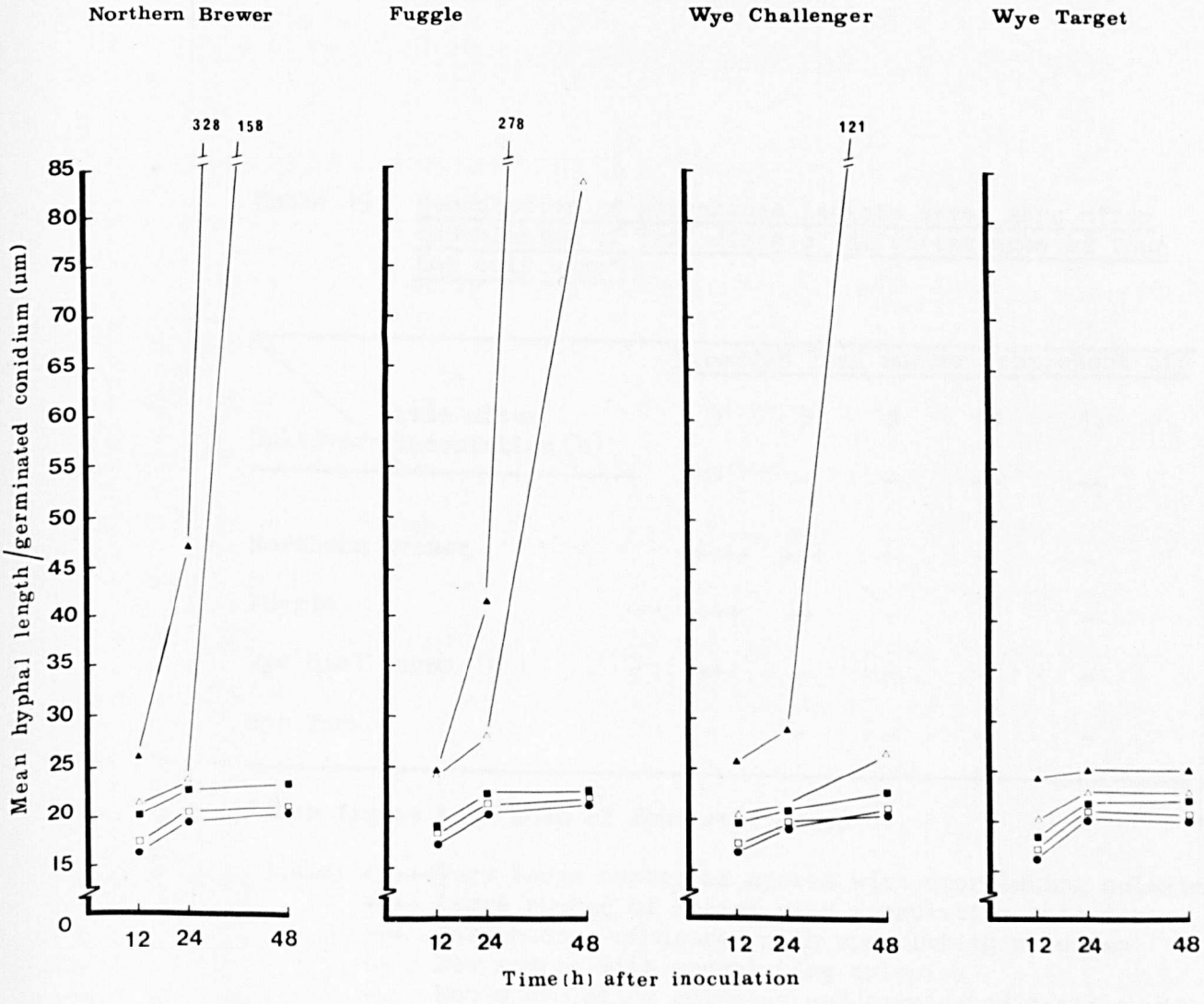

Northern Brewer
Fuggle
Wye Challenger
Wye Target
328
158
278
121
Mean hyphal length/germinated conidium (µm)
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
0
12
24
48
Time (h) after inoculation

Table 19. Sporulation of glasshouse isolate seven days after inoculation of leaf disks of different ages of four hop cultivars*

| Cultivar \ time after inoculation (h): | Expanded leaf number from shoot tip | | | | |
|---|---|---|---|---|---|
| | 3 | 5 | 8 | 11 | 14 |
| Northern Brewer | +++++ | +++ | ++ | - | - |
| Fuggle | ++++ | ++ | - | - | - |
| Wye Challenger | ++ | - | - | - | - |
| Wye Target | - | - | - | - | - |

*Each figure is a mean of four replicates

Scale: +++++ Very large number of spores with sporulating colonies
++++ Large number of spores with sporulating colonies
+++ Fair number of spores with sporulating colonies
++ Few spores with sporulating colonies
- Non-sporulating colonies and germinated conidia with short germ tubes

haustoria that were seen in sections of the third expanded leaves of Wye Challenger and eighth expanded leaves of Northern Brewer and Fuggle were abnormal and were embedded in granular cytoplasm.

(f) Light microscope observations of the post-penetration phases of infection.

Experiments so far have indicated that the first expressions of resistance in all genotypes coincide with the penetration of host tissues and the formation of haustoria. Further investigations therefore considered relationships between the pathogen and host within leaf tissues. Light microscopic studies were conducted with transverse sections prepared from inoculated leaf disks in two growthroom experiments. Leaves, naturally infected in the glasshouse and field were also used. In the first experiment. leaf disks of five cultivars/seedlings, including Wye Target, were each inoculated with the Glasshouse and East Farleigh isolates. Disks were removed for fixation each 2h up to 12h then 15h and 21h after inoculation, and then sections 10um thick prepared after wax-embedding. The results provided information on the time of haustorial formation, which was used in Fig. 1 and also on the response of the host cells after penetration. In the second experiment, six cultivars/seedlings, not including Wye Target, were each inoculated separately with all eight isolates and leaf disks were removed and fixed seven days after inoculation. Here, haustorial size and number were determined by measuring the longest and broadest regions of each haustorium encountered in the epidermal cells. Thirty haustoria for each host/pathogen combination were recorded in this manner, by examining every fifth section of about 20 on each slide. The frequency with which haustoria occurred was determined by a similar procedure in which the number of haustoria were counted within randomly-selected 0.5mm lengths of every fifth section.

Haustoria of the Glasshouse isolate were first seen in epidermal cells of Northern Brewer by 12h after inoculation but their formation was delayed until 15h with the East Farleigh isolate (Fig.1). In a host, reacting in a susceptible fashion to either the Glasshouse or East Farleigh isolate an infected epidermal cell usually contained a single haustorium (Plate 35) but two (Plate 36) or three were not uncommon, mostly with their long axes oriented vertically to the plane of the leaf. Each haustorium contained a nucleus in the haustorial body which was enclosed in a sac-like sheath; this is designated a 'normal' haustorium. Within the epidermal cell below the point of penetration of the host cell wall, the neck of the haustorium was surrounded by a collar. The nucleus of an infected epidermal cell was often found adjacent or close to the haustorium. The chloroplasts in the palisade cells immediately beneath the infected epidermal cells appeared to be unaltered.

Some abnormal haustoria of the Glasshouse isolate, which lacked distinct haustorial sheaths, collars and nuclei, were encountered 21h after inoculation in cells of 11/66/28 and Wye Challenger (Plates 37 & 38). However, 15h after inoculation of these hosts haustoria of this isolate were all apparently normal. In 14/66/50 haustoria of the Glasshouse isolate appeared to be normal at all stages of pathogen development. In all cultivar/seedlings except Northern Brewer only abnormal haustoria of the East Farleigh isolate were produced. The protoplasm of the epidermal cells containing abnormal haustoria was granular by 15h, this effect intensifying by 21h.

Also, several epidermal cells containing granular protoplasmic contents were often found together in the absence of haustoria (Plate 39). In this situation the haustoria appeared to have aborted following penetration of the epidermal cells (Plate 40). Alternatively, host cell disorganisation sometimes appeared to be due to a host reaction

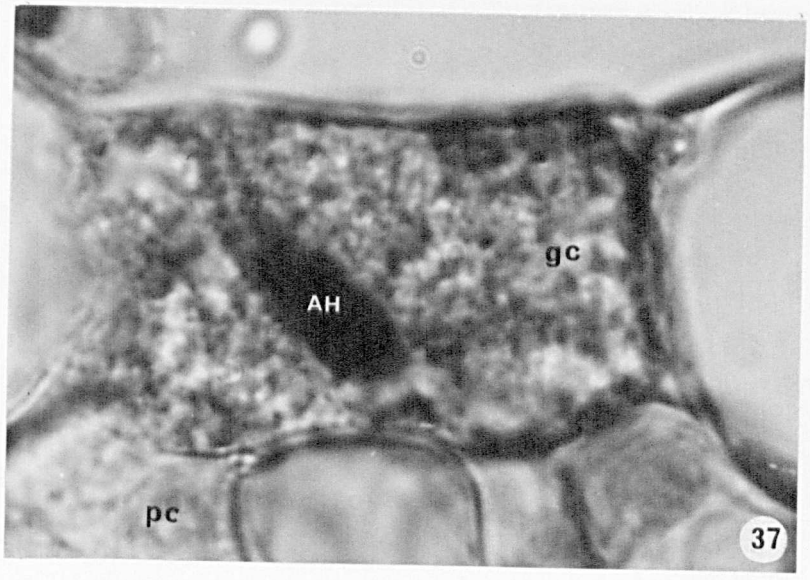
Plate 35 A single haustorium in the upper epidermal cell of cv. Northern Brewer which is connected to surface mycelium (M). Note the distinct sheath (S) surrounding the haustorial body which contains a single nucleus (N). The host nucleus (n) close to the haustorium. The palisade cells (pc) are unaffected as is the protoplasmic content of the affected epidermal cells. (x 2,520).

Plate 36 Two haustoria in a single epidermal cell of cv. Northern Brewer showing distinct sheaths (S) and nuclei (N). Note the fungal mycelium (M) on the leaf surface. (x 2,770).


M
S
n
N
pc
35


M
S
N
N
S
36

Plates 37 & 38 Epidermal cell of seedling 11/66/28 showing abnormal haustoria (AH) of the Glasshouse isolate, which have no sheaths nor haustorial nuclei. Note the granular nature of the epidermal cells (gc). Also the palisade cells (pc) (Plate 37) immediately beneath the affected cells were damaged. An adjoining cell also shows signs of cellular disorganisation (arrowed) and the palisade cells adjacent to the affected epidermis is damaged. (Plate 37, x 3,025; Plate 38, x 2,585).


gc
AH
pc
37

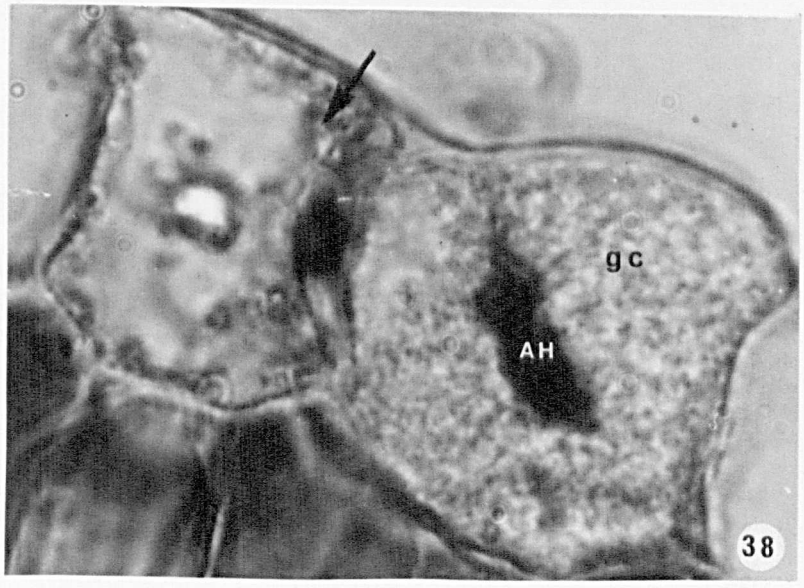
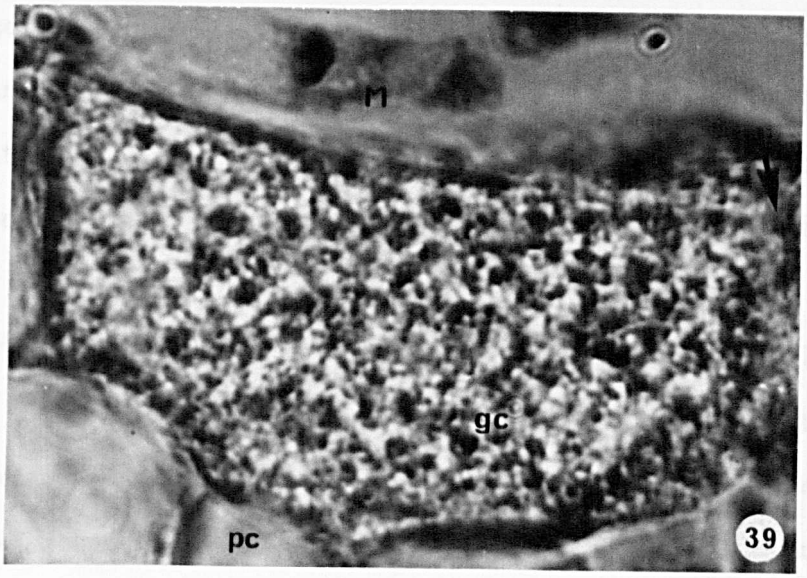
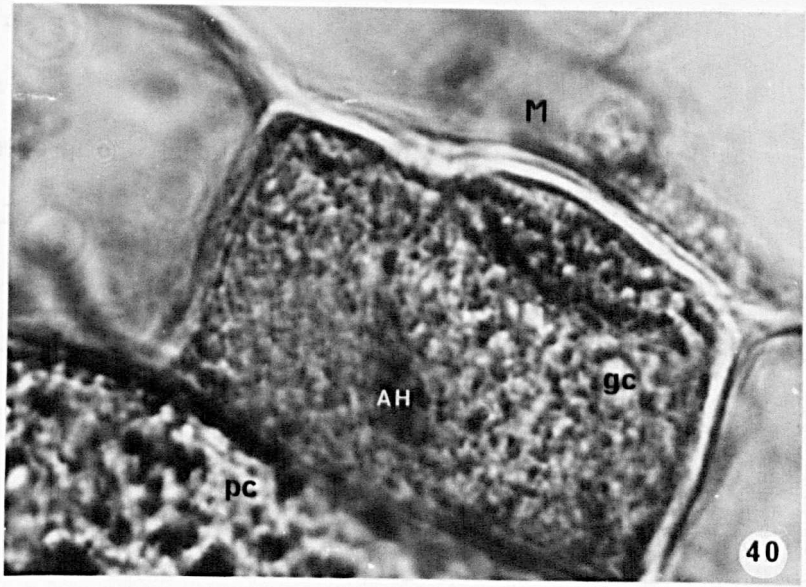

gc
AH
38

Plate 39 An epidermal cell of seedling 11/66/28 inoculated with the East Farleigh isolate showing only granular cytoplasm (gc) with no evidence of a haustorium. The adjacent cells (arrowed) were also similarly disorganised. The palisade cells (pc) beneath the infected cells appeared to be adversely affected also. Note the mycelium (M) on the surface of epidermal cells. (x 2,250).

Plate 40 An epidermal cell of seedling 14/66/50 inoculated with the East Farleigh isolate showing a degenerating haustorium (AH) embedded in granular cytoplasm (gc). Note the damage caused to the palisade cells (pc). (x 2,520).


M
gc
pc
39


M
AH
gc
pc
40

to an attempted penetration by the fungus. The chloroplasts of the palisade cells in contact with the infected epidermal cells appeared to be damaged (Plate 37).

No haustoria were detected in Wye Target at any stage of infection. Disorganisation of Wye Target protoplasm was first encountered 15h after inoculation with both Glasshouse and East Farleigh isolates. This lack of haustoria strongly suggests the host response was a reaction to attempted penetration of the fungus.

The haustoria produced in susceptible hosts by all the isolates were generally larger than those produced in resistant hosts (Table 20). It is of interest to note that East Farleigh isolate, which was earlier shown to be the least active of the isolates on all genotypes, in terms of hyphal growth and sporulation, also produced the smallest haustoria, whether normal or abnormal. Similarly, even the abnormal haustoria formed in 11/66/28 and Wye Challenger by the Glasshouse isolate were shown to be larger than those produced by East Farleigh and Marden isolates.

Generally, haustoria of all isolates were more abundant in Northern Brewer, Fuggle and 35/68/58 than in 11/66/28 and Wye Challenger (Table 21). Three haustoria were sometimes seen in epidermal cells of susceptible hosts with a few isolates, but no more than one of any isolate encountered was in Wye Challenger and 11/66/28.

In the field heavy leaf infection in susceptible hosts usually resulted in both leaf surfaces being colonised. Under these conditions abundant haustoria were present in almost every epidermal cell of both surfaces (Plate 41).

Under glasshouse conditions Wye Challenger and 11/66/28 showed a prominent blister with a convex upper and a concave lower surface. Usually the epidermal cells in the blister area appeared to be larger than the epidermal cells in the unaffected area. In the field only a few blisters

Table 20. Haustorial dimensions (μm) seven days after inoculation of six hop cultivars and seedlings with eight isolates*

| cv./Seedling: / Isolate | | Northern Brewer | Fuggle | 35/68/58 | 11/66/28 | Wye Challenger | 14/66/50 | Mean |
|---|---|---|---|---|---|---|---|---|
| East Farleigh | (a) | 16.9 | 17.1 | 16.9 | 13.6 | 13.0 | 12.6 | 15.0 |
| | (b) | 6.8 | 7.5 | 6.1 | 3.2 | 3.8 | 3.7 | 5.2 |
| East Malling | (a) | 17.9 | 17.3 | 16.9 | 13.0 | 13.5 | 13.0 | 15.3 |
| | (b) | 7.7 | 7.0 | 7.9 | 4.4 | 4.5 | 3.2 | 5.8 |
| Glasshouse | (a) | 19.0 | 16.6 | 17.8 | 15.1 | 18.1 | 17.4 | 17.3 |
| | (b) | 8.2 | 7.4 | 8.3 | 5.7 | 6.4 | 7.2 | 7.2 |
| Marden | (a) | 18.9 | 19.5 | 20.1 | 12.2 | 12.6 | 12.1 | 15.9 |
| | (b) | 7.5 | 8.1 | 7.8 | 3.3 | 3.8 | 3.9 | 5.7 |
| Mordiford | (a) | 18.6 | 18.9 | 17.8 | 11.5 | 14.6 | 11.7 | 15.5 |
| | (b) | 6.2 | 7.9 | 7.2 | 3.6 | 4.5 | 3.7 | 5.5 |
| Rutland | (a) | 17.3 | 18.3 | 19.8 | 12.3 | 15.0 | 13.1 | 16.0 |
| | (b) | 6.6 | 8.1 | 7.1 | 3.2 | 4.1 | 3.8 | 5.5 |
| Staplehurst | (a) | 17.5 | 17.9 | 18.5 | 13.9 | 11.9 | 13.2 | 15.5 |
| | (b) | 7.7 | 7.6 | 6.6 | 3.9 | 3.6 | 3.6 | 5.5 |
| Tenbury Wells | (a) | 20.0 | 18.2 | 18.2 | 15.4 | 15.4 | 19.0 | 17.7 |
| | (b) | 8.0 | 7.8 | 7.3 | 5.7 | 5.4 | 5.9 | 6.7 |
| Mean | (a) | 18.3 | 18.0 | 18.3 | 13.4 | 14.3 | 14.0 | |
| | (b) | 7.3 | 7.7 | 7.3 | 4.1 | 4.5 | 4.4 | |

*Each figure is a mean of thirty replicates

(a) = length (i) for isolate differences; S.E.$\pm$0.2, L.S.D.(5%)=0.6
(ii) for cultivar differences; S.E.$\pm$0.2, L.S.D.(5%)=0.5

(b) = breadth (i) S.E.$\pm$0.1, L.S.D.(5%)=0.3
(ii) S.E.$\pm$0.1, L.S.D.(5%)=0.3

Table 21. Number of haustoria per length (0.5mm) of leaf section seven days after inoculation of six hop cultivars and seedlings with eight isolates*

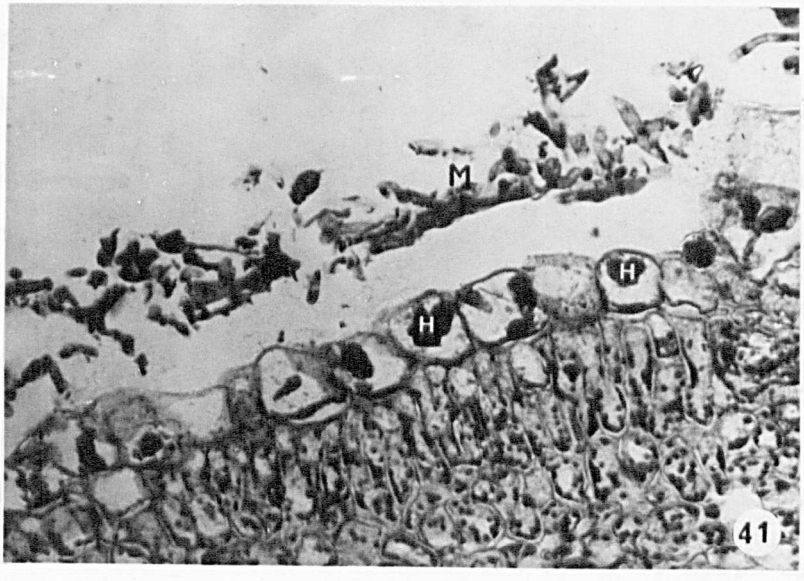
| cv./seedling : Isolate | Northern Brewer | Fuggle | 35/68/58 | 11/66/28 | Wye Challenger | 14/66/50 | Mean |
|---|---|---|---|---|---|---|---|
| East Farleigh | 2.00 | 1.33 | 1.47 | 1.00 | 1.00 | 1.00 | 1.30 |
| East Malling | 2.20 | 2.07 | 2.07 | 1.03 | 1.00 | 1.00 | 1.56 |
| Glasshouse | 2.28 | 1.30 | 2.87 | 1.10 | 1.33 | 2.63 | 1.92 |
| Marden | 1.47 | 2.43 | 1.73 | 1.03 | 1.00 | 1.10 | 1.46 |
| Mordiford | 1.60 | 1.30 | 1.50 | 1.00 | 1.00 | 1.03 | 1.24 |
| Rutland | 1.53 | 1.63 | 1.37 | 1.03 | 1.07 | 1.13 | 1.29 |
| Staplehurst | 1.87 | 2.13 | 2.40 | 1.00 | 1.00 | 1.03 | 1.57 |
| Tenbury Wells | 2.43 | 2.17 | 2.10 | 1.27 | 1.20 | 2.50 | 1.95 |
| Mean | 1.92 | 1.80 | 1.94 | 1.06 | 1.08 | 1.43 | |

*Each figure is a mean of thirty 0.5mm lengths.

(i) for isolate differences; S.E.$\pm$0.04, L.S.D.(5%)=0.10

(ii) for cultivar differences; S.E.$\pm$0.03, L.S.D.(5%)=0.09

Plate 41 A leaf of cv. Fuggle naturally infected in the field showing surface mycelium (M) detached on preparation of the tissue. The epidermal cells generally contain single haustoria, but sometimes two (H) were evident. Note the great frequency of haustoria. (x 320).


M
H
H
41

were formed. Under both glasshouse and field conditions the epidermal cells of the necrotic area of the blister were completely collapsed (Plate 42), while those of blisters bearing weak sporulation showed both normal haustoria and abnormal haustoria, the latter usually being embedded in the granular protoplasm of the cell. In transverse sections through chlorotic areas of Wye Target most epidermal cells were partially or completely collapsed with apparent damage to the palisade layer immediately beneath it (Plate 43). It appears from these results that resistance in Wye Target to all isolates tested was caused by a hypersensitive reaction induced in the host cell probably prior to penetration. On the other hand, the Glasshouse isolate produced normal haustoria initially in Wye Challenger and 11/66/28, a greater proportion of which eventually degenerated, showing a delayed hypersensitivity. As expected from earlier results, 14/66/50 behaved as a susceptible host to infection with the Glasshouse isolate while it reacted hypersensitively, immediately upon penetration by the East Farleigh isolate.

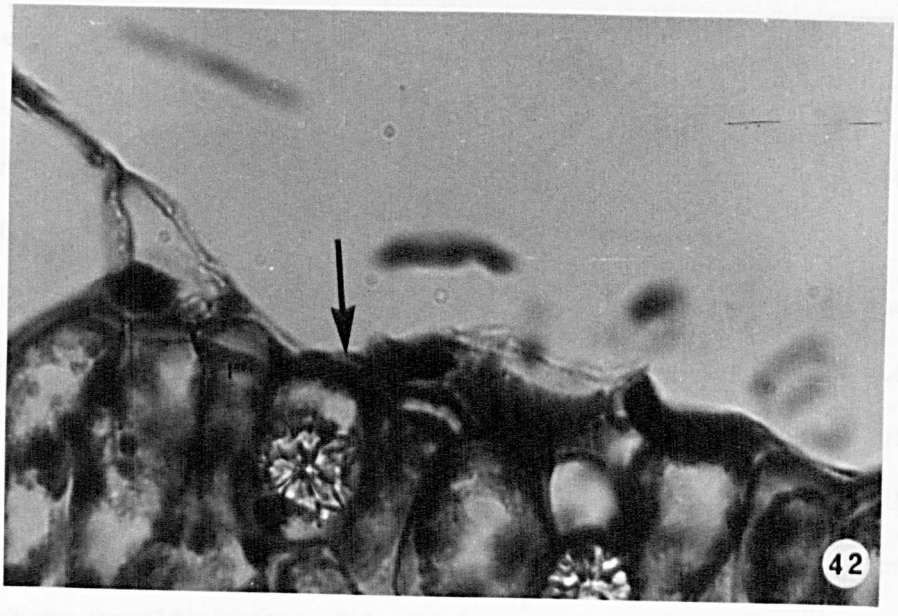
(g) Ultrastructural observations of S. humuli and its development in susceptible and resistant hop cultivars.

Light microscopic studies of the development of S. humuli on hop leaves indicated that the contents of penetrated cells of hosts exhibiting a resistant reaction became disorganised whether or not haustoria were in them. Damage to chloroplasts in the palisade tissue was also evident. However, in susceptible hosts, haustoria developed normally with no apparent damage to the host.

The increased resolution of the electron microscope was utilized to determine the changes that occurred in the protoplasmic organelles and membranes of resistant and susceptible hop cultivars as well as in the pathogen. Two principal reactions to infection by S. humuli were selected for detailed study - susceptible and immune, exhibited by Northern Brewer and Wye Target, respectively, to infection by the Glasshouse isolate.

Plate 42 A naturally-infected leaf of cv. Wye Challenger showing a collapsed epidermal cell (arrowed) of the blister. Note the large cell structure of the unaffected epidermal cell and the general disorganisation of the palisade cells (pc) (x 950).

Plate 43 A naturally-infected leaf of Wye Target showing a partially collapsed epidermal cell (arrowed) containing granular cytoplasm (gc). The contents of the palisade cells (pc) beneath the infected cells appear to be completely disorganised. (x 1,375).


42


gc
pa
43

Details of the experimental procedure are given in the Materials and Methods of this Section.

Conidia of S. humuli were barrel-shaped with curved end walls. The walls were relatively thin consisting of an electron dense outer layer and an electron transparent inner layer (Plate 44). Sometimes a mucilaginous layer was detected on the wall surface. The protoplasm occupied a thin layer lining the wall of the conidium and contained endoplasmic reticulum, several mitochondria of various shapes and sizes with their inner membranes invaginated as cristae. There were also plate-like structures (Plates 44, 45, 46 & 47), which Martin & Gay (personal communication) have shown to correspond with the fibrous bodies described, in conidia of other Sphaerotheca spp. (Blumer, 1933). The nucleus was irregular in shape and protected by a nuclear envelope containing scattered nuclear pores. The nucleoplasm usually appeared to be homogeneous, but the matrix contained dark areas, probably of chromatin (Plate 44). The most prominent feature of conidia of S. humuli was that more than 90% of the volume consisted of vacuoles. These usually contained electron dense particles, characteristic of vacuoles, and also light areas which may be glycogen (Plate 47).

The hyphal wall was delimited by an electron dense outermost layer. The cytoplasm contained abundant ribosomes, several mitochondria and profiles of the endoplasmic reticulum. There were also several lipid bodies but these were not enclosed by a membrane (Plate 49).

The epidermal cells of hop leaves vary in size and were seen to be bounded by a darkly staining cuticle which was either wavy (Plate 50) or smooth. Immediately beneath the cuticle was a thick, electron-transparent epidermal cell wall with microfibrils. The host protoplasm, arranged along the walls of the epidermal cells, was continuous between the host plasmalemma and the tonoplast. An epidermal cell contained a single

nucleus enclosed by a nuclear envelope (Plate 55). The protoplasm included numerous mitochondria of varying shapes and sizes with well developed tubular cristae and profiles of endoplasmic reticulum with or without ribosomes. The entire central area of the cell was occupied by a very large vacuole which contained small amounts of darkly staining electron-opaque material.

Primary penetration of the host is preceded by conidial germination, germ tube growth and appressorium formation on the leaf surface. The under side of the appressoria and contact zone with the leaf were coated with loose amorphous layers which are probably slimy mucilaginous substances cementing the appressorium to the leaf surface (Plates 50 & 51). Actual penetration of the pathogen was preceded by a morphological change in the host cell wall immediately beneath the appressorium. This involved the development of a disk-shaped ingrowth (papilla) between the host cell wall and the host plasmalemma. This ingrowth consisted of an electron-lucent material containing irregularly shaped and disposed electron-dense areas (Plates 50, 51 & 52). In later stages an additional densely staining deposit was usually arranged around the papilla material (Plate 52).

In Northern Brewer, the fungus penetrated into the cell lumen through the centre of the papilla. Further development caused invagination of the host plasmalemma and this was followed by the elongation of the neck, and the expansion of the terminal portion to form a globose haustorial body. Both the neck and the haustorial body were surrounded by a well defined fungal wall. The haustorial body and the adjoining portion of the neck were enclosed in a sac-like sheath which separated the haustorium from immediate contact with the host protoplasm. The tubular haustorial neck was slightly constricted at the point of penetration of the cell wall. Below this point the neck of the haustorium was usually surrounded by a collar. This consisted of an electron-lucent matrix identical in texture to

the papilla material. The neck was not closely invested by the collar and a channel containing electron dense material encircled it. About half way along the neck region four distinct zones of electron dense material were evident one pair inside and opposite the other (Plate 53). These observations suggest that they are part of two rings with the inner and outer rings embedding the fungal and host plasmalemmas respectively. Similar structures were observed in the neck region of haustoria of S. pannosa by Perera (1972), who suggested that the rings were lipidic in nature. The host-parasite interface in the neck region was partly obscured due to the accumulation of electron dense material which also may be lipidic. The host plasmalemma for the most part was lined by an electron dense material which became discontinuous towards the region where it connects to the haustorial body (Plates 53 & 54).

There were numerous profiles of haustorial lobes surrounding the haustorial body. The whole of the haustorium, i.e. the body and the lobes, were enclosed in a common sheath matrix bounded by the host plasmalemma or sheath membrane (Plate 55). These haustorial lobes were often connected to the haustorial body (Plates 56 & 57) and were sometimes branched and showed a curving of the branches through right angles (Plate 57). Haustoria presumed to be immature, possessed only a few lobes (Plate 58), while others (the mature ones) showed a large number of lobes (Plates 59 & 60). In transverse sections the arrangement of haustorial lobes on the opposite ends of the haustorial body suggested bipolarity (Plate 59) as shown for S. pannosa (Perera, 1972). The body of the haustorium contained a single nucleus surrounded by a double membraned nuclear envelope with nuclear pores. The nucleus usually occupied a relatively large area of the haustorial body (Plate 58). Most of the nucleoplasm was homogeneous but a nucleolus and several darkly stained bodies, probably of chromatin, were seen (Plates 55 & 58).

The haustorial body and the lobes were surrounded by a sheath matrix which contained very small granules. The sheath was bounded by the sheath membrane which was an invagination of the host plasmalemma. Thus the haustorium did not lie free in the host protoplasm (Plate 55 & 58). Immediately outside the wall of the haustorium, was the wide sheath-matrix, itself bounded by a sheath membrane contigous with the tonoplast of the host cell. The size of the sheath was considered by Bracker (1968) to be a function of haustorial age. Well marked tubular invaginations of the sheath membrane were conspicuous at the region where the haustorial body meets the neck (Plates 53, 54, 56 & 61), but much less so away from this region (Plates 55, 56, 59 & 62). Two haustoria sometimes occupied a single epidermal cell, but each haustorial sheath remained separate from the other, although they were pressed together (Plate 64). The electron-transparent layer around the haustorial body and the lobes was presumably the fungal wall (Plates 57 & 63). The cytoplasm of haustorial bodies and their lobes possessed numerous mitochondria of various sizes and shapes with well developed cristae in them. The cytoplasm also contained endoplasmic reticulum, abundant free ribosomes and vacuoles (Plates 55, 56 & 58). Some of the vacuoles contained electron-dense granules (Plate 60). Osmiophillic droplets, without boundary membranes found in the vacuoles in the body and the lobes, were probably lipids (Plate 59). Darkly stained granules were frequently found in the haustorial body and the lobes and were probably similar to glycogen deposits in other fungi (Coffey, Palevitz & Allen, 1972).

In an infected epidermal cell of Wye Target two types of defence mechanisms were observed in response to an attempted penetration by the fungus. Papilla like material became extensive and enveloped the lumen of an epidermal cell. The deposition of material occurred between the host cell wall and the host plasmalemma (Plates 65, 66 & 67). The failure

to observe, by any method, haustoria in the epidermal cells of Wye Target suggests that this encasement occurred only as a reaction of the host. This material was not confined to the invaded cells but was sometimes also found in the adjacent epidermal and palisade cells (Plates 66 & 68).

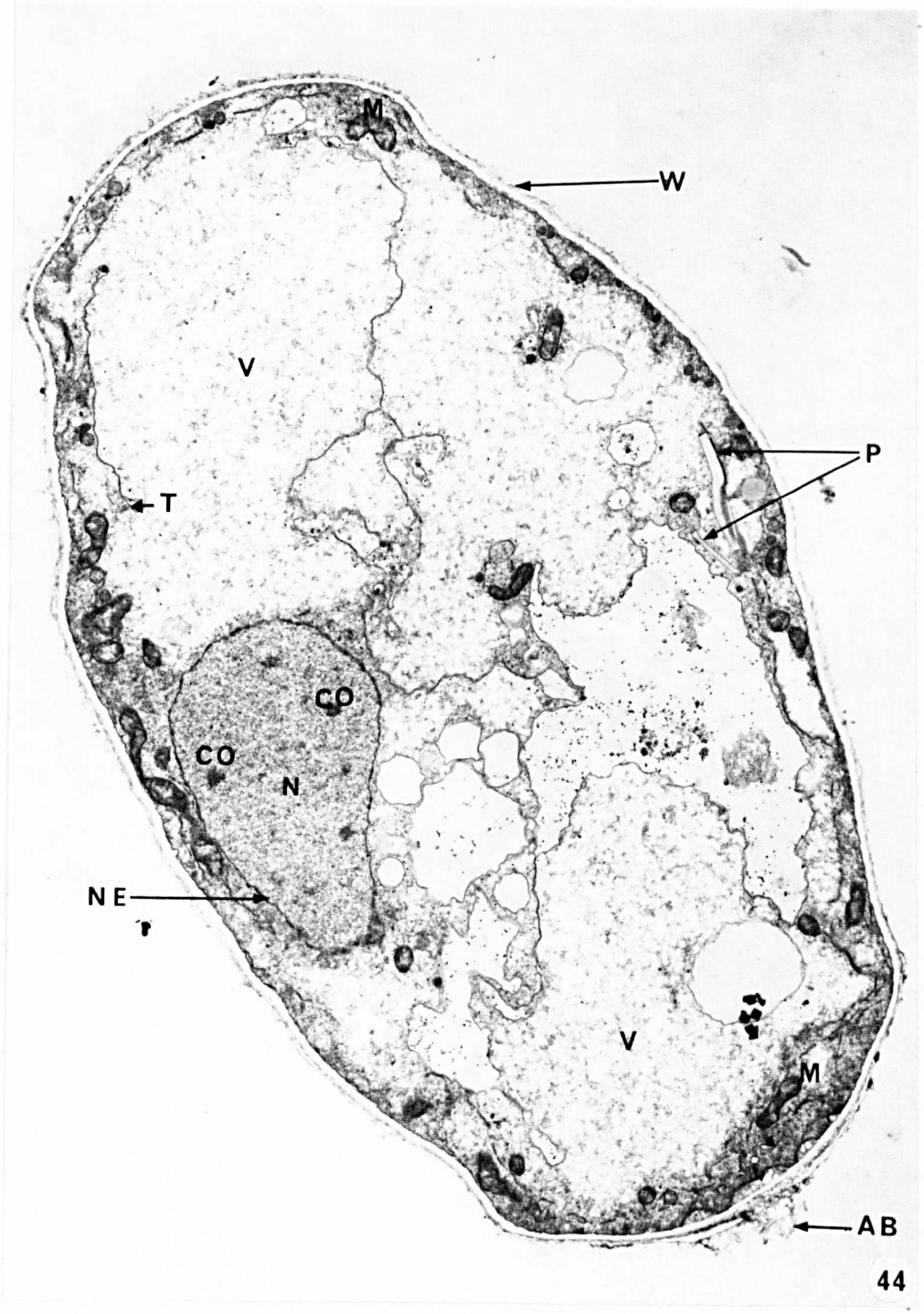
Additional response by the infected epidermal cells was a hypersensitive reaction where numerous changes were apparent in the host cell fine structure. Hypersensitivity also occurred in the neighbouring cells. The host tonoplast was disrupted and the protoplasmic contents were released into the vacuole where they assumed a very electron dense property. (Plates 66 & 69). Mitochondria in the affected cells retained their membraneous structure but did not appear to have well arranged cristae (Plate 70). The host nuclear envelope and the nucleolus had disintegrated (Plate 71). Chloroplasts of infected susceptible cells of Northern Brewer showed little abnormality in structure (Plate 72). Chloroplast degeneration seemed to be the first perceptible change in the cells beneath the infected epidermal cell of Wye Target (Plate 73). These investigations indicated that the development of lamellar structure in chloroplasts appear to be adversely affected in infected leaves. Lamellae in the abnormal chloroplasts in Wye Target did not show the clear grana structure common to normal lamellae (Plate 73). Chloroplasts of both healthy and infected cells contained lipid bodies, (Plates 72 and 73) but these appeared more numerous in inoculated Wye Target.

| | |
|---|---|
| A | Appressoria |
| AB | Abscission region |
| C | Collar |
| CH | Channel between haustorial neck and collar |
| CO | Chromatin |
| ER | Endoplasmic reticulum |
| FW | Fungal wall |
| G | Glycogen |
| HB | Haustorial body |
| HL | Haustorial lobes |
| HL/HB | Haustorial lobes connected to the body |
| L | Lipid bodies |
| M | Mitochondria |
| MAT | Sheath matrix |
| ML | Mucilaginous layer |
| N | Nucleus |
| NE | Nuclear envelope |
| NK | Neck |
| NP | Nuclear pore |
| NU | Nucleolus |
| OW | Original wall of conidium |
| P | Plate-like structure |
| R | Rings |
| RB | Ribosomes |
| SI | Sheath invagination |
| SW | Septal wall of conidium |
| T | Tonoplast |
| V | Vacuole |
| W | Conidial wall |

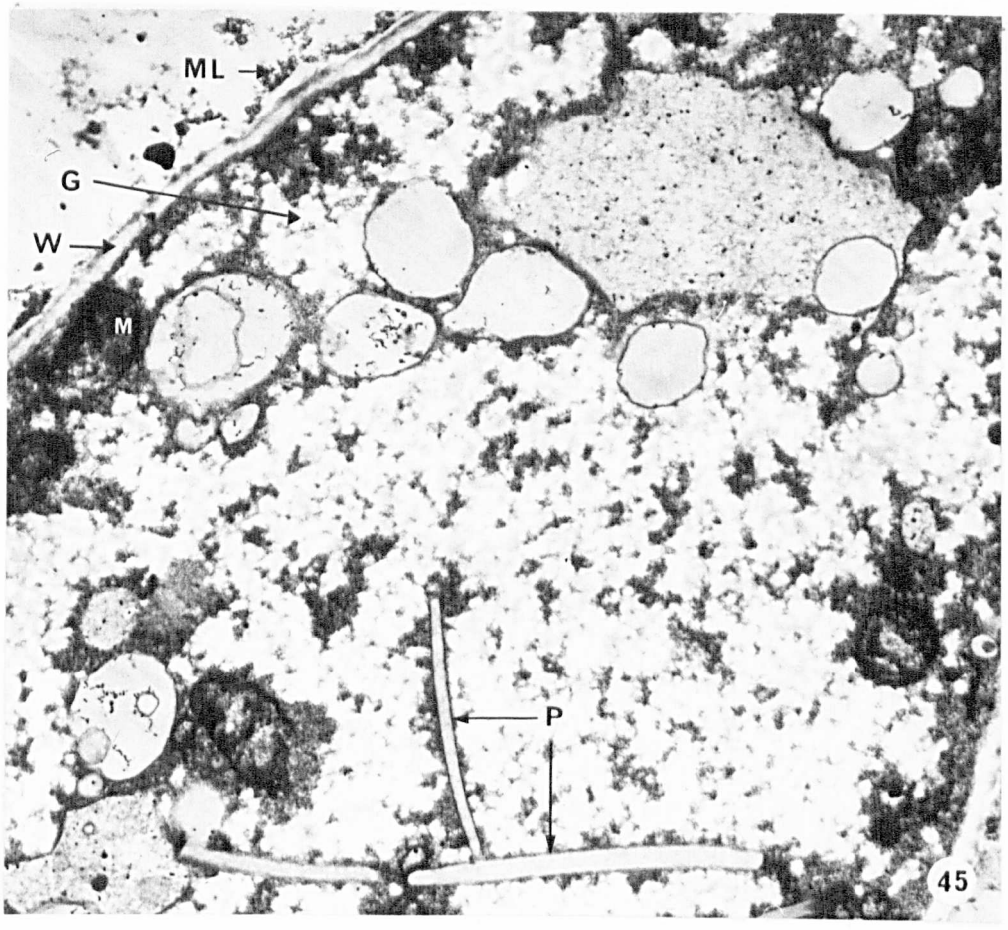
| | |
|---|---|
| cr | cristae |
| cu | cuticle |
| d | deposit |
| er | endoplasmic reticulum |
| hc | host cytoplasm |
| hp | host plasmalemma |
| hr | host reaction |
| hw | host wall |
| l | lipid bodies |
| lm | lamellae |
| m | mitochondria |
| mb | membrane body |
| mm | membrane |
| n | nucleus |
| ne | nuclear envelope |
| og | osmiophillic granules |
| pa | papilla like material |
| pc | palisade cells |
| r | ribosomes |
| sm | sheath membrane |
| t | host tonoplast |
| v | vacuole |

NB Capital and simple letters indicate structures of fungal and host origin respectively.

Plate 44 A median section through a conidium showing the barrel shaped structure of the spore of S. humuli. The abscission region (AB) which finally connected the conidium to another in the conidial chain. The conidial wall (W) consists of an electron lucent layer with an electron dense coating. There is a single nucleus (N) covered by a double membraned nuclear envelope (NE). The nucleus contains chromatin (co) like bodies. Note the presence of plate like structures (P) which probably correspond to fibrous bodies. There are several large vacuoles (V) occupying a large volume of the conidium. The tonoplast (T) was disrupted at some points (arrowed). (x 18,300).


M
W
V
P
T
CO
CO
N
NE
V
M
AB
44

Plates 45 & 46 Median section of a conidium showing abundant plate like bodies (P) which are probably fibrous. Usually these are present in the protoplasm but here, the tonoplast is damaged during preparation and all protoplasmic organells are mixed with vacuolar contents. The nucleus (N) also show signs of damage. A muciloginous layer (ML) is evident on the conidial wall (W). There are numerous unstained particles which are probably glycogen bodies (G). In plate 46 the lateral original wall (OW) is distinct from the walls which originated as part of a septum. The septal wall has split between adjacent spores and the septal wall (SW) shown here is actually a half septum.
(Plate 45, x 11,925; Plate 46, 7,640).


ML
G
W
M
P
45

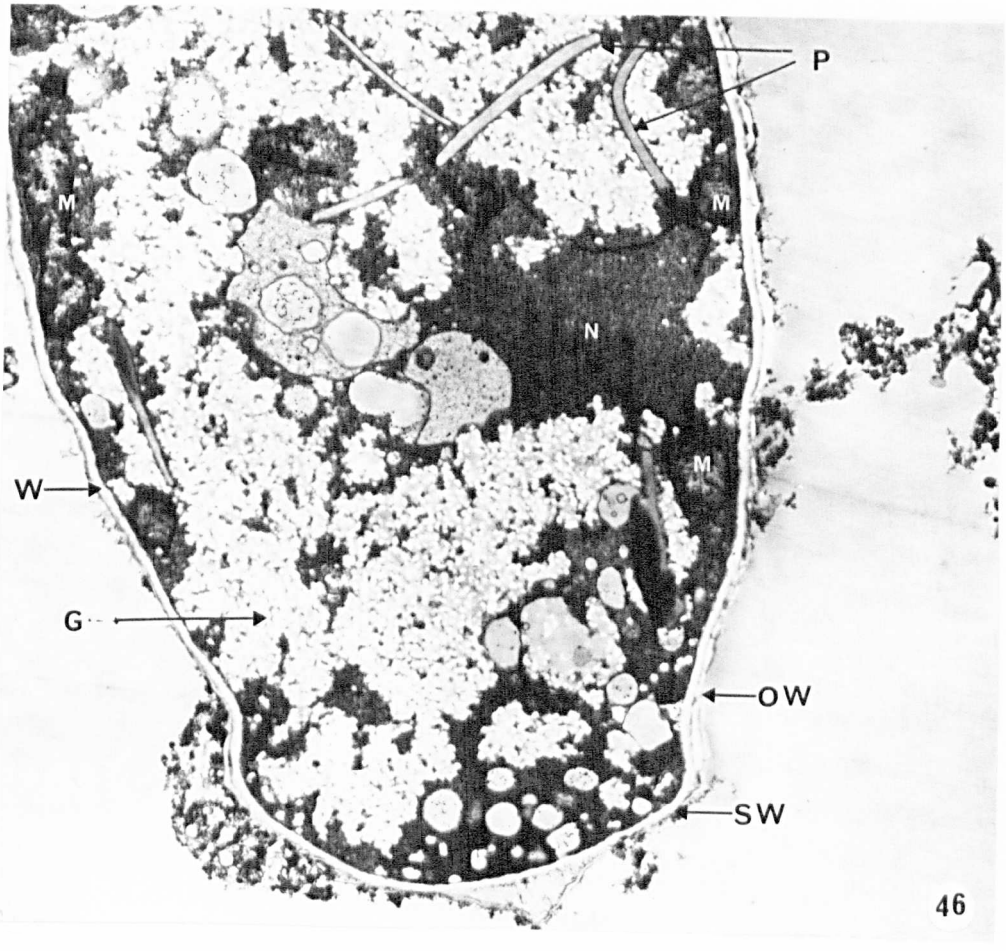
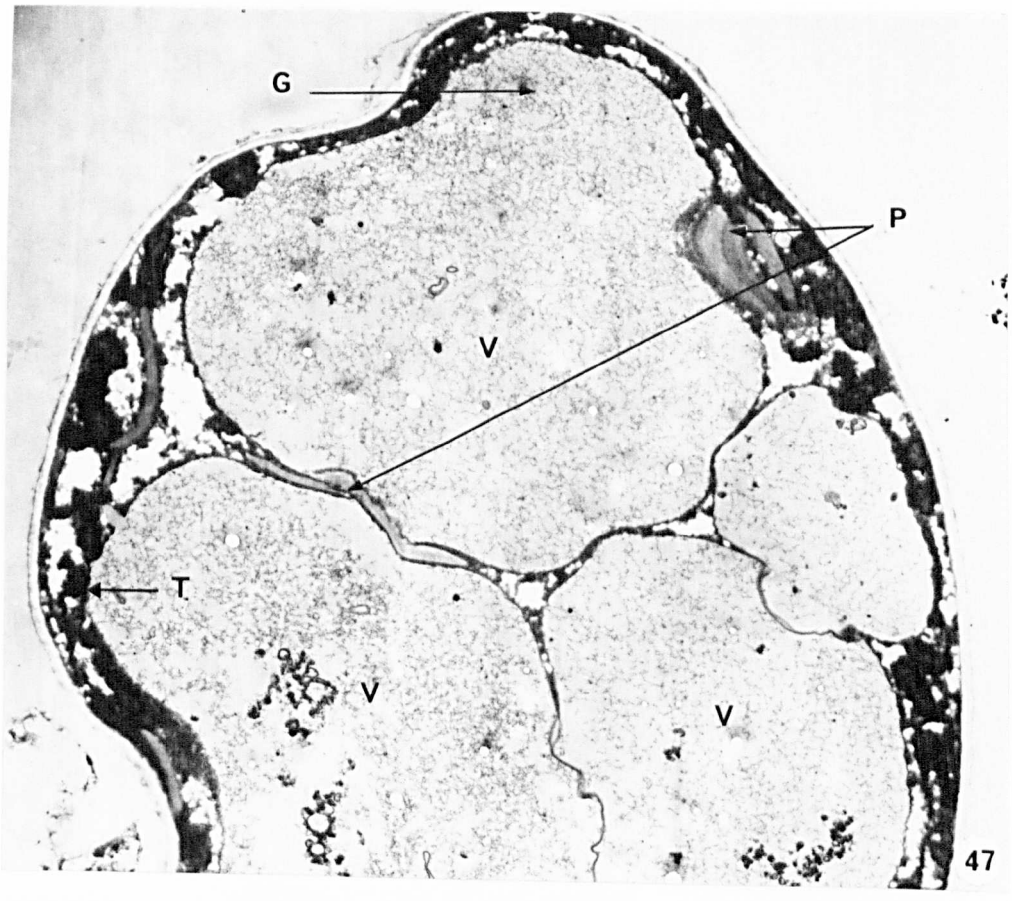
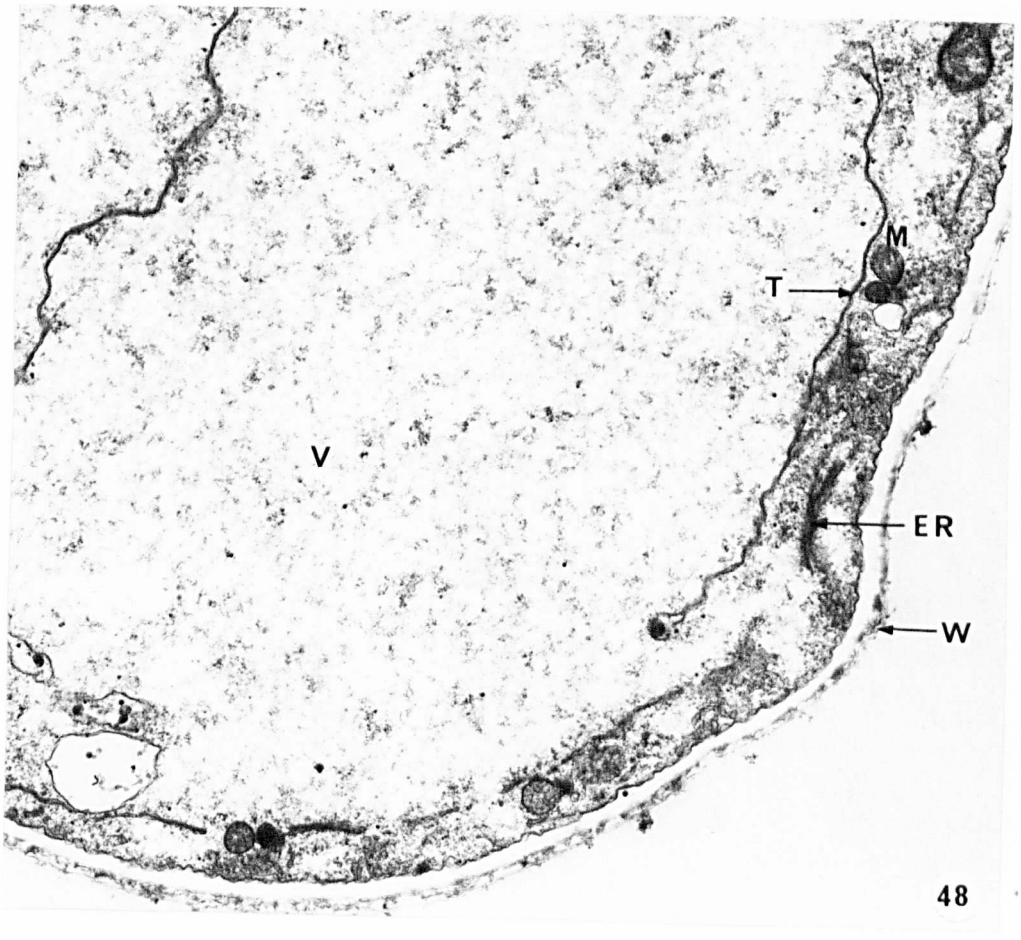

P
M
M
N
M
W
G
OW
SW
46

Plate 47 A median section through a conidium showing several large vacuoles (V) containing amorphous electron dense material. The plate-like bodies (P) appear to be closely packed and some were found in the protoplasm even between two vacuoles Several glycogen-like granules (G) were also present. (x 7,280).

Plate 48 Median section through a conidium showing the detailed wall structure. Mitochondria (M), and endoplasmic reticulum (ER) occurred in the protoplasm which lines the inner wall of the conidium and were separated from vacuolar contents (V) by the tonoplast (T), which is damaged at some points. (x 40,950).

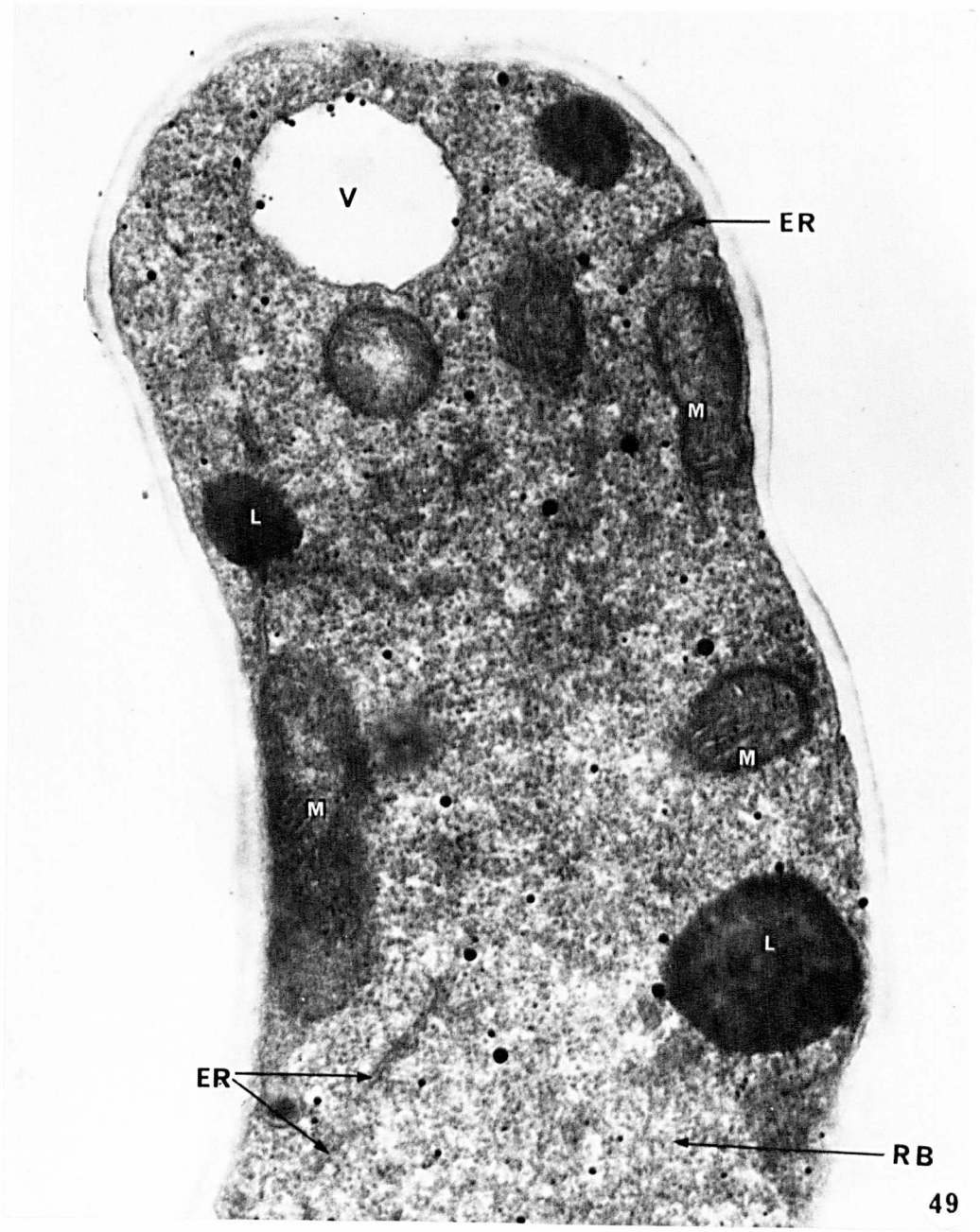



47



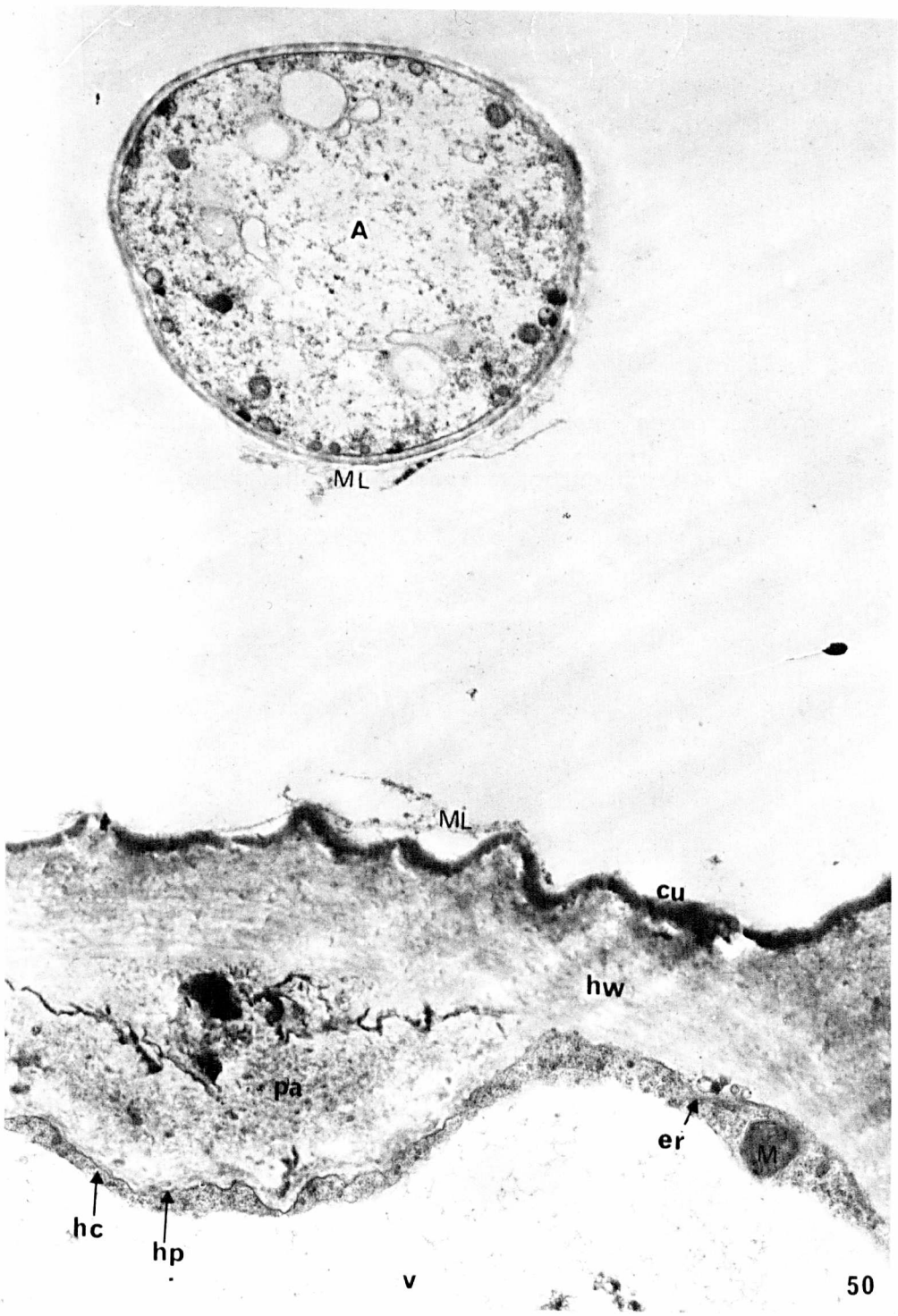

48

Plate 49 Transverse section through a hypha showing numerous ribosomes (RB), rough and smooth endoplasmic reticulum (ER), mitochondria (M) and lipid bodies (L). The lipid bodies lacked membranes. There was also a single vacuole (V). (x 37,900).

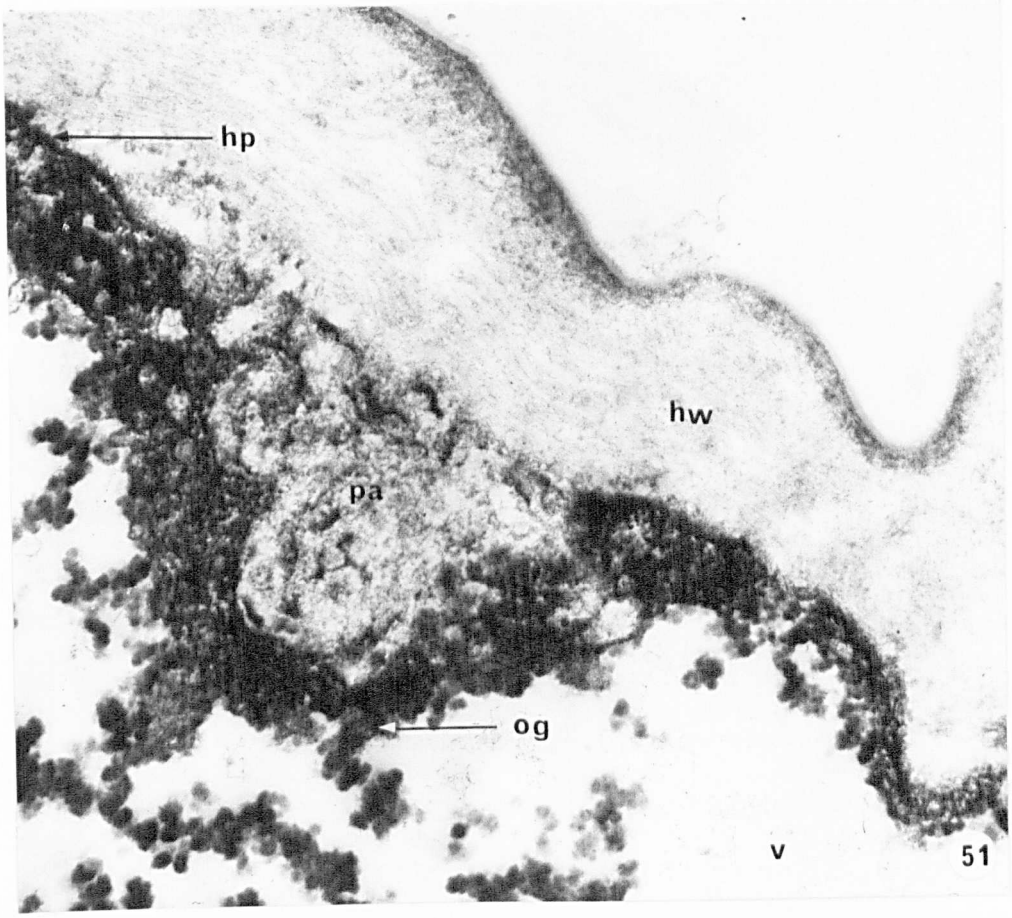



49

Plate 50 Section of the epidermal cell of cv. Northern Brewer showing its response prior to penetration of the fungus. Note the detached appressorium (A) which was probably cemented to the host wall by the mu cilaginous layer (ML). Note also the dome-shaped papilla (pa) between the inner surface of the host cell wall (hw) and the host plasmalemma (hp) immediately below the appressorium (A). This deposit has electron dense amorphous material and electron lucent membranous material. Note also the difference in texture of the fibrillar like material in the host cell wall and granular papilla material. (x 16,200).


A
ML
ML
cu
hw
pa
er
M
hc
hp
v
50

Plates 51 & 52 Section of epidermal cell through a susceptible host cv. Northern Brewer howing accumulation of reaction products into a papilla (pa) between the host wall (hw) and host plasmalemma (hp). This also showed a cluster of very osmiophillic granules (og) (Plate 51) which are quite different from ordinary vacuole contents. Osmiophillic material was sometimes tightly packed around the papilla (pa) (Plate 52). The papilla material is a mixture of electron lucent and dense material resembling the material of the collar seen later. Osmiophil ic granules may also be found later in the neck region around the collar. (Plate 51, x 43,520; Plate 52, x 16,200).


hp
hw
pa
og
v
51

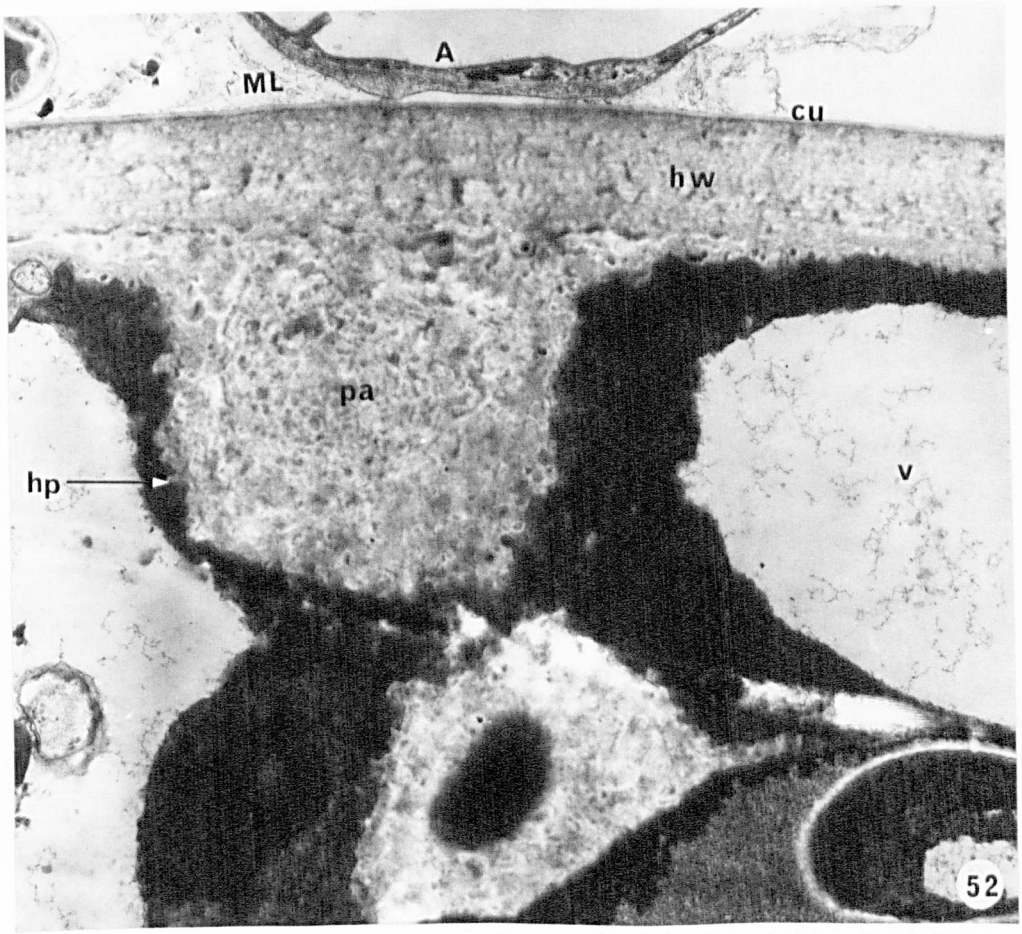

A
ML
cu
hw
pa
hp
v
52

Plate 53 Section through an infected epidermal cell of cv. Northern Brewer showing the connection of the neck to the body of the haustorium. The connection with the appresorium had been severed during preparation. Note that almost the entire length of the neck (NK) is surrounded by a collar(C) and near the constricted region of the neck the channel (CH) was occluded with electron dense material. About half way along the neck are profiles of two rings (R) obscuring the haustorial and host plasmalemma. Note the greatly infolded sheath membrane (SI) at the junction of the haustorial neck and body. The host protoplasm (hc) had several profiles of smooth and rough endoplasmic reticulum (er) and mitochondria (M). (x 28,600).

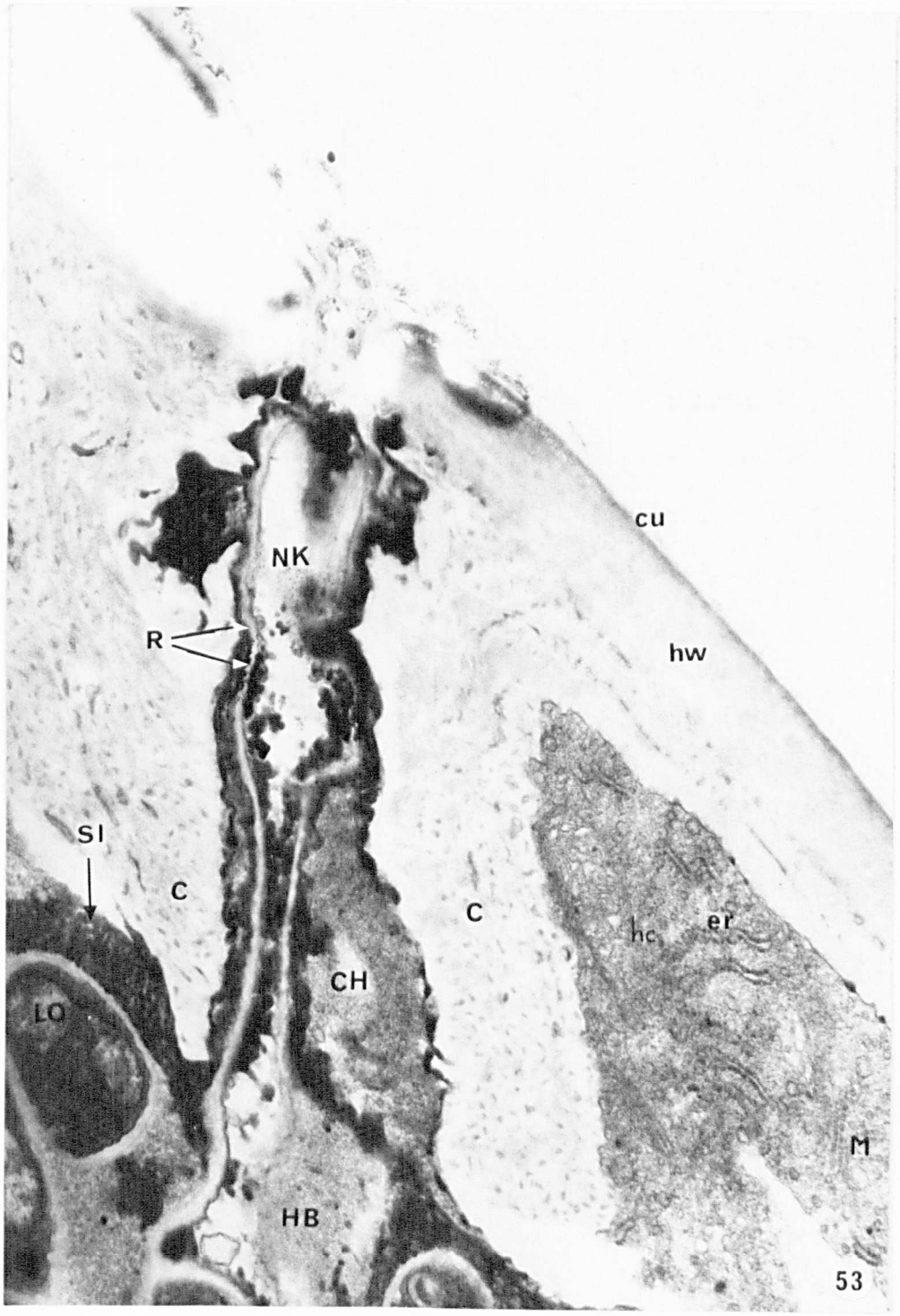

cu
NK
R
hw
SI
C
C
hc
er
CH
LO
M
HB
53

Plate 54 Oblique vertical section through the epidermal cell of cv. Northern Brewer showing the neck region and connection to the haustorial body. The haustorial neck (NK) is surrounded by the electron dense material in the channel (CH) which in turn is surrounded by the electron lucent collar (C). Note also the membrane body (mb) near the neck region. The protoplasm of the host contained profiles of endoplasmic reticulum (er) and mitochondria (m). (x 29,400).


hw
C
C
CH
NK
m
er
C
HB
er
hp
SI
mb
54

Plate 55 Transverse section of an infected epidermal cell of cv. Northern Brewer a mature haustorial body (HB) and profiles of haustorial lobes (HL) embedded in a common sheath matrix (MAT). Note the large nucleus (N) containing a nucleolus (NU) and bounded by a membranous nuclear envelope (NE). Mitochondria (M) and endoplasmic reticulum (ER) were found in both haustorial body and lobes. Vacuoles (V) in the haustorial body contained profiles of membranes. The transparent layer adjoining the fungal plasmalemma was probably the fungal wall (FW). The sheath matrix was electron lucent and contained fine granular material. The sheath matrix was limited by the sheath membrane (sm) or host plasmalemma which was the interface between the haustorium and the host cytoplasm. The sheath membrane showed small invaginations (SI). The host nucleus (n) was frequently seen in the proximity of the haustorium. (x 13,600).

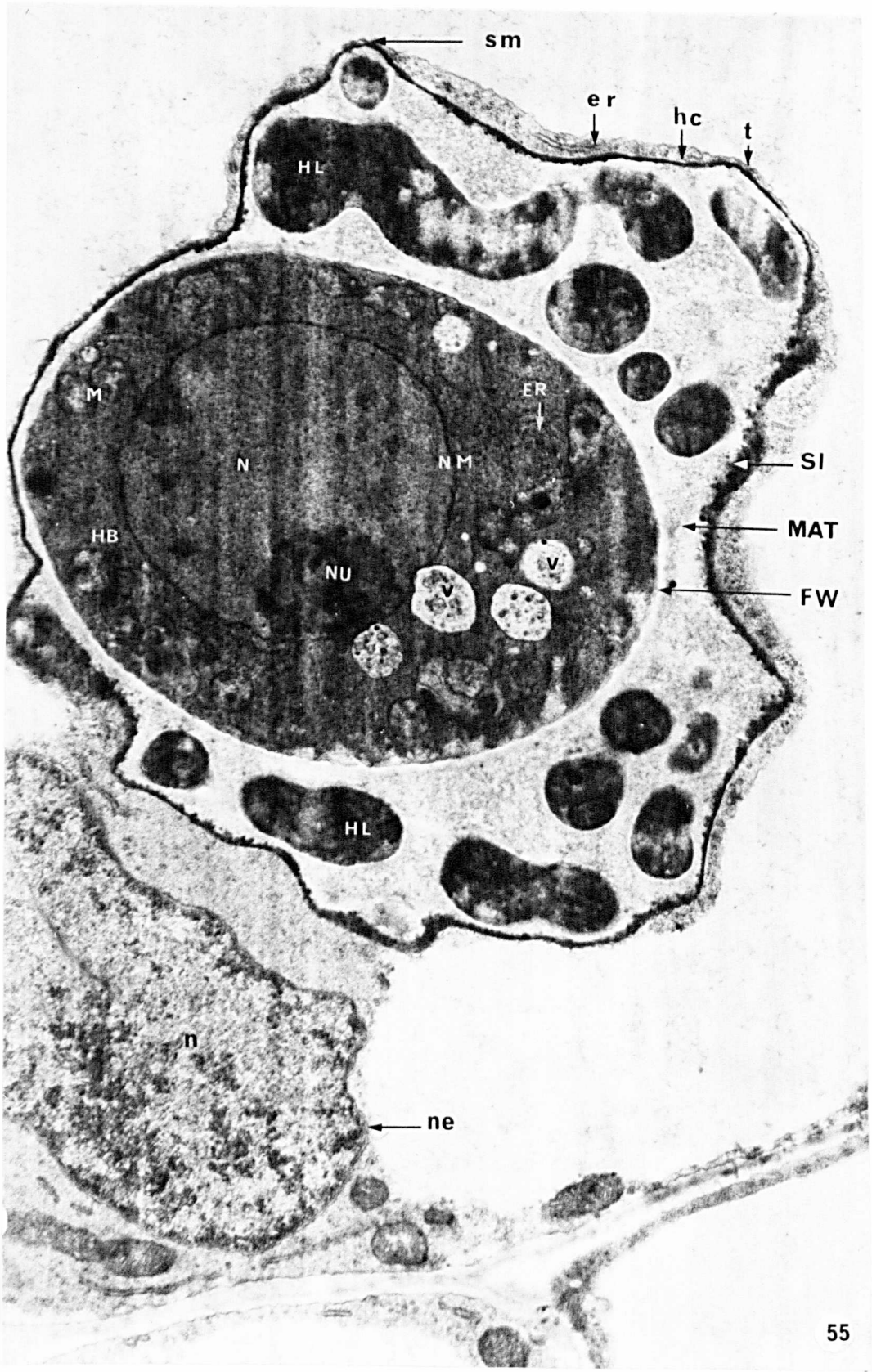

sm
er
hc
t
HL
M
ER
N
NM
SI
MAT
HB
NU
V
V
FW
HL
n
ne
55

Plate 56 A section through a haustorium in epidermal cells of cv. Northern Brewer. The electron lucent papilla material (pa) lies beneath the appressorium (A) and the membrane is heavily folded at the junction of the neck and body. Note also the connection of the haustorial lobe to the body. (HL/HB). (x 16,250).

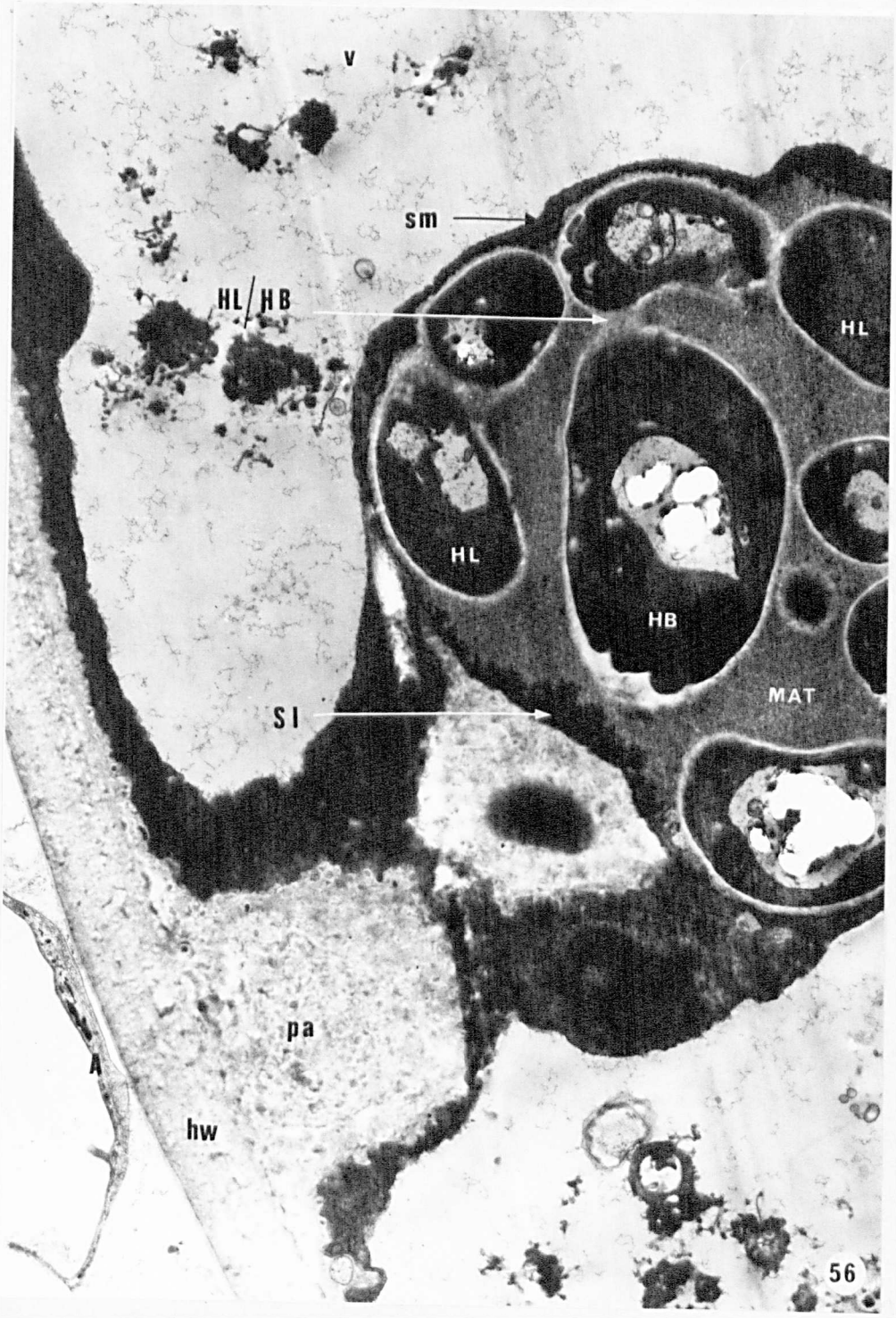

v
sm
HL/HB
HL
HL
HB
MAT
SI
pa
A
hw
56

Plate 57 Electron micrograph of section through the periphery of the haustorium in an infected epidermal cell of cv. Northern Brewer showing connection of haustorial lobes (HL) to the body (HB). Note the branching of lobes and their reflexed form. (x 18,400).

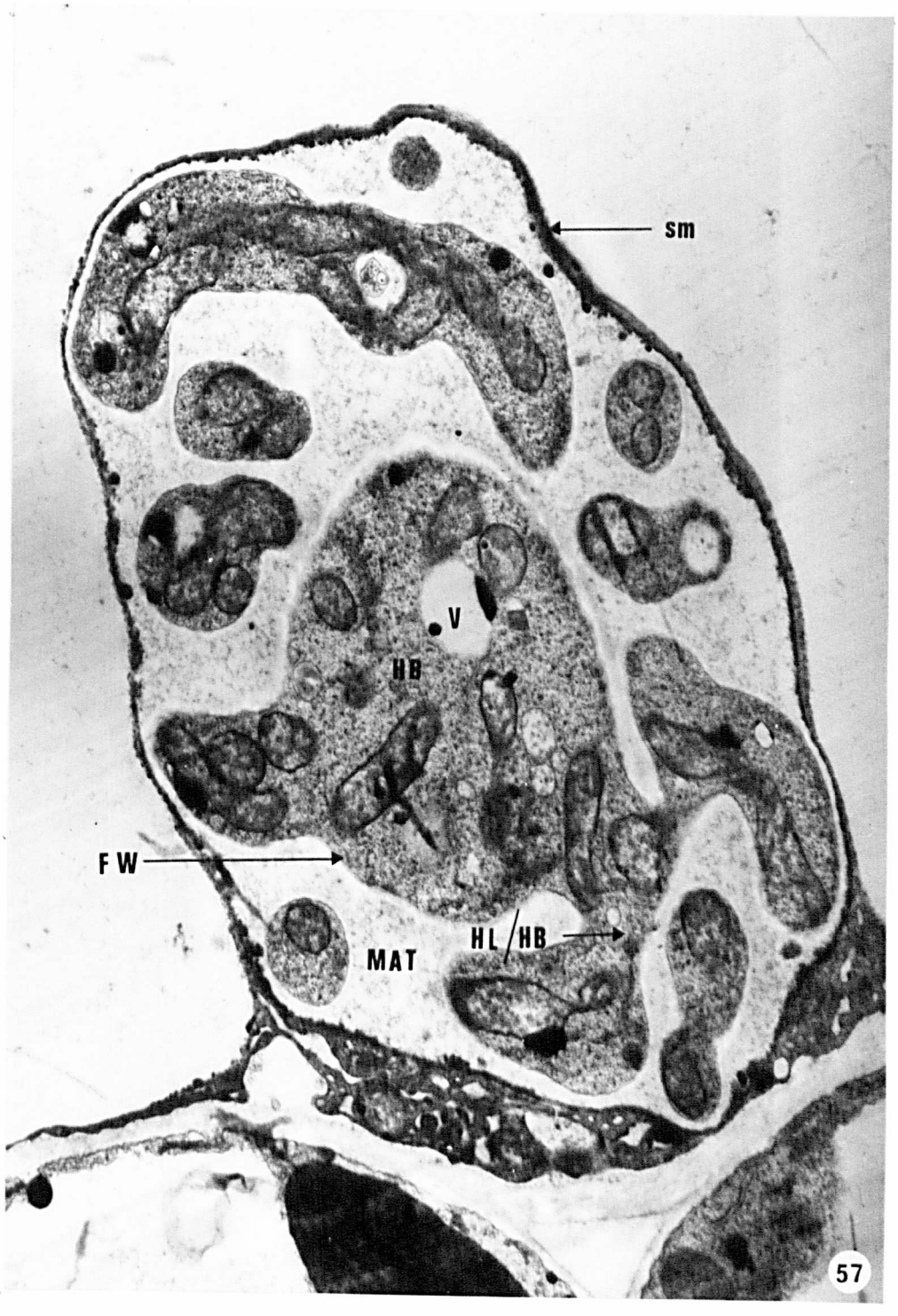
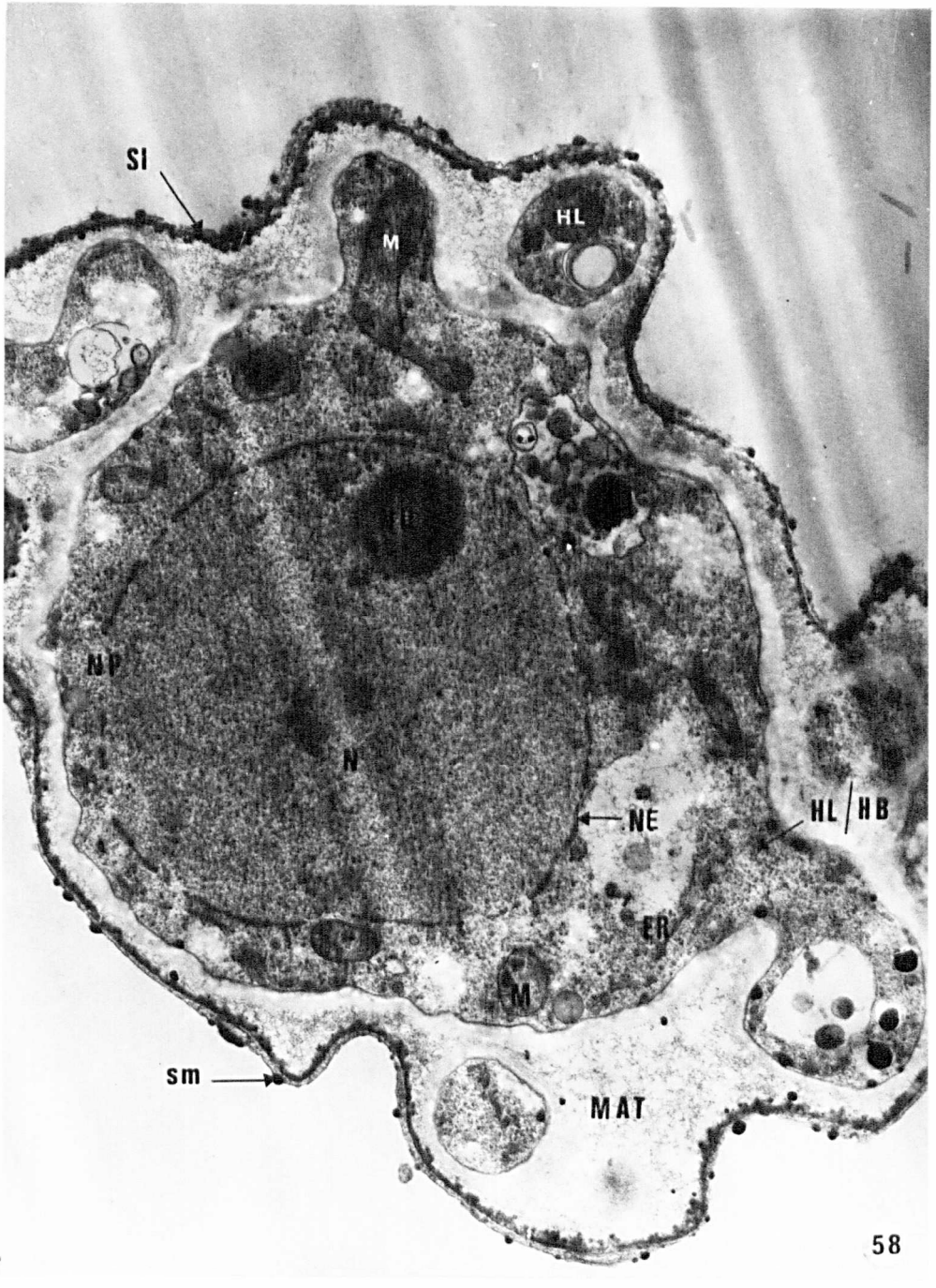
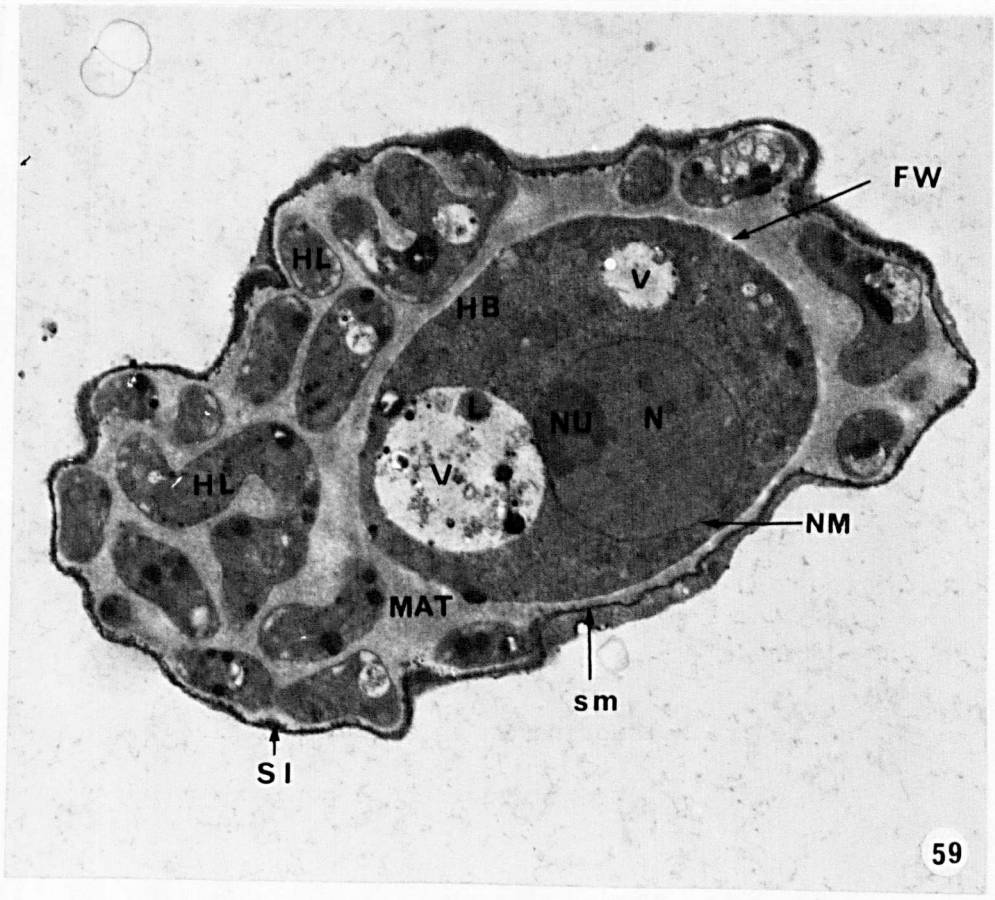

sm
V
HB
FW
HL/HB
MAT
57

Plate 58 A section through a haustorium presumed to be young in the cell lumen of cv. Northern Brewer. Only a few haustorial lobes (HL) were present and the sheath matrix (MAT) was poorly developed. The occurrence of haustorial lobes (HL) at the two ends of the haustorial body (HB) (some of which are connected to the body) suggest a bipolar arrangement. The haustorial body contained a large nucleus (N) with a nucleolus (NU). The nucelar envelope (NE) had quite distinct nuclear pores (NP). (x 13,800).


SI
M
HL
NP
N
NE
HL/HB
ER
M
sm
MAT
58

Plates 59 & 60 A mature haustorium in an epidermal cell of cv. Northern Brewer. A large globose haustorial body (HB) contained a nucleus (N) with a nucleolus (NU) (Plate 59) The vacuoles (V) in the haustorial body contained probably lipil (L) like bodies. The profiles of some of the haustorial lobes (HL) in the sheath matrix (MAT) suggest that they were branched. The sheath matrix is extensive accomodating a large number of lobes. There is some suggestion of bipolarity in plate 59. (Plate 59, x 7,640; Plate 60, x 12,880).


FW
HL
HB
V
L
NU
N
V
HL
NM
MAT
sm
SI
59

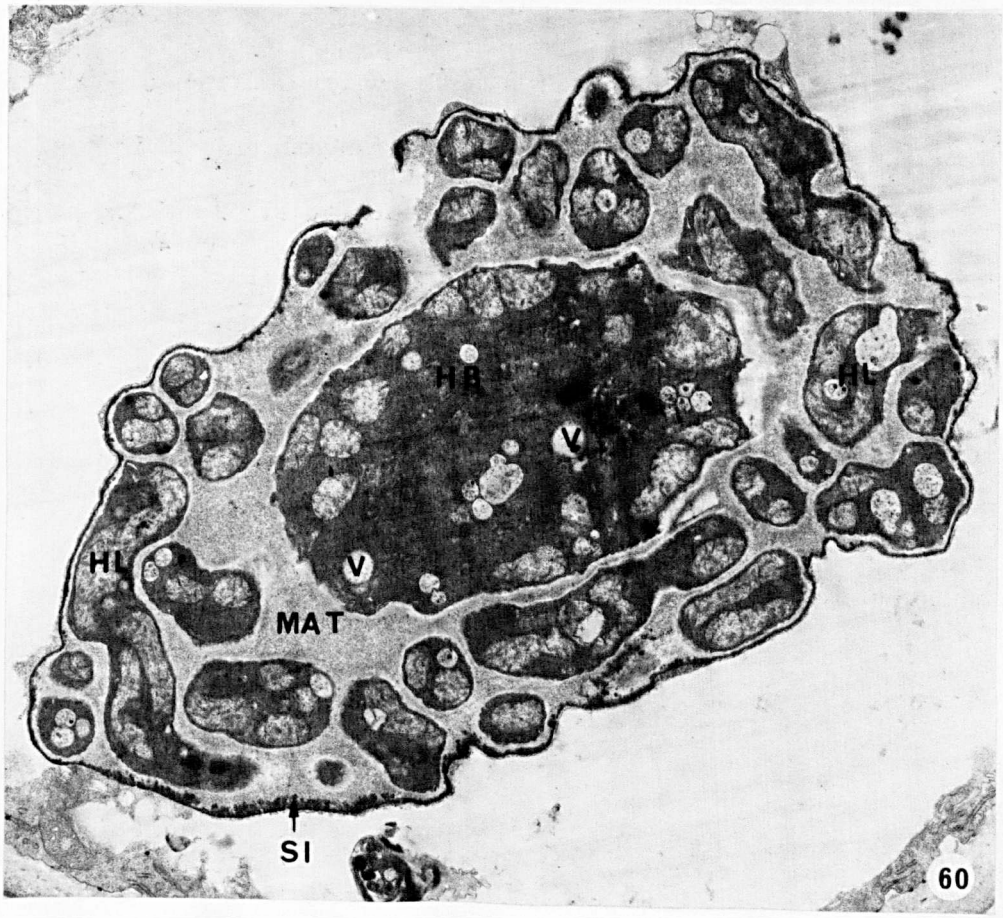
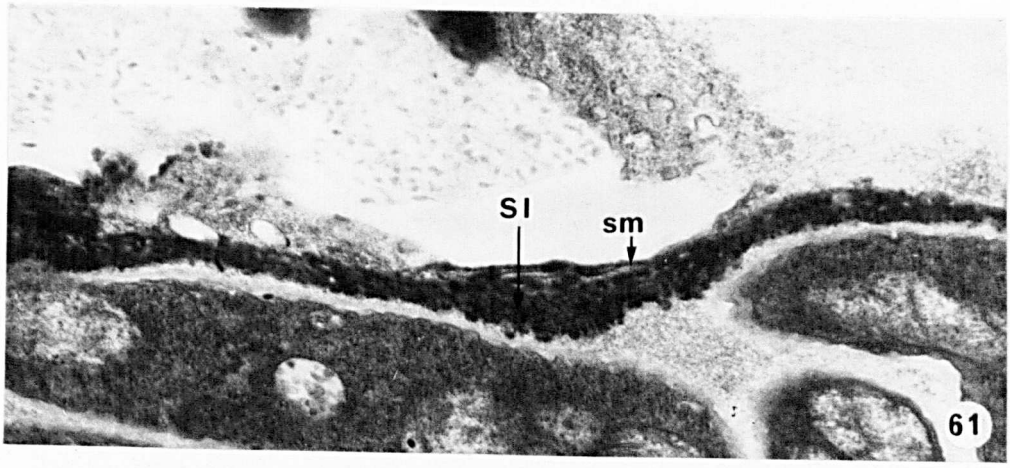
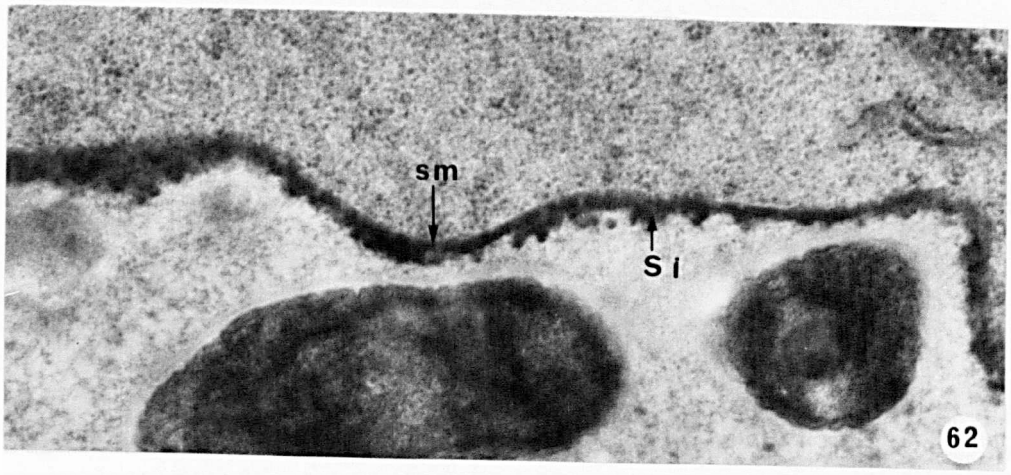

HB
HL
V
HL
V
MAT
SI
60

Plate 61 A portion of the junction between the neck and the body of a haustorium in an epidermal cell of cv. Northern Brewer. Note conspicuously folded invagination (SI) of the sheath membrane (sm). (x 27,500).

Plate 62 A portion of the sheath membrane (sm) distant from the neck region a haustorium in an infected epidermal cell of cv. Northern Brewer. Note the invaginations(SI) of the sheath membrane are less well developed than at the junction with the neck (Plate 61). (x 39,000).

Plate 63 Section of part of the body and lobes of an haustorium showing fibrillar material in the fungal wall (FW). Note that the vacuoles (V) in the lobes (HL) contained several lightly stained bodies. (x 46,560).


SI
sm
61


sm
SI
62

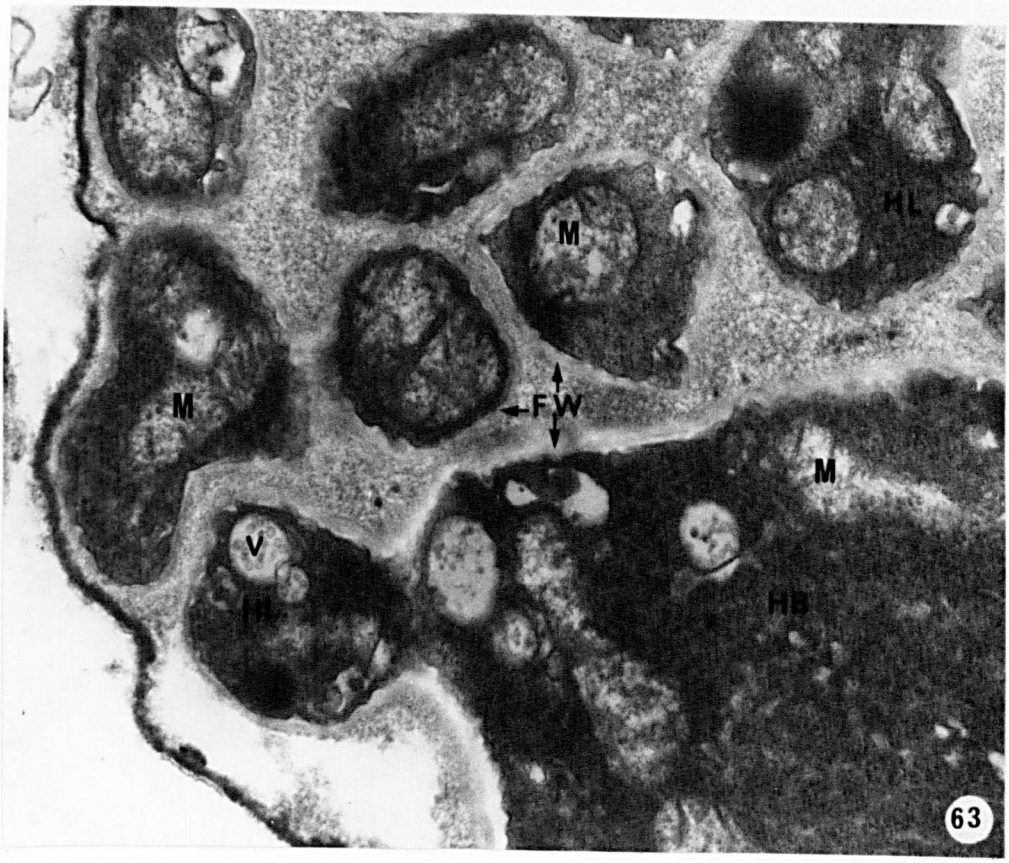

M
HL
M
FW
M
V
HL
HB
63

Plate 64 A section through an epidermal cell of ov. Northern Brewer showing two haustoria. Note that each sheath membrane (SM) retained its independent identity even though the two sheaths were pressed together (arrowed). Note also the connection of haustorial lobes to the body (HL\HB) and the characteristic curvature of the lobes. (x 9,800).

Plate 64 A section through an epidermal cell of cv. Northern Brewer showing two haustoria. Note that each sheath membrane (sm) retained its independent identity even though the two sheaths were pressed together (arrowed). Note also the connection of haustorial lobes to the body (HL/HB) and the characteristic curvature of the lobes. (x 9,800).

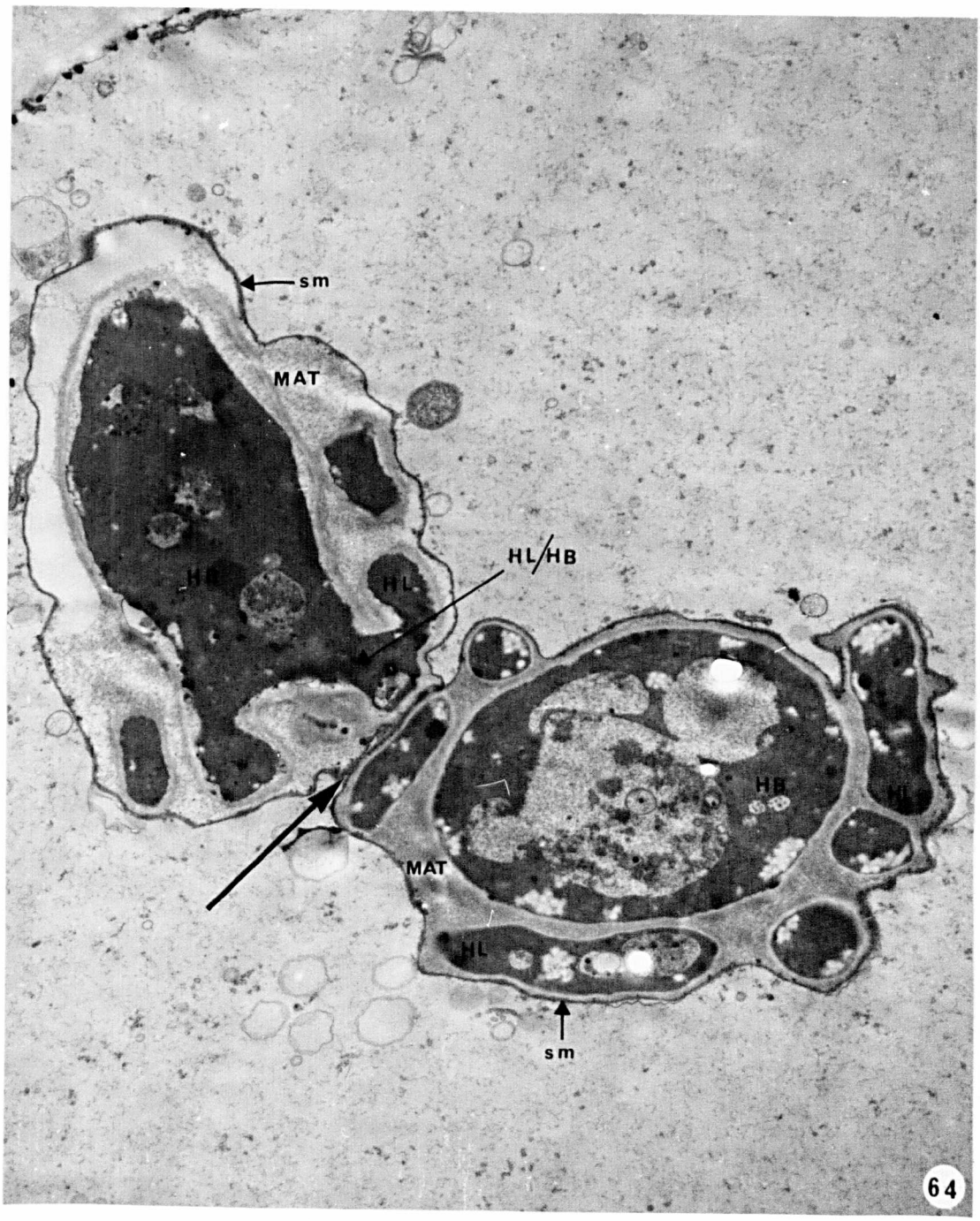

sm
MAT
HL
HL/HB
HB
HL
MAT
HL
sm
64

Plate 65 An epidermal cell of cv. Wye Target. Note the host reaction involving the formation of papilla like material (hw) between the fibrillar host (hw) wall and the host plasmalemma (hp). This generalized reaction is not confined to the infected cell but also to the adjoining cell. Hypersensitivity of the infected cell and the neighbouring cells was indicated by the presence of darkly staining osmiophillic granules (og). Note also the rupture of the host tonoplast (t) (arrowed). (x 5,400).

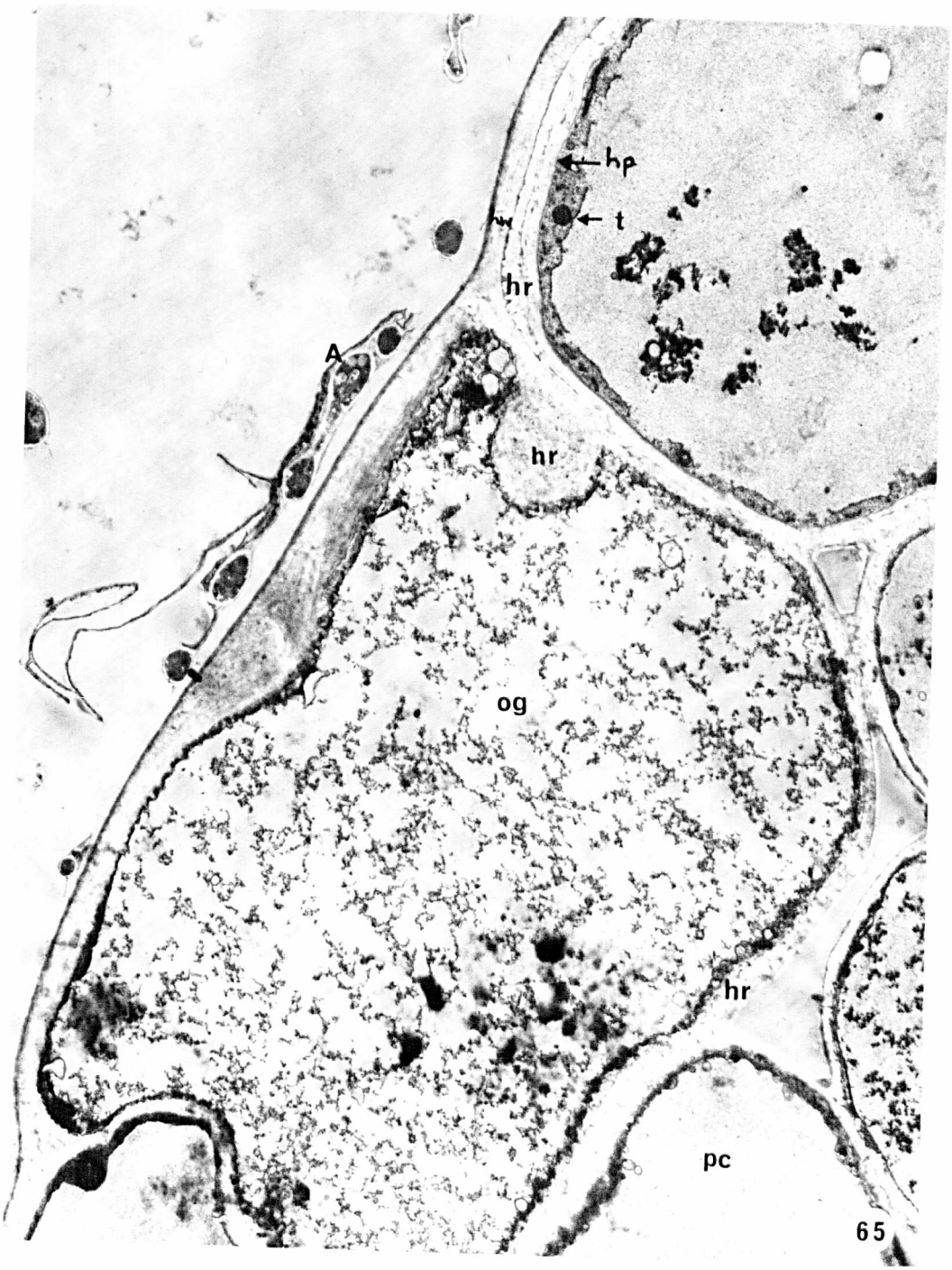

hp
t
hr
A
hr
og
hr
pc
65

Plate 66 An infected epidermal cell of cv. Wye Target showing the host response to an attempted penetration by the fungus. Note the accumulation of reaction material (hr) between the host cell wall (hw) and the host plasmalemma (hp) of the lower epidermal cell in the figure. Note also the accumulation of a similar deposit in the palisade cells (pc). The adjoining epidermal cell shows a hypersensitive reaction with damage to the tonoplast (t) and the protoplasmic organelles. (x6,325).

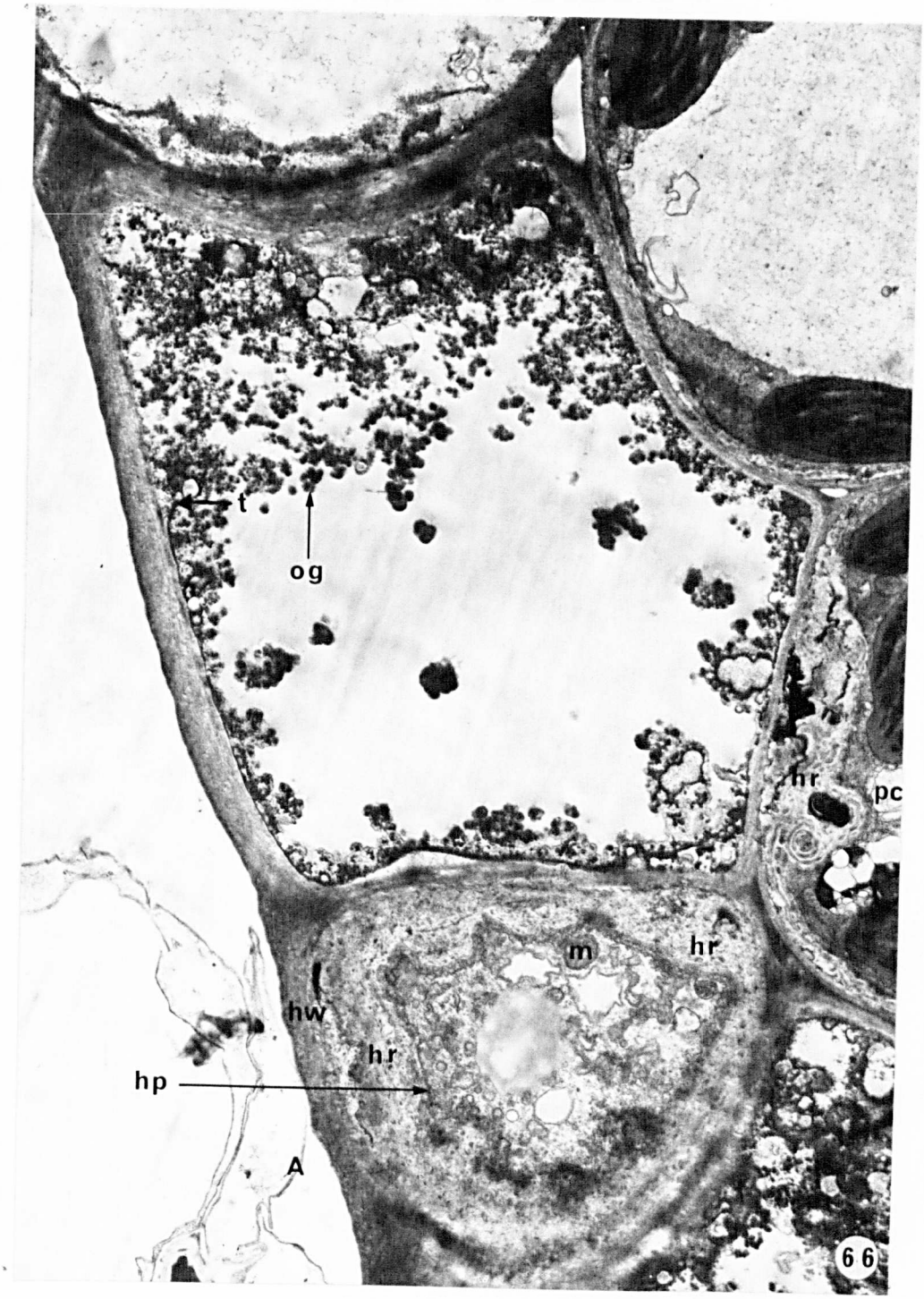
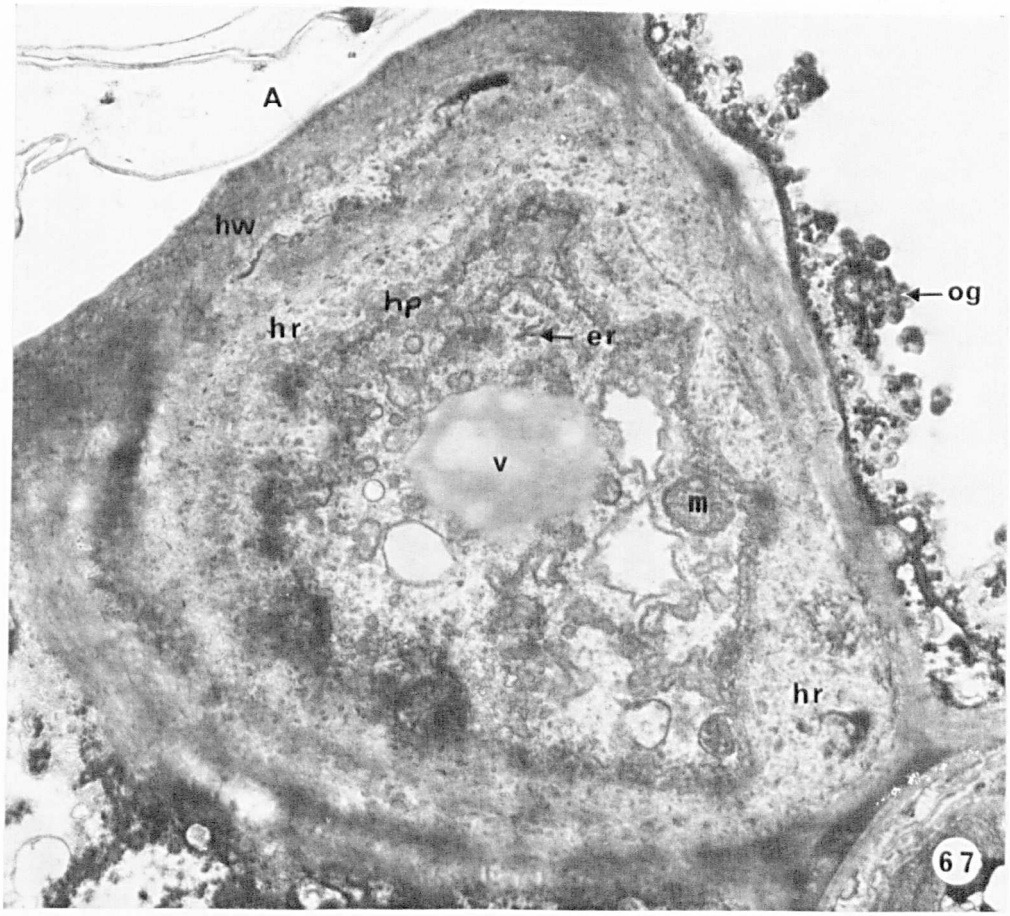

t
og
hr
pc
m
hr
hw
hr
hp
A
66

Plate 67 Enlargement of part of Plate 66 showing a cell of cv. Wye Target. Papilla-like material has been deposited between the host cell wall (hw) and host plasmalemma (hp). Note the similarity of the host reaction material (hr) to the papilla material and its difference from the cell wall. Note also the accumulation of osmiophillic granules (og) in the adjoining cell. (x 10,080).

Plate 68 A section through an epidermal cell of cv. Wye Target showing the accumulation of host reaction (hr) products in the palisade cells (pc) immediately adjoining an affected epidermal cell. (x 20,000).


A
hw
hp
hr
er
og
v
m
hr
67

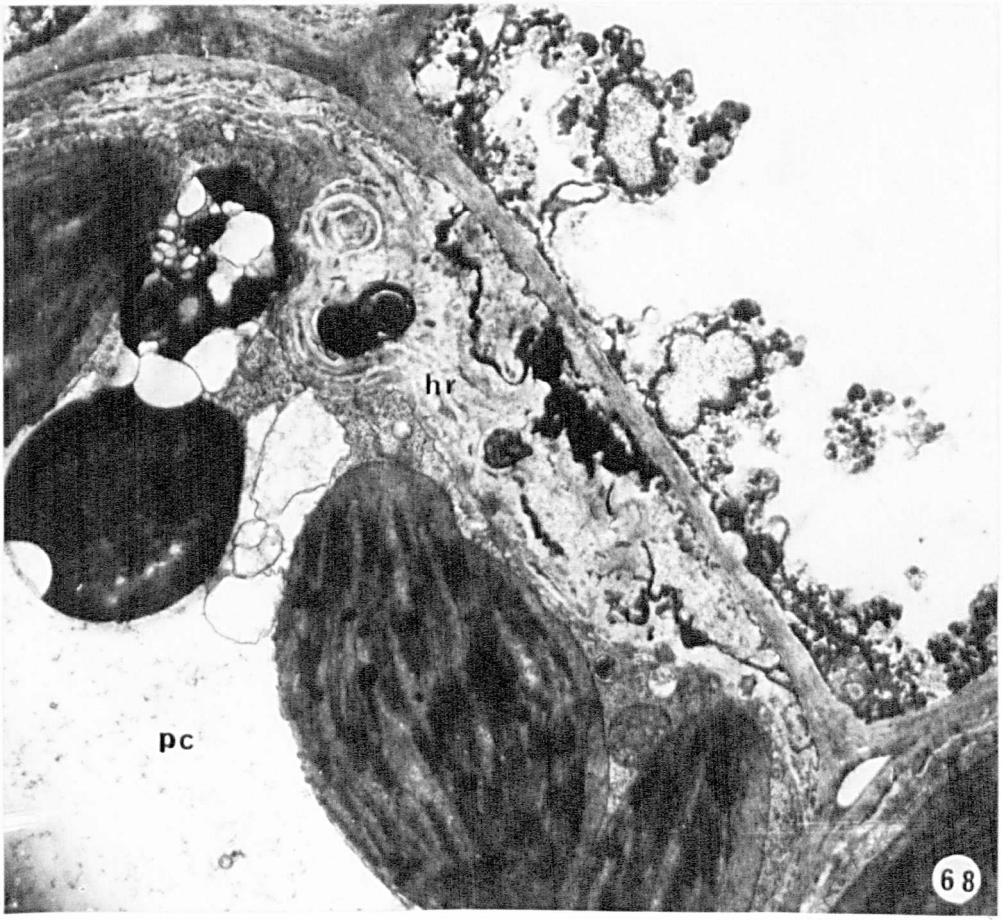
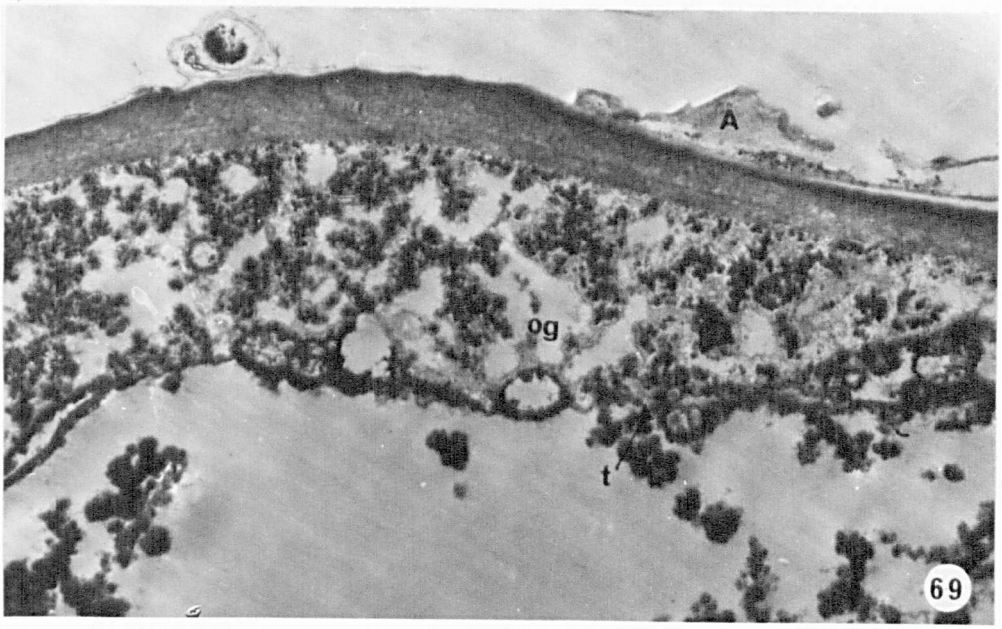
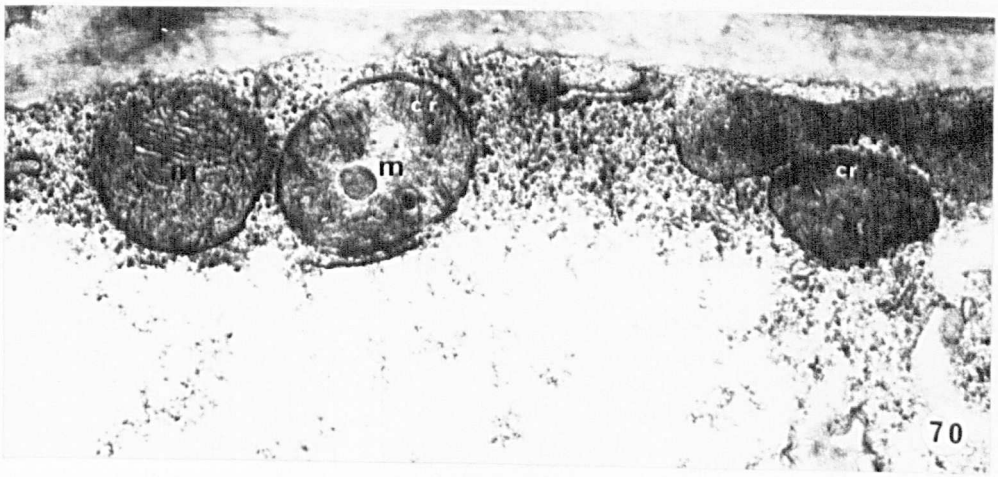

hr
pc
68

Plate 69 Epidermal cell of cv. Wye Target beneath an appressorium (A) showing disruption of the tonoplast (t) in response to infection. Note the highly osmiophillic granules (og). (x 10,360).

Plate 70 Mitochondria (m) in an affected epidermal cell of cv. Wye Target showing disorganisation in the membranes and cristae (cr). (x 31,500).

Plate 71 A nucleus (n) in an affected epidermal cell of cv. Wye Target showing alterations to the nuclear envelope (ne) and the nucleolus (nu). (x 13,680).


A
og
t
69


m
m
cr
cr
70

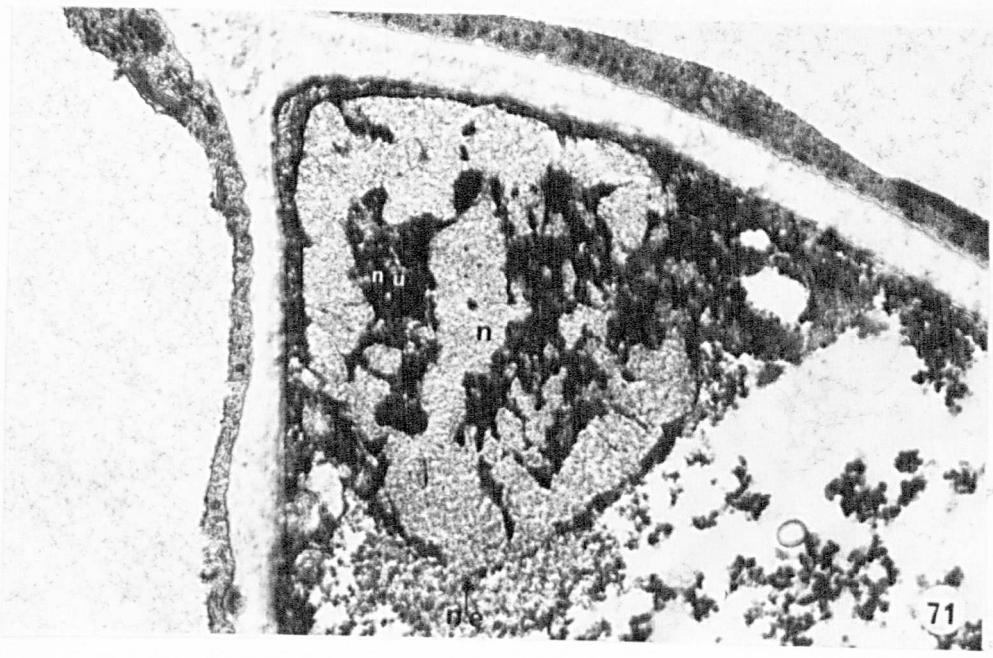
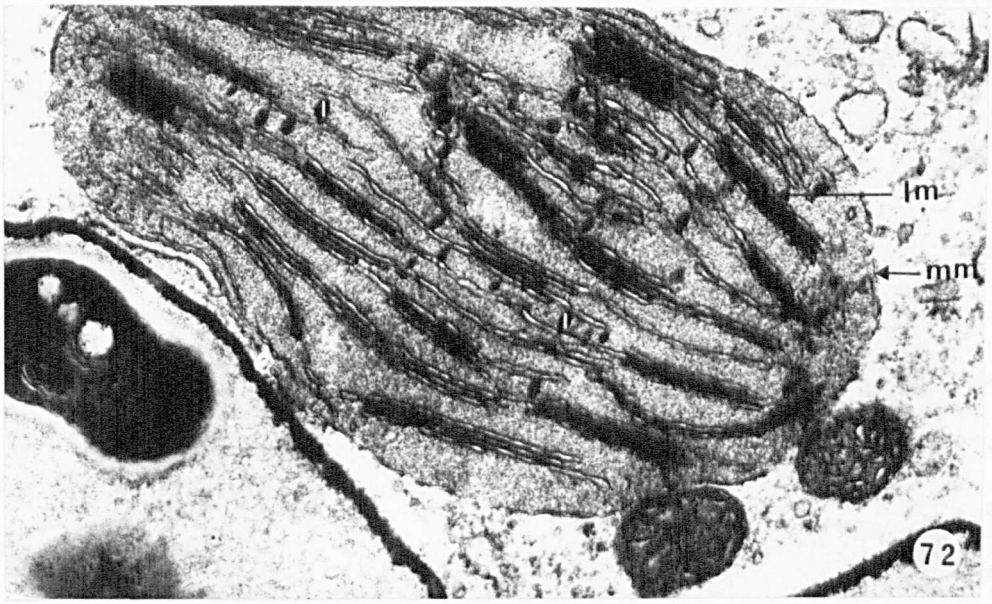
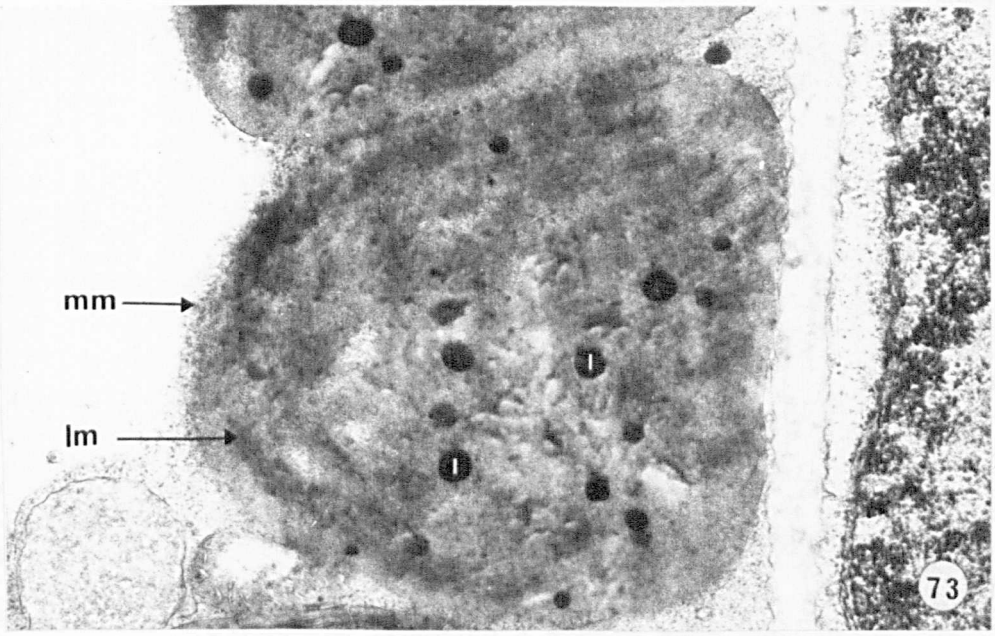

n u
n
n e
71

Plate 72 A chloroplast in an infected susceptible cv. Northern Brewer showing intact membrane (mm) wall conspicuous lamallae (lm). Note the presence of lipid bodies (l). (x 28,100).

Plate 73 Chloroplasts of resistant cv. Wye Target showing disruption of the chloroplast membrane (mm) little evidence of lamellae (lm) structure and an abnormal number of lipid bodies (l). (x 23,310).


lm
mm
72


mm
lm
73

## DISCUSSION

Although, in the past, several other powdery mildews have received considerable attention, there havebeen virtually no studies of host-parasite relationships in hop powdery mildew, whether concerning the effects of environmental factors or of resistant host plants. For convenience, in the present investigation, several phases in the development of the pathogen were recognised and defined. Each phase could differ in its response to environmental factors. However, since nothing is known of these responses, many of the present experiments were conducted under one set of standardised conditions, which, though probably not optimum, were known to be suitable for infection and development of symptoms. Since major genes were controlling resistance of the genotypes examined in this study, environmental changes were not expected to affect substantially the basic host/pathogen interrelationships.

Conidia of S. humuli contain several vacuoles which occupy more than 90% of the conidial volume. Conidial vacuoles of other powdery mildews are known to accumulate various metabolic products in the form of true solutions, crystalloids or colloids (Buvat, 1963). Also several workers, including Yarwood (1950), Jhooty & McKeen (1965) and McKeen, Mitchell & Smith (1967), have shown that powdery mildew conidia can withstand dry atmospheric conditions by virtue of their large water content. Most of the conidia which germinated in these experiments produced a single germ tube which, on a susceptible host, grew over the leaf surface before penetration occurred. Energy for this growth was probably produced by the mobilisation of much of the reserve food contained in the vacuoles. Particles, probably of glycogen, were found in the conidia of S. humuli. This glycogen may have been produced by the conversion of sugars and lipid reserves in the conidia,

possibly to be used in the infection process. The rate of germ tube growth declined progressively during the first 15h after inoculation, probably due to gradual exhaustion of the endogenous energy reserves within conidia. When haustoria were established in susceptible hosts, 12-15h after inoculation, hyphal growth rate increased rapidly but when haustoria failed to establish in certain resistant hosts, almost stagnant growth resulted. Several lipid-like bodies were found also in the mycelium of S. humuli. McKeen (1970) postulated that large quantities of lipid were oxidized during respiration, eventually resulting in the production of water which was utilized during the infection process. Thus, lipids also could serve as reservoirs of energy.

Conidial germination increased on all genotypes during the first 15h after inoculation, thereafter only a slight increase was noticed. Conidia germinated equally well on both susceptible and resistant genotypes, eventually over 80% of them germinating. Germination does not therefore, appear to be a component in inter-varietal resistance in hop powdery mildew, as it is in certain other powdery mildews. Woodward (1927), for instance, reported differential amounts of conidial germination in Podosphaera leucotricha on susceptible and resistant apple varieties. On the other hand Peries (1961b) showed that conidia of S. macularis, closely related to S. humuli, germinated equally well on the leaves of both susceptible and resistant varieties of strawberries as well as on the surfaces of non-host leaves. There are several reports attempting to explain the differences observed in conidial germination in resistant and susceptible leaves to be due to stimulatory or inhibitory substances. Thus, Roberts, Martin & Peries (1961) found the surface waxes of rose leaves to stimulate conidial germination in S. pannosa while absorbed waxes and cutin acids were inhibitory. They also showed that the fungitoxic

activity of cutin acids could be reduced by dilution with surface waxes. Conidial germination and hyphal growth in S. humuli was shown here to decrease with increase in leaf age. Apart from physical factors like cuticle thickness, components of leaf surface fractions may contribute to this increased resistance. An investigation of these factors could therefore, be useful in understanding changing response with age of leaf tissue.

Following conidial germination and germ tube extension on susceptible hosts, appressoria, often indistinct, were formed anywhere on the leaf surface. This observation contrasts with that of Preece, Barnes & Bayley (1967), with Erysiphe polygoni and that of Stanbridge, Gay & Wood (1971) with E. graminis f. sp. hordei where appressoria form only over the junction areas of the epidermal cells. Resistant hop genotypes encouraged the production of fewer appressoria than susceptible ones. Also the number of appressoria formed by, for example, the Glasshouse isolate, was greater than the number produced by other isolates, e.g. East Farleigh. Thus the number of penetration points may be a factor responsible for the different measurements of hyphal length.

Ultrastructural studies on the cv. Northern Brewer showed that, prior to penetration of an epidermal cell, there is a marked host reaction particularly in the region immediately beneath the appressorium. This consisted of a disk-shaped ingrowth (papilla) in the area between the host cell wall and the host plasmalemma. A similar morphological change beneath the appressorium has been noted during the infection process in other powdery mildews and is generally attributed to the secretion of enzymes, e.g. cutinase, pectinase and cellulase, by the pathogen (Akai, Kunoh & Fukutomi, 1968; Kunoh & Akai, 1969; Leong, McKeen & Smith, 1970; McKeen, Smith & Bhattacharya, 1969). Edwards & Allen (1970) suggested that the ingrowth arises at the surface of the host plasmalemma by biosynthetic activity of the host cell cytoplasm before penetration of the host cell wall has been completed. Furthermore,

Ellingboe (1968) and McKeen & Bhattacharya (1969) suggested that papilla formation commences as soon as the enzymes which appear to change the nature of the cell wall at the point of penetration reach the host plasmalemma.

In a susceptible host normal haustoria of S. humuli showed a structure resembling closely the haustoria of obligate pathogens in general. Thus there is no penetration of the host plasmalemma and therefore no direct contact with the host cytoplasm. The haustorial neck is usually surrounded by a collar, the origin of which is not completely understood. It was assumed that the papilla material formed prior to penetration, constituted the beginning of the collar in E. graminis (Stanbridge, Gay & Wood, 1971), though this was based purely on similarities in their texture. In the present study, both the collar material and that of the papilla were of a similar texture also. The two rings in the haustorial neck region of S. humuli that occupy parts of the host and fungal plasmalemmas, constitute a notable feature since a similar ring structure has been previously recorded only by Perera (1972) with S. pannosa in rose leaves. Ring structures are commonly found in association with haustoria of several rust species (Zimmer, 1970; Hardwick, Greenwood & Wood, 1971; Coffey, Palevitz & Allen, 1972). However, those in S. humuli differ by possessing distinctive staining properties and being clearly defined. The rings in the rusts have a diffuse structure (Hardwick et al, 1971). It is possible that these two rings may function together as a barrier in preventing the escape of fluid from the sheath matrix to the exterior of the cell. Moreover, the rings may mark the division between the phase of penetrating the cell and the phase of absorption of nutrient supplies.

The finger-like protrusions arising from the haustorial body are curved and lie close to the body unlike in E. graminis, where they project from it. Similar protrus ions have been seen in some haustoria of S. fuliginea on cucumber though they were not always present (Dekuijzen

& van der Scheer, 1969). Their function may possibly be to increase the surface area of the haustorium for efficient nutrient absorption.

The sheath matrix represents the interface between the host and the pathogen. Its ability to change shape with the growth of the haustorium suggests that it contains either a gel or a fluid. As such it may function as a reservoir of material being transported between host and pathogen. Little is known about the transfer of metabolites from the host to the haustoria. The exchange of metabolites is probably not unidirectional but may also include a transfer of material from the parasite to the host.

In compatible cultivars some conidia produced between 1-5 germ tubes, the range of germ tube number depending upon the particular pathogen/host combination. Whereas up to 5 germ tubes per conidium were encountered in susceptible combinations, only one was observed in the most resistant situation. The greater the number of conidial germ tubes produced the greater was hyphal development on the host surface. Thus, the number of germ tubes could be used as a more rapid and convenient parameter for larger scale comparison of isolate/host compatibility during the early stages of colonization. This situation is quite unlike that in Erysiphe graminis where production of more than one germ tube indicates the failure of conidia to establish infection (Wolfe, personal communication). Mount & Slesinski (1971) observed that conidia of E. graminis f. sp. tritici formed only one germ tube when the environmental conditions were optimum but more when conditions were suboptimal.

Development of the pathogen on the leaf surface was affected by the particular isolate/genotype combination only after the first penetration or attempted penetration had occurred, some 12h after inoculation. Thereafter a measure of the total length of hyphae produced by germinated conidia provided a good indicator of the level of resistance in operation. For comparative purposes, measurements were usually made 48h after inoculation when conidia, on a susceptible host, had produced several germ tubes and had begun to initiate colonies.

Three different resistance mechanisms among the genotypes examined could be recognised as expressions of each of the known major genes for resistance. Each mechanism is based on a form of hypersensitivity by the host cells and is as follows:-

(1) When the $I_1$ gene was expressed as, for example, when 14/66/50 or Wye Challenger were inoculated with the East Farleigh isolate, abnormal haustoria were exclusively produced 15h after inoculation. They were associated with disorganised epidermal cells containing granular cytoplasm. This effect was intensified at 21h. An immediate hypersensitive reaction was therefore produced in response to host cell penetration.

(2) When the B gene was expressed as, for example, when Wye Challenger was inoculated with the Glasshouse isolate, normal haustoria were initially formed, by 15h, but by 21h after inoculation, a large proportion had degenerated and were associated with disorganised epidermal cells. In later stages there was always a proportion of normal haustoria among abnormal haustoria. This was a type of delayed or incomplete hypersensitivity again in response to host cell penetration.

(3) When the $I_2$ gene was expressed as, for example, when Wye Target was inoculated with either the Glasshouse or East Farleigh isolates, no penetration of the host cell wall appeared to occur, but during attempted penetration, by 15h, the contents of epidermal cells were disorganised and granular and became more so by 21h. This again, was an immediate hypersensitive reaction but in response only to attempted penetration. The ultrastructure of these disrupted epidermal cells was examined only 7 days after inoculation when a deposition of papilla-like material was found around the cell, between the cell wall and plasmalemma. It is not known whether a similar host cell reaction occurs during the resistance expression of the $I_1$ and B genes.

In the resistant reactions in general the fungus initiated degenerative changes, since both host cellular structure and fungal haustoria became disorganised. The hypersensitive reactions were confined not only to the affected epidermal cells but sometimes also to adjacent epidermal and mesophyll cells. On occasions, haustoria which appeared abnormal 15-21h after inoculation eventually diffused into the matrix of the infective cell and disappeared.

The apparent lack of further hyphal growth after penetration in some resistant reactions indicates that host cells, before or after responding adversely to the presence of the pathogen may lack some factor essential for haustorial establishment. Alternatively, host cells may contain some factor toxic to the fungus. Unsuccessful establishment of the fungus could result in the production of fungal substances that kill the cells of resistant hosts. Another possibility is that a fungal factor which elicits no effect in a susceptible host cell is toxic to the resistant host cell or that the fungus produces different substances in the two types of cells. The basis of the resistance mechanisms is probably very complicated. These complexities cannot be resolved easily with the methods developed for studying post-infectional compounds, e.g. phytoalexins, in facultative pathogens, as the powdery mildew fungus is restricted only to the epidermal cells.

The chloroplasts in the palisade cells adjacent to the affected epidermal cells in a resistant reaction usually showed an adverse reaction to the pathogen also. The observed rupture of the chloroplasts' boundary membrane may be due to the apparent increase in chloroplast size. The entire membrane system of the infected host cell ultimately may break down resulting in death, as in premature senescence.

The high statistical significance of the interactions between cultivars/seedlings and hyphal growth of isolates means that the isolates differ markedly in their pathogenicity (Van der Plank, 1968). Among the eight isolates of

S. humuli studied, four physiologic races could be recognised on the basis of their differential behaviour to selected genotypes. Salmon (1919) sought an explanation for the sudden breakdown of immunity of some of the hop seedlings in group I in his classification. Although he attributed this breakdown to the evolution of new strains of the fungus, he failed to furnish experimental evidence for this. More recently Neve (1968) showed that the complete resistance shown by seedling 14/66/50 broke down during 1968. In the present investigation, the isolate prevalent in the Hop Department glasshouses in 1970, and designated the Glasshouse isolate, caused a susceptible reaction in 14/66/50 which has the $I_1$ gene. An isolate obtained from wild hedgerow hops in Rutland, a locality far from any hop growing area, was found to attack 14/66/50. This behaviour was quite unlike that exhibited by the population that prevailed in Wye prior to 1968. This may suggest that the $I_1$ gene could be more widespread than originally supposed and may be present in many wild hedgerow hops. An investigation of the reactions of wild hops to the various recognised strains of S. humuli would be interesting. Two of the eight isolates collected from around the country, East Farleigh and Marden, induced an immune response in 14/66/50 and therefore appeared to be of the same strain to that prevalent in the Department glasshouses before 1968.

The fact that four races have been identified from a fairly random collection of only eight isolates points to the extreme pathogenic variability within the population of S. humuli present in this country. This is in spite of virtually no selection pressure with respect to genotype except in the environment of the Hop Research Department where all the breeding for resistance in the country is carried out. Elsewhere, up to 1970, all cultivars grown commercially were susceptible. It seems quite likely that, if further genes for resistance are discovered, a distinct possibility, new isolate collections will reveal still more strains of the pathogen. The dangerof collapse of the resistance in new cultivars in commerce, e.g. Wye Challenger and Wye Target.

which is based on single genes, is therefore a very real one. Wye Target, which showed an immediate hypersensitive reaction to isolates of the fungus, showed evidence of breakdown in the glasshouse in 1973. Although the attack was not very virulent the breakdown might suggest the appearance of another pathogenic race of S. humuli.

Hyphal growth and sporulation characteristics of the Glasshouse and Tenbury Wells isolates showed that they were consistently the most virulent, not only on susceptible but on resistant hosts as well. It was interesting to note that these isolates generally formed larger haustoria, and there were a greater number of haustoria per unit area of infected leaf. On the same basis the East Farleigh isolate was consistently the least virulent and produced fewer and smaller haustoria.

These studies permitted a better understanding of the genetic constitution of Wye Challenger and 11/66/28. It was shown that, in addition to the B gene, Wye Challenger also possesses the $I_1$ gene, present also in 14/66/50; Wye Challenger now exhibits the blister reaction to infection of the prevailing population of the pathogen at Wye College. Presumably, therefore, it was immune before 1968. Also the East Malling, Mordiford and Rutland isolates grew more vigorously and sporulated more profusely on Wye Challenger than on 11/66/28 although both were known to contain the B and $I_1$ genes. This behaviour could be due to the modifying effects of additional minor genes or to the existence of a hitherto unknown major gene in 11/66/28. This aspect requires further investigation.

The formation of blisters on hop leaves is apparently unique among powdery mildews. The blisters formed initially on susceptible cultivars were almost completely covered with sporulating colonies, resulting in loss of identity of the blister. However, blisters formed by Wye Challenger were prominent since little mycelium grew on them. A histological study revealed

that the epidermal cells on the blister were larger than normal. This cell enlargement could be due to a hormonal stimulation by the parasitising fungus. Blisters were usually formed in great abundance in infected plants on the open bench of a glasshouse, few were observed in the growthroom, in isolation boxes in the glasshouse, and in the field. This behaviour could possibly be due to changes in the physiology of the plant brought about by different environmental conditions.

It was shown that certain environmental conditions, particularly higher temperatures, were lethal to disease establishment when plants were exposed immediately after inoculation. However, once the disease was established relatively high temperatures enhanced expression of disease symptoms. Also, changes in environmental conditions during the incubation period influenced the production of symptoms. A detailed investigation of the influence of environmental conditions is of great importance to a better understanding of the build-up of disease epidemics.

Under field conditions fungicide applications and fluctuating weather conditions could alter the phylloplane microflora. Under high humidity conditions in the growthroom conidia of S. humuli were colonised by a fungus, possibly a Fusarium. This aspect needs careful investigation because of its wider implications as a possible method of biological control.

## SUMMARY

1. From studies of the development of eight isolates of S. humuli, collected from various localities, on leaves of various cultivars/seedling hops, containing either no resistance genes or one or more of three known major genes for resistance, four principal interactions were recognised:

a) On host plants with no resistance genes, infected epidermal cells showed no adverse reaction to the presence of haustoria which were normally formed and apparently functional. As a result there was extensive hyphal development which supported profuse sporulation on the leaf surface. Young leaves responded to infection by forming raised blisters which became smothered in sporing mycelium. Older leaves failed to produce blisters.

b) When the $I_1$ gene was expressed, epidermal cells reacted hypersensitively immediately after their penetration, within 15h after inoculation. The haustoria were degenerated, permitting no hyphal development on the leaf surface.

c) When the B gene was expressed, hypersensitivity was relatively delayed until after the formation of haustoria, a proportion of which, by 21h after inoculation, had degenerated. Remaining, functional haustoria supported a limited amount of hyphal growth and sporulation. In this reaction, infected leaves of various age produced blisters which were prominent and remained so because of little superficial fungal growth. Such blisters were commonly produced only in glasshouse conditions.

d) When the $I_2$ gene was expressed, an immediate hypersensitivity occurred in response only to attempted penetration by the pathogen, within 15h from inoculation. No haustoria, however, were seen and there was no hyphal development.

2. The extent of hyphal growth, 48h after inoculation, was used as a convenient parameter for assessing the relative compatibility of the various genotype/isolate combinations. Hyphalgrowth was correlated with the number of germ tubes produced by conidia which, on susceptible hosts, was up to 5, and on the most resistant hosts, only 1-2.

3. Consistently about 70-80% conidia germinated on leaf surfaces irrespective of the level of resistance. Germination declined, however, on leaves of increasing age.

4. Haustoria were fewer and smaller in hosts expressing the $I_1$ or B gene than in susceptible ones.

5. The eight isolates could be grouped into four strains on the basis of their behaviour on the various genotypes. Three isolates represented a strain which was prevalent at Wye College before 1968 and by which the $I_1$ genotype could be recognised. Selection caused a new strain to arise to which hosts of the $I_1$ genotype were susceptible; this strain has been prevalent at Wye ever since and was included in these studies.

6. Irrespective of their behaviour on genotypes, the isolates broadly showed a range of virulence. Consistently, an isolate from East Farleigh, Kent showed less hyphal growth. and sporulation and possessed smaller haustoria, fewer in number than the other isolates.

7. Wye Challenger was known previously to contain the B gene. Experiments revealed that it also possesses the $I_1$ gene and as such can express either resistance reaction depending on the strain of S. humuli used.

8. Studies indicated that, in addition to the $I_1$ and B genes, the seedling 11/66/28 also may contain either an additional, unknown major gene for resistance or several minor genes which modify the expression of the major genes.

9. While failing to suggest an explanation for variation in expression of the blister symptom in B-gene hosts, an experiment in which inoculated plants were exposed to two temperature regimes showed that, at 24°C, infection fails to occur, while established infections will sporulate quicker than at lower temperatures.

10. Resistance to colonisation by S. humuli increased with age of leaves but this relationship was influenced markedly by the cultivar used.

11. Ultrastructural studies of host/parasite relationships were conducted in some detail with a susceptible reaction (cv. Northern Brewer) and on a preliminary scale with the $I_2$ genotype (cv. Wye Target).

a) Penetration of the epidermal cell of a susceptible host by the pathogen was preceded by the formation of a disk-shaped ingrowth (papilla) between the host cell wall and the host plasmalemma. The collar which surrounds the neck of the haustorium was comprised of material identical in texture to the papilla material.

b) In the haustorial neck region two ring structures were detected on the fungal and host plasmalemmas.

c) The arrangement of haustorial lobes on the opposite ends of the haustorial body suggested bipolarity.

d) In Wye Target, the epidermal cell immediately beneath an appressorium showed a deposition of papilla-like material between the host cell wall and plasmalemma, around the cell. A similar material was sometimes found in the adjacent palisade mesophyll cells.

12. Conidia were sometimes parasitised by hyphae probably of a species of Fusarium.

## SECTION - II

## REVIEW OF LITERATURE

In temperate regions powdery mildew fungi are relatively inactive during the winter when they persist by a number of methods. They are known to survive the winter as mycelium on leaves and shoots or within dormant buds of their hosts, as cleistocarps and on alternate hosts.

The hop powdery mildew fungus, Sphaerotheca humuli, could survive the winter in England in three possible ways, as cleistocarps, as mycelium in dormant buds or on alternate hosts. The hop plant is a herbaceous perennial and the entire bine is pruned at the base near the soil level after the crop is harvested. It is therefore, not possible for powdery mildew to survive the winter as mycelium on host leaves. Little research has so far been conducted on overwintering in hop powdery mildew. This is now reviewed in the context of similar work on overwintering of other powdery mildew fungi.

### As mycelium on leaves or shoots

Survival of Erysiphe graminis f. spp. tritici and hordei in Russia as dense, brown mycelial mats on the lower leaves of wheat and barley respectively has been reported by Gorlenko (1940, 1942). The presence of active mycelium of Sphaerotheca pannosa (Wallr.) Lēv. var. rosae Wor. on young leaves of rose plants led Yarwood (1939) and Howden (1968) to assume that infection in the spring was initiated by the rejuvenation of the dormant mycelium in buds. Gilles (1960) suggested that Sphaerotheca macularis (Wallr. ex Fr.) Jaczewski, on strawberry overwinters in the mycelial stage on old leaves, but he could not identify the fungus positively in this form due to failure of the mycelium to sporulate. Confirmatory evidence that the mycelium of strawberry mildew survived on the green, overwintering leaves to produce conidia capable of infection of young leaves in the spring was provided by Peries (1961).

As mycelium in buds

Studies carried out on several powdery mildew species over the years by many workers have suggested that in many cases mycelium can survive the winter in dormant buds to initiate infection in the following spring, but the evidence advanced by them to support this view varies greatly. Suspected American gooseberry mildew mycelium was detected within dormant blackcurrant buds and was thought by Porreye (1963) to cause initial infection in the spring. He could not, however, identify the fungus in buds positively as S. mors-uvae and he also failed to observe primary infections from such buds. Therefore, the detection of a mycelium in dormant buds should not be regarded as proof that the fungus overwinters there. Failure to detect ascospores in the air in the spring was sufficient evidence for Preece (1965) to conclude that survival of S. mors-uvae was by means of persistent mycelium in gooseberry buds. On evidence based mainly on field observations at bud burst in the spring when young expanding leaves appeared already covered with mildew, Ballard & Volck (1914), Fisher (1918), Yarwood (1939) and Howden (1968) all suggested that survival of Podosphaera leucotricha (Ell. & Ev.) Salm.) and S. pannosa var. rosae was by means of mycelium in the buds. Höstermann (1922) collected infected apple branches in winter, sprayed them with various fungicides in the laboratory and found that, on opening, buds were infected with mildew, thus providing strong circumstantial evidence for survival in buds. Recent evidence that S. mors-uvae perennates in buds of gooseberry was obtained by dissecting buds and by inducing buds on surface sterilized shoots to burst under conditions which precluded chance infection (Merriman & Wheeler, 1968). Using similar methods, Weinhold (1961), Korokani (1969) and Price (1970), showed that rose mildew, S. pannosa var. rosae and peach mildew, S. pannosa var. persicae also perennated in infected buds. Detailed anatomical studies carried out by Woodward (1927) showed that mycelium of P. leucotricha lying within the

buds had produced haustoria in the leaf tissue enclosed by bud scales, showing conclusively that the fungus was sufficiently established to obtain nutrients from host tissue for its survival during the winter. Production of primary mildewed shoots by infected buds in the field, labelled in the previous autumn, showed that, once within the bud, the hyphae of P. leucotricha could remain dormant until the following spring when the fungus spread, rapidly smothering the emerging leaves.

As cleistocarps

Cleistocarps, sometimes called perithecia in powdery mildew fungi, are the fruiting bodies whose function is thought to be to carry the fungus in a dormant condition through the winter (Salmon, 1900). In Sphaerotheca each cleistocarp has a single ascus containing eight ascospores but in Erysiphe there are several asci containing 2-8 ascospores. In some species these ascospores are known to be responsible for causing primary infection in the spring.

The literature on the formation and the role of cleistocarps in overwintering is sometimes contradictory. Many workers, e.g. Salmon (1900) and Blumer (1933, 1967), are of the general opinion that in Europe cleistocarps are rarely formed, whereas they are considered to occur frequently in America by Longree, (1939), Yarwood, (1957) and Schnathorst, (1965). Salmon (1900) presumed that cleistocarps were capable of overwintering whenever they were formed. However, Yarwood (1944, 1957), in reviewing literature on this subject, inferred that the role of cleistocarps in overwintering may have been over-emphasized since, in many cases, they are either not formed at all or are non-functional when formed. He was, however, of the opinion that, in some species, cleistocarps have a definite function to perform. Both Yarwood (1957) and Schnathorst (1965) commented that the formation of cleistocarps is no assurance that they function in nature.

Cleistocarps were first recognised as overwintering bodies by Smith (1884) in E. graminis. His work was largely overlooked until Salmon (1903) showed that cleistocarps of this fungus could dehisce accompanied by the ejection of the ascospores. Since this observation, the role of cleistocarps of several powdery mildew fungi has been investigated.

There are several reports to show that cleistocarps play no role in overwintering. Those formed on infected apple shoots in the field were examined in the laboratory by Cunningham (1923). He observed that the ascospores in the cleistocarps collected in October disintegrated under a coverslip when released by pressure. Also he found no evidence that ascospores were released naturally from wetted cleistocarp samples. He concluded therefore, that cleistocarps have no part to play in the perennation of apple mildew. These findings were confirmed by Woodward (1927) and Burchill (1958). A similar observation was made by Peries (1962) who found only deformed asci with disintegrating ascospores or no ascospores at all in overwintered cleistocarps of S. macularis on strawberry. When rose shoots bearing cleistocarps were overwintered on soil under natural conditions, a decline in the number of ascospores occurred as the winter progressed. The ascospore content of other cleistocarps which were overwintered on the rose bushes also declined. This appeared to be due to atrophy of the ascospores. Thus, Price (1970) concluded that cleistocarps of S. pannosa are unlikely to survive the winter. Similar observations were made for gooseberry mildew by Merriman & Wheeler (1968).

Although Salmon (1914a) recorded a discharge of ascospores from artificially wetted cleistocarps of S. mors-uvae kept dry during the winter, he did not carry out inoculation experiments with them. When he (1914b) examined cleistocarps that had overwintered naturally on gooseberry he could find none with ascospores. These observations illustrate the dangers

of drawing conclusions from laboratory experiments unless infection can be caused by ascospores of overwintered cleistocarps.

Hop cones bearing cleistocarps of S. humuli were collected in January by Salmon (1907) from bines lying in a hop garden. These cleistocarps were kept dry until April, when he soaked them in distilled water and placed them on a filter paper in several Petri dishes. He kept one set in the laboratory and another on the ground in the hop garden. He observed several ascospores on the lids of the Petri dishes about eight hours after wetting. Many of these were germinating, and some, when transferred to hop leaves, were able to cause infection resulting in the formation of sporulating colonies within seven to eight days. Similar results were obtained when cleistocarps, kept dry during the winter, were placed on upper surfaces of hop leaves after soaking them in water. However, he did not carry out similar experiments with naturally overwintered cleistocarps and so these findings could not be considered as conclusive evidence that cleistocarps can survive the winter to cause infection in the spring. Blodgett (1915) collected cones and leaves bearing cleistocarps of S. humuli in April from bines that were lying in the hop garden during the winter. When these were soaked in water and placed in a saturated atmosphere in the laboratory, they dehisced, readily liberating ascospores.

Two groups of cleistocarps, one from the glasshouse and the other from the field, were exposed to natural conditions during the winter 1913-14. Cleistocarps of the first group, brought into the laboratory in January, released ascospores after wetting, but the second group showed dehiscence for the first time in February and the succeeding samples showed a steady increase in the number released; they also dehisced very readily. Blodgett also brought infected cones into the laboratory after a period of exposure to outdoor conditions and left them for several weeks under dry conditions. No improvement in the dehiscence was found. In addition,

Blodgett also carried out experiments using cleistocarps overwintered under natural conditions to inoculate whole plants in the glasshouse. These plants were inoculated with ascospores which were caught on glass slides kept over cleistocarps, and also by placing cleistocarps on moist filter paper inside bell jars directly above plants. Infected colonies were seen within eight days of setting up the experiment. However, he did not attempt to show microscopically that the colonies arose from ascospores. The possibility of a foreign infection cannot be excluded, however, as these plants in the glasshouse could have been infected before covering with the bell jars.

Systematic studies to investigate the role of cleistocarps in powdery mildews were first made by Wheeler's school at the Imperial College Field Station, Silwood Park, and by Jordan and his co-workers at Long Ashton Research Station, Bristol. They largely used refinements of methods adopted by the early invesigators, e.g. Salmon (1907), but developed methods for quantifying cleistocarp behaviour. Existing techniques to trap airborne ascospores in the field were also used to understand the patterns of ascospore release and relate them to meteorological factors (Jordan, 1966, 1967). Leaf-clearing techniques were used to identify the origin of colonies. Overwintering of Erysiphe cichoracearum DC. on leaves of burdock was investigated by counting the number of dehisced cleistocarps at intervals and assessing the ability of the remainder to dehisce when transferred to the laboratory. The viability and infectivity of the ascospores were determined by allowing them to be discharged on to leaf disks of blackcurrant in the laboratory (Cook & Wheeler, 1967). Later Merriman & Wheeler (1968) and Smith & Wheeler (1969) showed that cleistocarps of S. mors-uvae and Erysiphe polygoni DC. respectively, could successfully overwinter to cause infection in the spring. Likewise, natural release of ascospores in the spring was demonstrated for Uncinula.

salicis (DC. ex Mérat) Wint. on willow and aspen and Microsphaera penicillata (Well. ex. Fr.) Lév. on wild sweet pea, after overwintering under extreme conditions of the Canadian winter (Smith, 1971).

Alternate hosts

Powdery mildew fungi generally exhibit a very high degree of specilisation of parasitism, each species of which infects only a single host species. However, there are exceptions to this general rule. A few species are limited in their choice of host plants to certain families, e.g. S. pannosa on Rosaceae, E. graminis on the Gramineae (Salmon, 1900). On the other hand, many species occur on a large number of very different host plants. E. polygoni was recorded on 190 different species of host plants belonging to 89 genera (Salmon, 1900). Stone (1962) found that E. cichoracearum is not specific to cucumber, being capable of infecting also Sonchus aspen and S. arvensis which can serve as sources of inoculum for glasshouse crops.

Successful transfer of S. humuli from hops to Taraxacum officinale, a host of S. humuli var. fuliginea, was reported by Magnus (1899). However, Salmon (1900) never recorded S. humuli on Taraxacum spp. and subsequently Blumer (1933) identified cleistocarps of S. humuli var. fuliginea on herbarium material left by Magnus and concluded that he had recorded a foreign infection.

Salmon (1904b) showed that conidia of S. humuli on Potentilla reptans were unable to infect Alchemilla vulgaris, A. arvensis, Fragaria sp., Spiraea ulmaria, Agrimonia eupatoria and Poterium officinale, all of which are host plants of the S. humuli. The same specialisation of parasitism has been proved to exist in the case of the variety fuliginea of S. humuli. Inoculation of S. humuli ascospores from cleistocarps of hops to P. reptans and S. ulmaria by Salmon (1907) was not successful. Bouwens (1927) found that hop mildew could not be transferred to strawberry. S. macularis

from Geum urbanum failed to infect other species of Geum and Fragaria vesca, Sanguisorba spp., P. reptans, Filipendula ulmaria, Aruncus silvestris and Rubus spp., all reported to be host plants of S. macularis. Peries (1961b) succeeded in transferring S. macularis from strawberry only to Potentilla fragariastrum after inoculating twenty five different species of weed and cultivated plants which included hops.

## MATERIALS AND METHODS

### (a) Collection of material.

Cones and leaves bearing cleistocarps were collected from seedling varieties of hops in the Nursery garden, Wye College in the beginning of October in 1970 and 1972. In October 1971 cleistocarps were not found in abundance in this garden and therefore collections were made from a planting of highly susceptible cv. Northern Brewer hops growing on a farm at East Farleigh, Kent. The cones and leaves collected in the field were scanned in the laboratory using a binocular microscope (x50), in order to select those having large numbers of cleistocarps on the bracts and bracteoles of cones and laminae of the leaves.

The infected hop material was divided into several portions each of which was placed in a terylene-net bag (625 mesh/$cm^2$, measuring approximately 24 x 21cm). In 1970 and 1972, immediately after collecting the material, the bags were laid on a ridge of bare soil in the Nursery garden. In 1971, the bags contained cleistocarps from the farm at East Farleigh which is affected with the hop Verticillium wilt fungus. To avoid introduction of Verticillium to the College hop gardens, the bags were laid on the surface of a garden soil at a private residence in Wye, one quarter mile from the nearest field of hops. All bags were held in place by string attached to eight metal pegs driven into the soil.

### (b) Assessment of cleistocarp maturity.

A bag containing cleistocarps was brought to the laboratory once each month from the time of collection, October, until May the following year. In 1972 assessments were made at fortnightly intervals and, in 1973, at weekly intervals from the beginning of April. No further records were taken after May each year as most cleistocarps had dehisced naturally in

the field by this time. Several cones and leaf disks were removed at random from a bag and areas bearing abundant cleistocarps were then wetted with water, using a camel-hair brush. One thousand cleistocarps, in about fifty samples, were then removed with a dissecting needle under a binocular microscope (x50). This process facilitated easy removal of cleistocarps without damage as they tended to adhere to the needle by surface tension. The cleistocarps so removed were then placed in a drop of distilled water on a microscope slide and a cover slip was placed over them. Those cleistocarps which had already dehisced naturally in the field showed a rupture of the wall and could be recognised by careful microscopic examination. In order to reveal their contents, intact cleistocarps were burst open by applying gentle pressure to the coverslip with a dissecting needle. Samples assessed throughout the winter provided information on the nature of cleistocarp contents, their colour and also the proportion which had dehisced naturally in the field. All the observations were made in the laboratory using a light microscope at x 105 or x 420.

(c) Natural dehiscence of overwintered cleistocarps and ascospore infectivity.

The ability of overwintered cleistocarps to dehisce spontaneously under artificial conditions was examined on the same sampling occasions. Bracts and bracteoles containing cleistocarps were removed at random from cones taken from bags exposed to outdoor conditions, immersed in distilled water for half an hour and then placed in the middle of a moist Whatman No. 1 filter paper in a 9cm plastic Petri dish. A clean microscope slide, 7.5 x 3.8cm, was placed in the middle of the lid of a Petri dish which was also lined with moist filter paper. The bottom half of the Petri dish containing the cleistocarps was inverted over the lid so that the cleistocarps were placed directly above the glass slide in a saturated atmos phere. Six inverted Petri dishes were incubated for five days in a growthroom

operated at 18° ±2°C, 4,500 lx from warm white fluorescent tubes and a 16h photoperiod. From April onwards similar samples were also exposed outdoors, but in the shade. Ascospores discharged onto the slides were counted under the microscope, (x105 and x420), 24h and 48h after setting up the experiment by scanning the entire slide. To obtain an estimate of the total number of cleistocarps in each sample replicate, 5% of the bracts and bracteoles and leaf disks used were removed at random after each experiment in 1973 and the total number of cliestocarps were counted. This gave approximately the total number of cleistocarps in each replicate, so that the total ascospores caught on microscope slides could be related to this figure. In 1970/71 and 1971/72 cleistocarps were not counted.

Other experiments were carried out simultaneously to test the viability and infectivity of ascospores released from overwintered cleistocarps. The same procedure used for the dehiscence experiments described above was followed except that ten 12mm leaf disks or two entire young leaves, obtained from mildew-free Northern Brewer plants grown in isolation in an air-cooled glasshouse, were substituted for the microscope slides. The leaf material was placed adaxial surface uppermost on moist 'Airoporena' so that ascospores could be discharged onto them. It was then incubated in the growthroom at 18⁺-2°C. As controls, similar leaf material was incubated in dishes in the same conditions but with no cleistocarps. After 5 days leaf disks were then removed, decolourized, stained, mounted in Euparal and then examined for the presence of mildew colonies.

Procedures for the investigations on overwintering in dormant buds and on alternate hosts are more specific to individual experiments and are therefore included with the Results.

## RESULTS

1. Overwintering as cleistocarps.

(a) Incidence of primary field infections probably initiated by ascospores.

A planting of cv. Northern Brewer in a non-cultivated area of the Withersdane hop garden at Wye was surveyed on 18 May 1971. A random plot of 365 plants was selected and the young foliage of each plant carefully examined for signs of infection. Discrete powdery mildew colonies were found on the leaves of 22.7% of the plants examined (Fig. 6). There was no obvious source of this infection other than cone debris containing cleistocarps from a heavy outbreak of disease the year before, which littered the garden. A few seedling plants, probably having arisen from seeds shed from the previous year's cones were also infected.

A second survey was carried out in a field of the cv. OT 48 at Trottenden Farm, Goudhurst, Kent on 24 April 1972. Due to a severe attack of powdery mildew the year before only one half of this area was harvested; in the remaining half the disease was allowed to develop further, producing cleistocarps on the cones which later fell to the ground. The bines were eventually cut down, removed from the garden and burnt. When the buds were beginning to produce shoots the following year, 712 plants were individually examined for powdery mildew infection from an area embracing the picked and unpicked hops. Of 25.3% plants infected 22.8% were within the area unpicked the previous year (Fig. 7). Profuse discrete colonies were apparent on both surfaces of expanded leaves close to the soil surface. The infected plants (2.2%) in the picked area were all very close to the adjacent unpicked area. Abundant cone debris on the ground in the unpicked zone made a clear demarcation between the two areas. Laboratory examination of the debris showed the presence of abundant, mature cleistocarps. Some of the

Fig. 6 Part of a non-cultivated area of the Withersdane hop garden, Wye College, in May 1971, showing distribution of plants cv. Northern Brewer with discrete, white powdery mildew colonies thought to have originated from ascospores. 22.7% of the plants were infected.

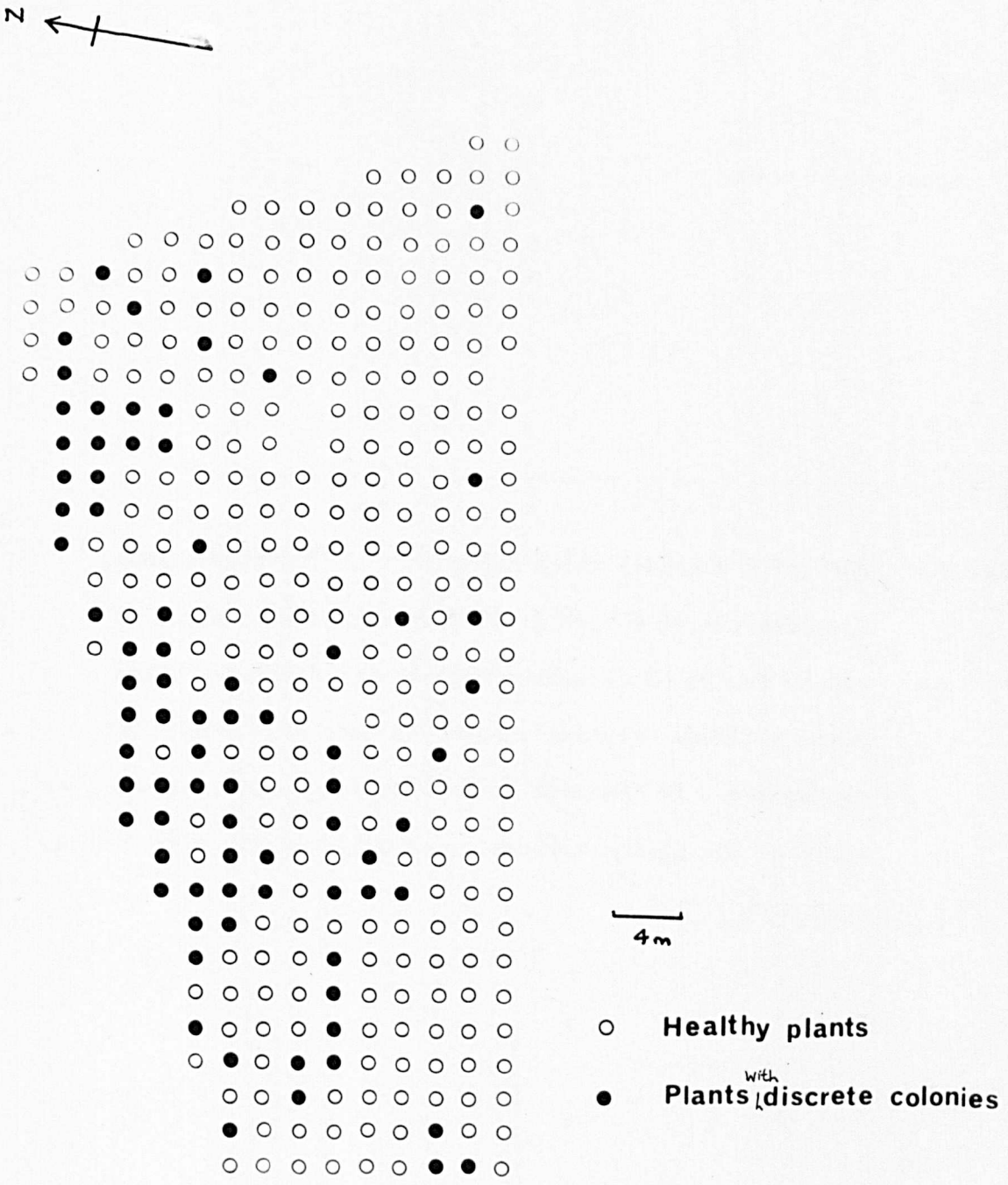
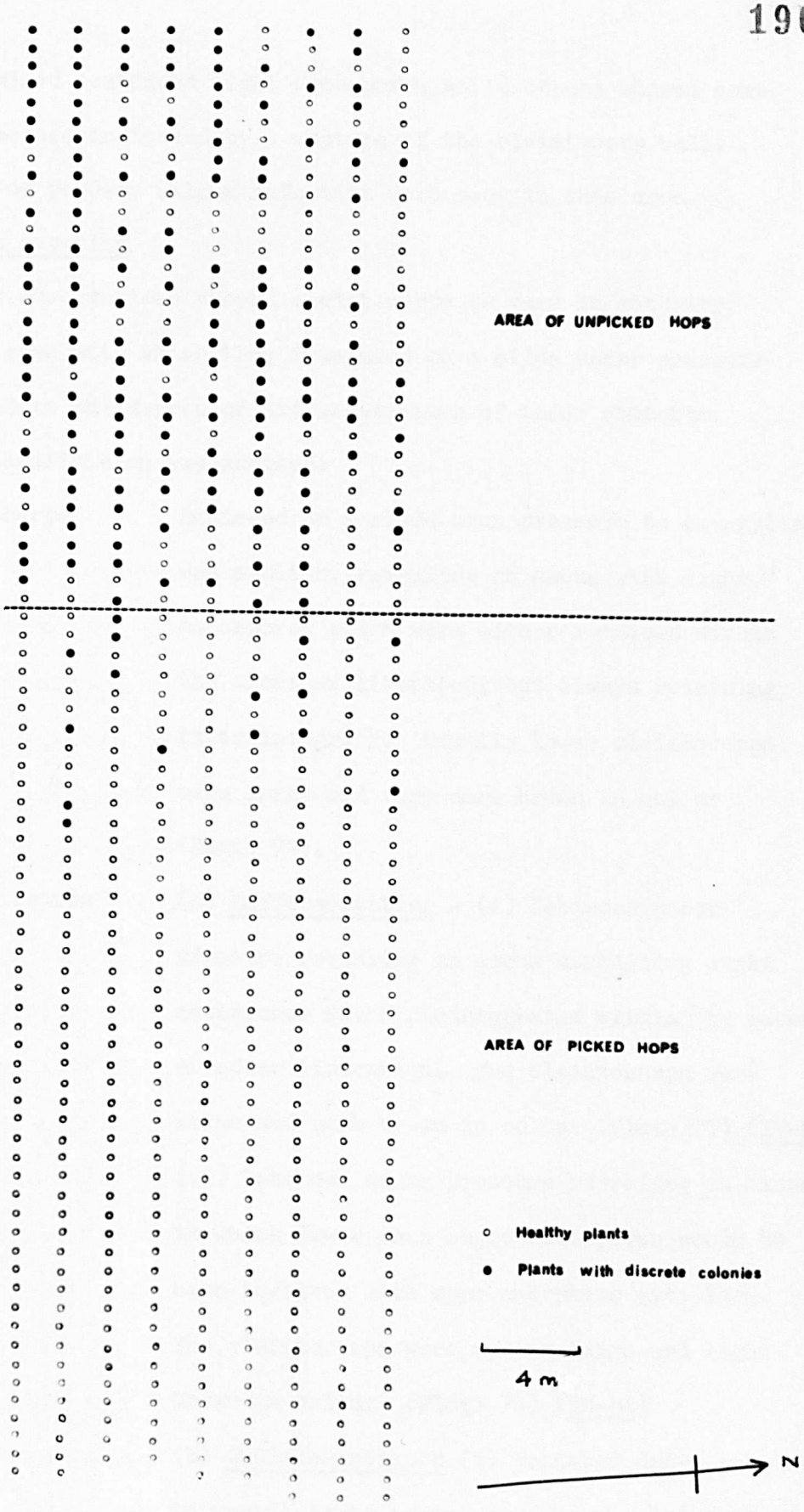

N
4m
Healthy plants
Plants with discrete colonies

Fig. 7 Part of a non-cultivated hop garden at Trottenden Farm, Goudhurst, Kent in April 1972, showing distribution of plants cv. OT 48 (Bramling Cross) with discrete, white powdery mildew colonies thought to have originated from ascospores. In the area of hops unpicked the previous year 22.8% of the plants were infected while in the picked area, only 2.2% .


AREA OF UNPICKED HOPS
AREA OF PICKED HOPS
Healthy plants
Plants with discrete colonies
4 m
N

cleistocarps examined contained eight ascospores while others showed some evidence of dehiscence indicated by a rupture of the cleistocarp wall. No other sources of powdery mildew infection were seen in this area.

(b) Cleistocarp maturity.

Preliminary observations showed cleistocarps to vary in maturity according to the ease with which they fractured on a slide under pressure on a coverslip and to the degree of differentiation of their contents. The following classification was adopted:

1. Mature cleistocarps (M) — Dehisced on a slide when pressure to coverslips was applied, revealing an ascus with eight ascospores which were either retained within the ascus or liberated; but always retaining their integrity. Usually these cleistocarps were large and very dark brown in colour (Plate 74).

2. Immature cleistocarps (IM) — (a) Differentiating - (i) Dehisced under pressure revealing an ascus containing eight ascospores which disintegrated within the ascus or after liberation. The cleistocarps were large and dark brown in colour (Plate 75) (IM-D1)
(ii) Dehisced under pressure revealing an ascus in which fewer than eight ascospores could be seen together with some amorphous cytoplasm. The cleistocarps were medium-large and light brown in colour (Plate 76) (IM-D2).
(b) Undifferentiated (i) Dehisced under pressure to reveal ascus containing amorphous cytoplasm with no signs of differentiation into ascospores.

The cleistocarps were small and light brown in colour. (Plate 77) (IM-UD1).

(ii) Dehisced under pressure to reveal no contents. The cleistocarps were small and very light brown in colour (Plate 78) (IM-UD2).

(iii) Failed to dehisce under persistent pressure on coverslip. The cleistocarps were very small and very light brown in colour. (Plate 79) (IM-UD3).

Records of the maturity of cleistocarps sampled from overwintering cone and leaf material are given in Tables 22, 23 & 24 for 1970/71, 1971/72 and 1972/73, respectively.

In samples from each winter season cleistocarps varied in their state of maturity and, on the same sampling dates, the proportion at each stage of maturity varied between years. The differences most probably result because the period in the late summer when cleistocarps form varies from year to year.

The system of classifying cleistocarps into stages of maturity is a subjective one, since little is known about what constitutes a 'mature' cleistocarp. Data in Tables 22-24 show that in the samples collected in May, and sometimes in April also, there was a decline in the proportions of (M) and (IM-D1) cleistocarps accompanied by the appearance of already dehisced cleistocarps in the samples. It is therefore probable that both cleistocarps classified as (M) and (IM-D1) were capable of dehiscing under certain natural conditions. For this reason Figs. 8 & 9 are both given to compare seasonal fluctuations in the proportions in the samples of (M) and (M)+ (IM-D1) cleistocarps, respectively.

Plates 74-79 Cleistocarps in different stages of maturation as encountered in the samples exposed in a hop garden during the winter. For details of classification, see text.

74 Mature (M) cleistocarp, with 8 differentiated ascospores in the ascus, dehisced artificially. (x 495).

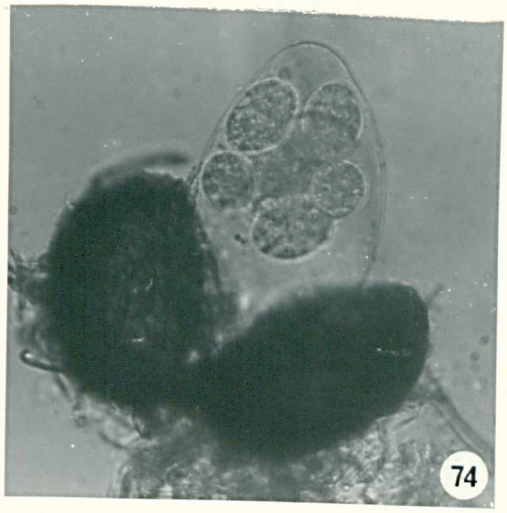
75 Immature (IM-D1) cleistocarp, with 8 partially differentiated ascospores in the ascus, dehisced artificially (x 495).

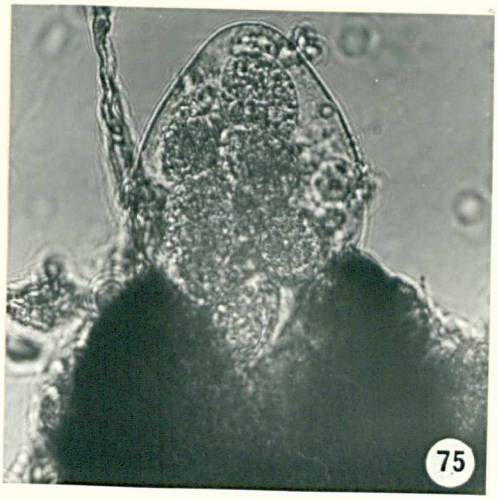
76 Immature (IM-D2) cleistocarp, with incomplete ascospore development, dehisced artifically. (x 495).

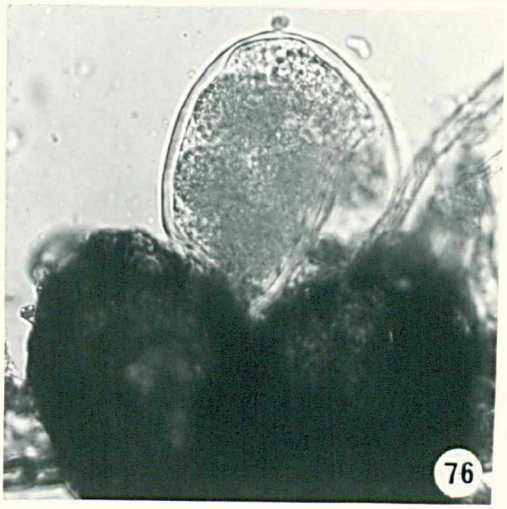
77 Immature (IM-UD1) cleistocarp, with an amorphous mass of cytoplasm in the ascus, dehisced artifically. (x 1,415).

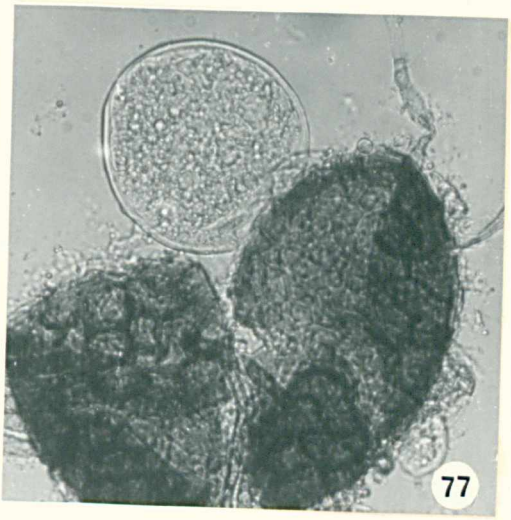
78 Immature (IM-UD2) cleistocarp, dehisced artificially, but with no contents. (x 545).

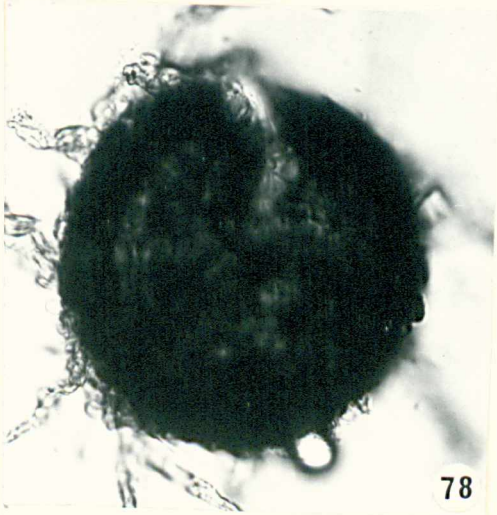
79 Immature (IM-UD3) cleistocarp, which failed to dehisce. Note the much smaller size and short appendages. (x 1,600).


74


75


76


77


78

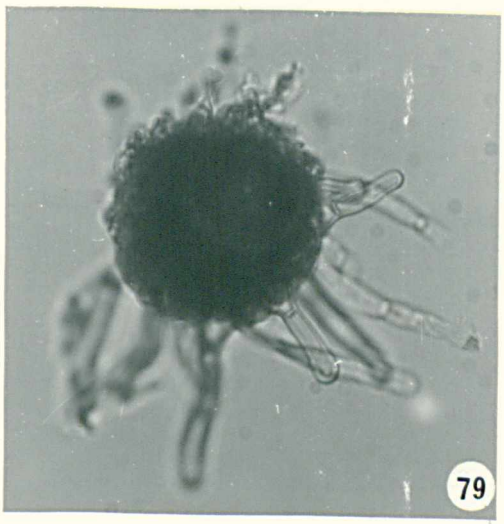

79

Table 22. Percentage cleistocarps in various stages of maturity in cone and leaf samples overwintered 1970-71*

| Date of sampling | Cleistocarp condition** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mature(M) | Immature(IM) | | | | | | |
| | | D1 | D2 | UD1 | UD2 | UD3 | Naturally dehisced |
| 17/18 October | 28.9 | 20.4 | 16.4 | 22.7 | 7.5 | 4.1 | 0 |
| 11/12 November | 23.0 | 44.8 | 10.3 | 6.8 | 0.7 | 14.4 | 0 |
| 9/10 December | 25.4 | 24.8 | 17.3 | 16.2 | 7.0 | 9.3 | 0 |
| 11/12 January | 30.7 | 19.0 | 24.5 | 9.6 | 3.7 | 12.5 | 0 |
| 10/11 February | 38.2 | 29.8 | 5.9 | 6.5 | 1.6 | 18.0 | 0 |
| 9/10 March | 28.1 | 51.8 | 4.2 | 2.4 | 3.0 | 10.5 | 0 |
| 12/13 April | 31.0 | 37.9 | 6.9 | 3.7 | 5.9 | 8.3 | 6.3 |
| 3/4 May | 0 | 8.3 | 5.5 | 10.2 | 11.7 | 17.6 | 46.7 |

*1000 Cleistocarps examined at each sampling

**For explanation of symbols, see text

Table 23. Percentage cleistocarps in various stages of maturity in cone and leaf samples overwintered 1971-72*

| Date of sampling | - Cleistocarp condition** - Mature(M) | Immature (IM) D1 | D2 | UD1 | UD2 | UD3 | Naturally dehisced |
|---|---|---|---|---|---|---|---|
| 18/19 October | 38.1 | 15.6 | 11.7 | 8.9 | 10.5 | 15.2 | 0 |
| 17/18 November | 22.2 | 41.7 | 4.9 | 11.0 | 1.9 | 18.3 | 0 |
| 18/19 December | 23.6 | 39.8 | 13.0 | 6.5 | 2.0 | 15.1 | 0 |
| 17/18 January | 11.1 | 31.9 | 9.3 | 25.9 | 3.3 | 1 .5 | 0 |
| 16/17 February | 25.7 | 24.4 | 7.8 | 20.0 | 5.4 | 16.7 | 0 |
| 17/18 March | 25.3 | 32.1 | 4.3 | 14.2 | 3.4 | 20.7 | 0 |
| 4/5 April | 26.6 | 18.8 | 3.9 | 31.8 | 4.4 | 14.5 | 0 |
| 18/19 April | 0 | 0.2 | 0 | 34.2 | 19.6 | 18.6 | 27.4 |
| 2/3 May | 0 | 0 | 0 | 21.7 | 20.0 | 21.2 | 37.1 |

*1000 cleistocarps examined at each sampling

**For explanation of symbols, see text

Table 24. Percentage cleistocarps in various stages of maturity in cone and leaf samples overwintered 1972-73*

| Date of Sampling | - Cleistocarp condition** - Mature(M) | Immature(IM) D1 | D2 | UD1 | UD2 | UD3 | Naturally dehisced |
|---|---|---|---|---|---|---|---|
| 17/18 October | 33.2 | 8.2 | 8.9 | 32.5 | 6.4 | 10.8 | 0 |
| 15/16 November | 57.3 | 20.0 | 5.6 | 10.1 | 0.9 | 6.1 | 0 |
| 15/16 December | 47.0 | 15.5 | 7.1 | 21.6 | 1.1 | 7.7 | 0 |
| 16/17 January | 20.7 | 26.8 | 5.8 | 24.6 | 5.1 | 17.0 | 0 |
| 15/16 February | 23.5 | 10.8 | 17.0 | 26.2 | 11.3 | 11.2 | 0 |
| 15/16 March | 37.8 | 14.3 | 11.3 | 29.4 | 2.2 | 5.0 | 0 |
| 2/3 April | 29.1 | 13.7 | 19.1 | 11.7 | 11.8 | 14.6 | 0 |
| 9/10 April | 35.3 | 10.8 | 10.3 | 18.7 | 5.7 | 19.2 | 0 |
| 16/17 April | 22.3 | 10.1 | 10.4 | 19.7 | 11.2 | 16.1 | 10.2 |
| 23/24 April | 9.7 | 11.9 | 9.4 | 21.6 | 7.9 | 12.2 | 27.3 |
| 1/2 May | 0 | 6.4 | 7.5 | 18.7 | 21.4 | 15.9 | 30.1 |

*1000 Cleistocarps examined at each sampling

**For explanation of symbols, see text

Fig. 8 Proportions of mature (M) cleistocarps among samples overwintered in a hop garden during 1970/71, 1971/72 and 1972/73 and examined at intervals. For definitions of cleistocarp maturity, see text.

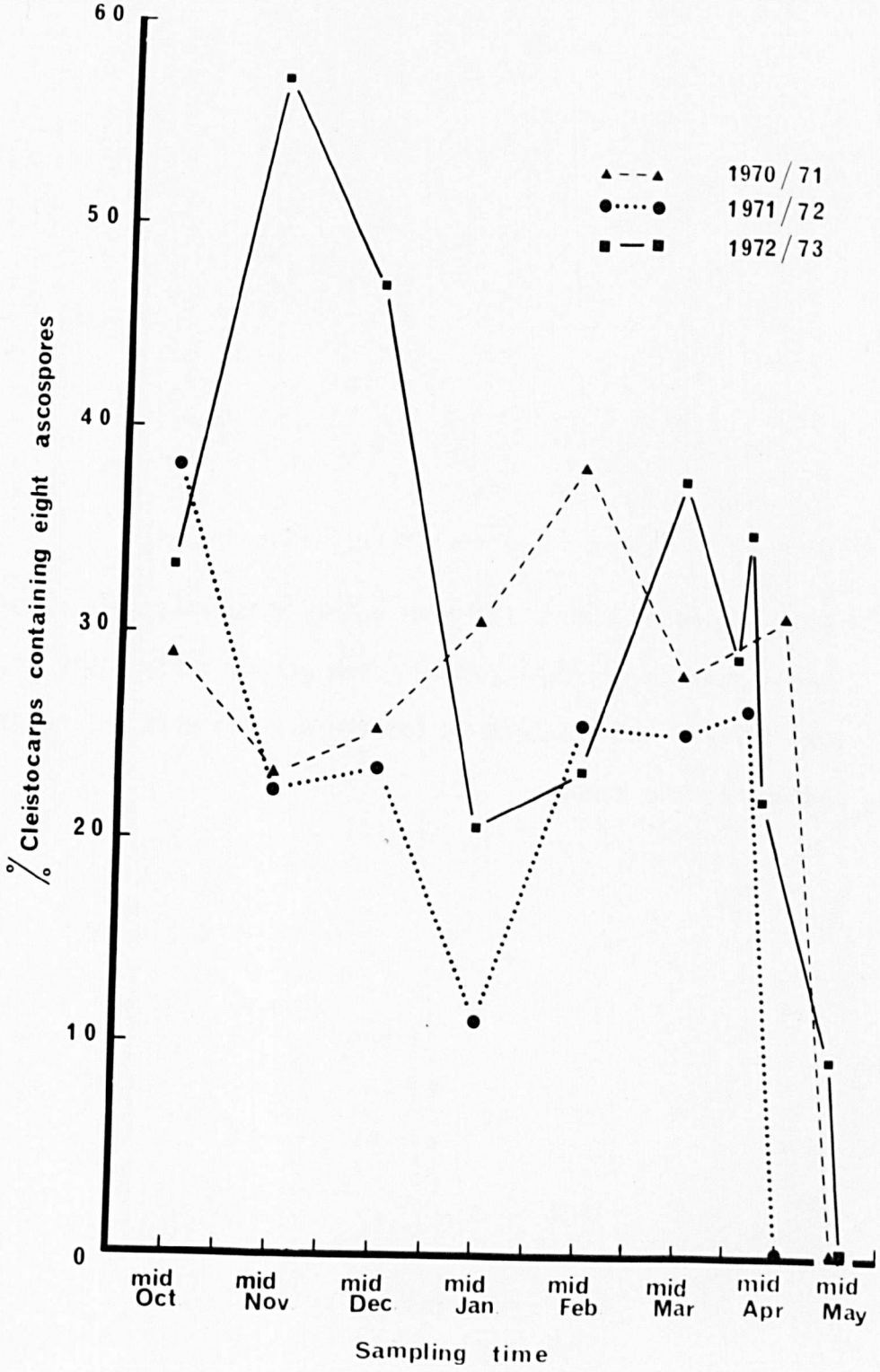
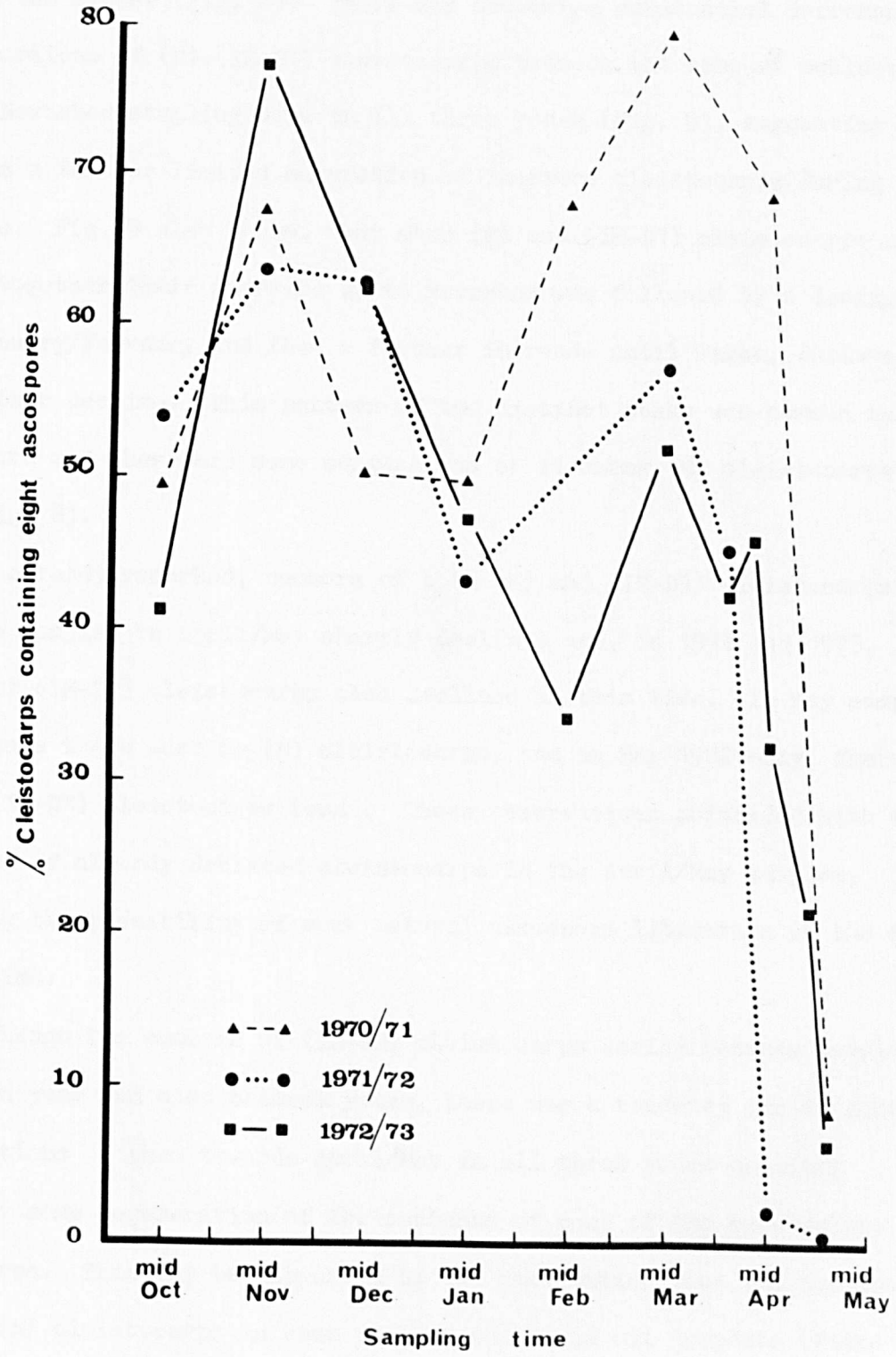

60
50
40
30
20
10
0
% Cleistocarps containing eight ascospores
1970/71
1971/72
1972/73
mid Oct
mid Nov
mid Dec
mid Jan
mid Feb
mid Mar
mid Apr
mid May
Sampling time

Fig. 9 Proportions of mature (M) plus nearly mature (IM-D1) cleistocarps among samples overwintered in a hop garden during 1970/71, 1971/72 and 1972/73 and examined at intervals. For definitions of cleistocarp maturity, see text.


80
70
60
50
40
30
20
10
0
% Cleistocarps containing eight ascospores
1970/71
1971/72
1972/73
mid Oct
mid Nov
mid Dec
mid Jan
mid Feb
mid Mar
mid Apr
mid May
Sampling time

Although the proportions of (M) cleistocarps in comparable samples varied between the years there was no consistent increase in their numbers early in the winter (Fig. 8). There was however, a substantial increase in the proportions of (M)+(IM-D1) cleistocarps between the time of collection and the November sampling date in all three years (Fig. 9), suggesting that there was a further limited maturation of immature cleistocarps during this time. Fig. 9 also shows, that when (M) and (IM-D1) cleistocarps are grouped together their increase up to November was followed by a decline until January/February and then a further increase until March, followed by a further decline. This pattern of two distinct peaks was common to all three years and there are some suggestions of it among (M) cleistocarps alone (Fig. 8).

As already remarked, numbers of both (M) and (IM-D1) cleistocarps in all years' samples in April/May sharply declined and, in 1972 and 1973, numbers of (IM-D2) cleistocarps also declined at this time. In May samples of all years there were no (M) cleistocarps, and in May 1972 only, there were no (IM-D1) cleistocarps found. These observations coincided with the appearance of already dehisced cleistocarps in the April/May samples, indicating the possibility of some natural ascospore liberation in the field at this time.

Although the numbers of (IM-UD) cleistocarps varied between samples in a given year and also between years, there was a tendency for an increase in proportions of them towards April/May in all three years possibly indicating some degeneration of the contents of some of the more mature cleistocarps. This may be supported by the observation that the ascospores of a few (M) cleistocarps in some samples contained oil droplets (Plate 81).

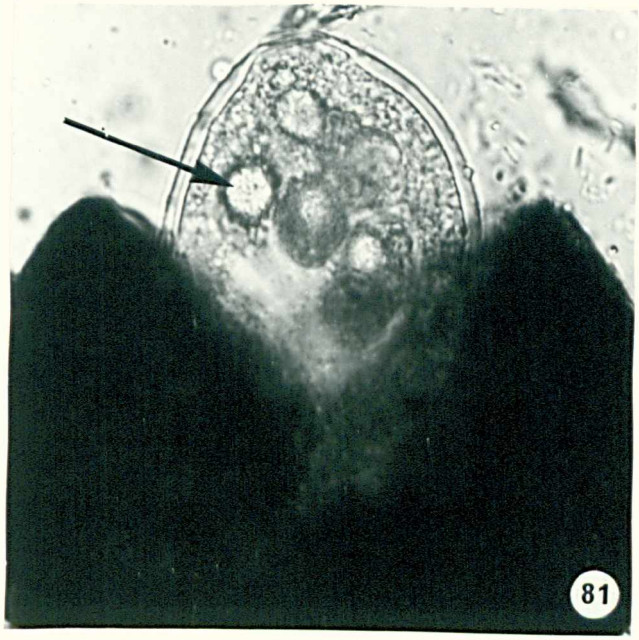
Cleistocarps were discovered within the alimentary canals of earthworms in abundance in samples obtained in April/May (Plate 80).

Plate 80 Cleistocarps within the digestive tracts of an earthworm. (x 141).

Plate 81 Artificially dehisced cleistocarp containing ascus with degenerating ascospores. Note the oil droplets in the ascospores (arrowed). (x 762).

Plate 82 Electron micrograph of the wall of an overwintered cleistocarp showing bacteria. (x 15,100).


80


81


82

These cleistocarps had no contents. Also, insects of Collombola sp. were abundant in these samples but contained no cleistocarps.

In some instances, particularly in the samples observed in April/May, cleistocarps thought to be mature (M) by virtue of their colour and size, did not appear to have any contents in them when examined microscopically. This might be due to the breakdown of ascospores through the activity of soil micro-organisms. Electron micrographs of overwintered cleistocarps showed bacteria between the cells of the cleistocarp wall (Plate 82).

(c) Natural dehiscence of overwintered cleistocarps and infectivity of ascospores.

The capacity of overwintering cleistocarps to dehisce and release ascospores naturally was examined in experiments carried out with samples collected from the nylon bags being exposed in the field as above. The cleistocarps were placed in Petri dishes, as already described, which were then placed either in the controlled environment of the growthroom or outdoors.

The results using three years' samples (Tables 25 & 26) show that ascospore release first occurred from cleistocarps collected in April of each year. Cleistocarps therefore appear to require a period of dormancy before they are able to dehisce. In 1973, when samples were examined more frequently in April, ascospores were first released from cleistocarps collected on 3/4 April, both in the growthroom and outdoors. The number released in the growthroom was slightly higher on the next sampling date (9/10 April) as was the proportion of dehisced cleistocarps. Thereafter, in both conditions, the number of released ascospores and of dehisced cleistocarps steadily increased reaching a peak on 23/24 April. During May ascospore release declined, suggesting that those cleistocarps capable of releasing ascospores had already largely done so. The proportion of cleistocarps which dehisced in the growthroom was higher than that outdoors.

Table 25. Number of ascospores released and germinated 24h after subjecting overwintered cleistocarps to prescribed conditions** in a growthroom and outdoors in 1970-71 and 1971-72*

| | growthroom | | | | | | outdoors | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No released | | No germinated | | % germinated | | No released | | No germinated | | % germinated | |
| October-March 1971 - 1972 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 April 1971 | 389 | | 5 | | 1 | | 94 | | 2 | | 2 | |
| 5 April 1972 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 |
| 18 April 1972 | | 10 | | 2 | | 20 | | 8 | | 2 | | 25 |
| 3 May 1971 | 24 | | 3 | | 13 | | 7 | | 0 | | 0 | |
| 2 May 1972 | | 4 | | 1 | | 25 | | 2 | | 0 | | 0 |

*Each figure is a mean of six replicates

**For details, see text

Table 26. Number of ascospores released and germinated 24h after subjecting overwintered cleistocarps to prescribed conditions**in a growthroom and outdoors in 1972-73*

| Date of sampling | growthroom | | | | outdoors | | | |
|---|---|---|---|---|---|---|---|---|
| | No released | No germinated | Estimated no cleistocarps exposed | Estimated % cleistocarps dehisced | No released | No germinated | Estimated no cleistocarps exposed | Estimated % cleistocarps dehisced |
| October-March | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3/4 April | 2 | 0 | 35,221 | 0.001 | 3 | 0 | 37,554 | 0.001 |
| 9/10 April | 6 | 0 | 31,277 | 0.002 | 1 | 0 | 32,981 | 0.000 |
| 16/17 April | 377 | 6 | 28,947 | 0.163 | 12 | 1 | 29,679 | 0.005 |
| 23/24 April | 447 | 16 | 26,556 | 0.210 | 105 | 7 | 33,562 | 0.039 |
| 1/2 May | 27 | 5 | 33,713 | 0.010 | 20 | 1 | 30,735 | 0.008 |
| 7/8 May | 3 | 1 | 29,768 | 0.001 | 4 | 1 | 29,573 | 0.001 |

*Each figure is a mean of six replicates

**For details, see text

The number of cleistocarps dehisced under both growthroom and outdoor conditions, were very small with the least recorded under the latter conditions.

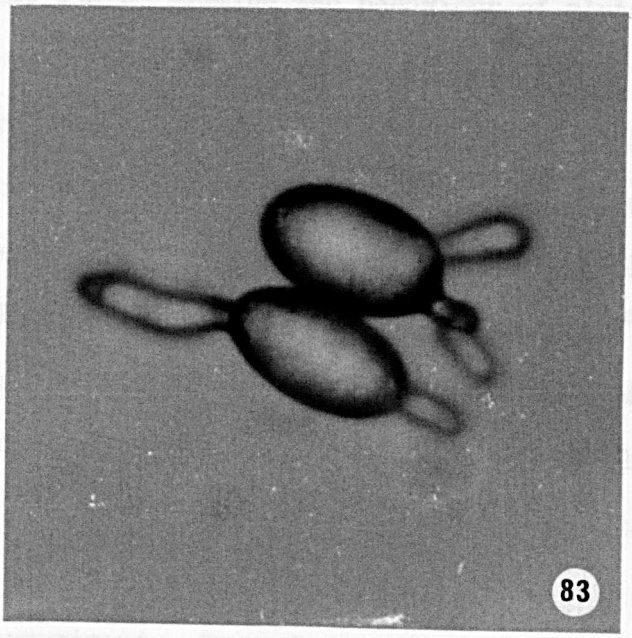
Consistently more of the cleistocarps were able to release ascospores when subjected to conditions in the growthroom. Also, a greater number of ascospores germinated in the growthroom.

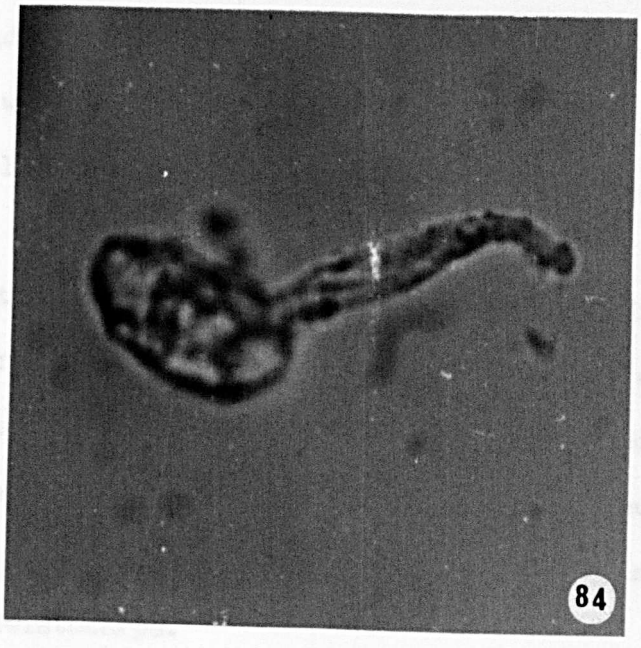
In 1971 and 1972 the first liberation of ascospores in April were accompanied by some germination, but in 1973, ascospores released from the two samples examined on 3/4 and 9/10 April failed to germinate, even after keeping them wet for 48h. In the more detailed experiments of 1973 ascospore germination was greatest at the time of greatest ascospore release, on 23/24 April, in both experimental conditions. Typical ascospore germination on glass is illustrated in Plate 83. In some cleistocarps, crushed on glass slides examined in May 1971, some ascospores emerged from the asci already germinated (Plate 84).

Experiments were conducted in April 1971 and 1972 to examine the ability of ascospores to infect leaves and leaf disks of cv. Northern Brewer on to which they were allowed to be released naturally. After release the leaves were kept in the same moist Petri dishes for seven days before removal but no mildew colonies developed. When the leaf disks were decolourized and stained ascospores could be recognised, some with short germ tubes, but there were no colonies sporulating or otherwise. With no knowledge of the conditions controlling infection of hop leaves by ascospores, it is possible that the conditions provided here were not conducive for colony development. Also, under these conditions other fungi were encouraged to colonise the surfaces of leaf disks. In 1972/73, therefore, an experiment was carried out at three temperatures: $4\pm 1^{\circ}C$; $8\pm 2^{\circ}C$ (incubator) and $18\pm 2^{\circ}C$. Ascospores still failed to produce powdery mildew colonies, however, although some germination was recorded under all temperature regimes.

Plate 83 Ascospores germinating on a glass slide within 24h of natural discharge. Both ascospores have two germ tubes (x 2,330).

Plate 84 Ascospore which was artifically released from a cleistocarp in a germinated condition.
(x 1,995).


83


84

(d) Survival of cleistocarps in different environments.

Previous results showed that ascospores were liberated only after overwintered cleistocarps had undergone a resting period. The object of this experiment was to examine the behaviour of cleistocarps when they were kept in a range of near constant environments over the winter period.

Cones and leaves bearing cleistocarps were collected from infected field plants in October, 1972. They were placed in small terylene net bags and then placed in three environments: refrigerator ($4\pm1^{\circ}C$, in darkness), incubator ($8\pm2^{\circ}C$, in darkness) and growthroom ($18\pm2^{\circ}C$, with a 16h photoperiod. A sample of 1,000 cleistocarps was examined for their state of maturity from each treatment immediately after collection and at the end of the experiment, in May. The ability of cleistocarps to dehisce naturally was examined as before at $18^{\circ}C$, at monthly intervals from the start of the experiment. On each occasion cleistocarps were held over six glass slides as replicates in six Petri dishes for each regime. The methods used were identical to those used previously, but the number of ascospores released was not related to the number of cleistocarps finally dehisced.

The initial range of cleistocarp maturity immediately after collection (Table 27) varied somewhat between samples placed in different regimes. There was some suggestion that, at $4^{\circ}C$, some cleistocarps were becoming more mature over the seven months of the experiment but, at higher temperatures, there were suggestions of a reduction in the numbers of the more mature cleistocarps.

Naturally dehisced cleistocarps were never found among these samples which were kept under constantly dry conditions, providing some evidence that free water is needed for dehiscence.

Table 27. Percentage ascospore differentiation of cleistocarps kept under different environments 1972/73*

| Temperature to which cleistocarps subjected (°C) | Date of sampling | Cleistocarp condition** | | | | | |
|---|---|---|---|---|---|---|---|
| | | Mature(M) | Immature(IM) | | | | |
| | | | D1 | D2 | UD1 | UD2 | UD3 |
| Refrigerator (4 ) | 29 October | 37.9 | 14.0 | 10.2 | 15.3 | 11.8 | 10.8 |
| | 2 May | 52.4 | 21.1 | 10.6 | 10.1 | 2.6 | 3.2 |
| Incubator (8 ) | 29 October | 51.2 | 24.4 | 9.3 | 7.9 | 4.3 | 2.9 |
| | 2 May | 48.6 | 11.4 | 10.2 | 12.6 | 3.9 | 13.3 |
| Growthroom (18 ) | 29 October | 47.8 | 14.3 | 7.3 | 25.9 | 1.7 | 3.0 |
| | 2 May | 41.3 | 15.4 | 15.3 | 21.7 | 2.7 | 3.6 |

*1000 cleistocarps were examined at each sampling

**For explanation of symbols, see text

At monthly intervals cleistocarps from the different treatments were soaked in water for 1h prior to being placed above glass slides in moist chambers in the growthroom (18°C). Ascospore release again only occurred from those cleistocarps from all three regimes sampled after April (Table 28). This showed that the period of dormancy required by cleistocarps before being able to release their contents seems to be independent of the temperature during the overwintering period. Very little germination of ascospores occurred under these conditions, and only when the ascospores had been released from cleistocarps kept at 18°C.

(e) Effect of different temperatures on ascospore discharge.

Results of the experiments just described showed that cleistocarps are capable of liberating ascospores only after April, each year. This coincided with the appearance of naturally-dehisced cleistocarps in the infected cone samples kept outdoors throughout the winter. Although temperature over the range 4-18°C appears not to influence cleistocarp dormancy, temperature could have an effect on the extent of cleistocarp dehiscence.

An experiment was therefore set up on 19 April 1973 to examine the effect of temperature on ascospore discharge from cleistocarps overwintered outdoors or in the growthroom during the previous winter. The experimental procedure was similar to that previously described. Four different temperatures were examined: 4±1°C in a refrigerator, 8±2°C in an incubator, 18±2°C in the growthroom and 24±0°C in an incubator. Except in the growthroom where 16h photoperiod was imposed all other treatments were in darkness. The total number of cleistocarps used in each treatment was estimated from a 5% sample of the infected bracts, bracteoles and leaf disks exposed for dehiscence. The number of ascospores released on to glass slides was counted 24h after wetting the cleistocarps (Table 29).

Table 28. Number of ascospores released and germinated 24h after submitting cleistocarps kept at different temperatures to prescribed conditions,** 1972/73*

| Date of sampling | Environment in which cleistocarps were kept during the winter | | | | | |
|---|---|---|---|---|---|---|
| | Refrigerator (4°C) | | Incubator (8°C) | | Growthroom (18°C) | |
| | No released | No germinated | No released | No germinated | No released | No germinated |
| October-March | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 April | 61 | 0 | 62 | 0 | 72 | 1 |
| 2 May | 41 | 0 | 36 | 0 | 77 | 1 |

*Each figure is a mean of six replicates

**For details, see text

Table 29. Effect of temperature on ascospore release within 24h after wetting cleistocarps which had been overwintered in a growthroom or outdoors, 1973*

| Temperature to which cleistocarps subjected (°C) | outdoors | | | | growthroom | | | |
|---|---|---|---|---|---|---|---|---|
| | No released | No germinated | Estimated no cleistocarps exposed | Estimated % cleistocarps dehisced | No released | No germinated | Estimated no cleistocarps exposed | Estimated % cleistocarps dehisced |
| 4 | 60 | 1 | 29,742 | 0.025 | 112 | 2 | 30,375 | 0.046 |
| 8 | 128 | 3 | 28,486 | 0.056 | 272 | 5 | 31,434 | 0.108 |
| 18 | 324 | 30 | 27,747 | 0.146 | 530 | 45 | 28,438 | 0.233 |
| 24 | 55 | 1 | 29,775 | 0.023 | 200 | 1 | 30,420 | 0.082 |

*Each figure is a mean of four replicates

In both samples cleistocarp dehiscence was greatest at 18°C and was least at 4°C and 24°C. Once again more cleistocarps released ascospores in the samples overwintered in the growthroom than in those kept outdoors during the winter. Ascospore germination followed the same pattern, poor germination being recorded at all temperatures except 18°C.

(f) Observations on overwintering of hop powdery mildew in the field in relation to the role of cleistocarps.

The survey (Fig. 6) of leaf infection on plants growing in a non-cultivated area in the Withersdane hop garden at Wye College in May, 1971, showed discrete powdery mildew colonies on expanding young leaves which had possibly originated from ascospores. Although ascospores germinated on glass and on leaves in a growthroom they failed to give rise to sporulating colonies. Therefore, experiments were set up in the field in October 1971 and 1972 to obtain more information of the role of ascospores in initiating spring primary infections.

Clay pots (14 cm diameter), each containing two healthy strap cuts of cv. Northern Brewer in sterile compost, were buried in soil up to their rims in a circle (1.4m diameter) in the centre of the Nursery garden. The strap cuts were taken from rootstocks from beneath the soil surface where they had never been exposed to powdery mildew infection. Cones bearing abundant cleistocarps were placed on the surface of the compost within each pot to a depth of 3 cm. They were also distributed between and around the pots over the garden soil to provide a potentially concentrated source of ascospores. A Burkard volumetric spore trap was sited in the centre of the plant circle and was operated each year from early March onwards (Plate 85). In 1971/72, its orifice was 0.7m from the ground but, in 1972/73, much closer to the cleistocarp debris, 15 cm above ground level. By the spring, 1972 much of the infected cone debris had been blown by wind from the site and annual weeds

grew over the area in which the cleistocarp debris was distributed. Therefore, for the 1972/73 experiment the cleistocarp debris was distributed over a terylene net (625 squares/$cm^2$) stretched over the ground. A nylon net (1 square/$cm^2$) was then used to retain the cone debris.

A similar group of pots containing strap cuts acted as controls and was buried in an area in the same hop garden about 100m away from the experimental site. They were surrounded by seedling hops, resistant to powdery mildew. These pots also remained outdoors until the following spring.

Records of temperature, relative humidity and rainfall in the hop garden were obtained during both experiments. Leaf wetness at the soil surface was recorded using a Casella wetness recorder from 16 April to 6 May 1973.

During April the strap cuts developed leafy shoots which were then examined for the presence of powdery mildew infection at two-day intervals from 1 May to 6 May 1973. Then, thirty leaves were removed on each occasion which were decolourized and stained. Both upper and lower surfaces were then examined for evidence of mildew colonies.

The results (Table 30) show that only those shoots arising from amidst cleistocarp debris became infected (Plate 86). A careful examination of shoots of all the permanent hop plants (approximately 10,500) in the Nursery garden failed to reveal any powdery mildew which could have been a source of the infection of strap cuts. In both years, discrete white pustules occurred in greater abundance on the lower surfaces of the leaves. It was not uncommon to find colonies on the upper surface of leaves or on both surfaces but rarely on stems. Powdery mildew colonies were present on leaves mostly near the ground but occurred also up to about 7 cm away from the soil surface, these symptoms probably arising from infection when the leaves were close to the ground before shoot extension. The first symptoms were observed on 28 April 1972 and on 30 April 1973. On leaves

Plate 85 Experimental plot in an area of unsprayed hops in the Nursery Garden. A deep circular bed of cone debris bearing cleistocarps surrounds a Burkard suction trap. Around the periphery of the bed are sunken pots containing plants being exposed to ascospore infection in April, 1973.

Plate 86 Powdery mildew colonies initiated by ascospores (arrowed) on a plant of cv. Northern Brewer, being exposed on the site illustrated above.


85


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Table 30. Percentage leaves and stems of exposed plants of cv. Northern Brewer infected

| Period of experiment | Treatment | Upper surface | Lower surface | Upper & lower surface | Stem |
|---|---|---|---|---|---|
| 1971/72 | with cleistocarps | 3.9 | 10.5 | 1.2 | 0 |
| | control | 0 | 0 | 0 | 0 |
| 1972/73 | with cleistocarps | 1.8 | 10.9 | 1.0 | 0.2 |
| | control | 0 | 0 | 0 | 0 |

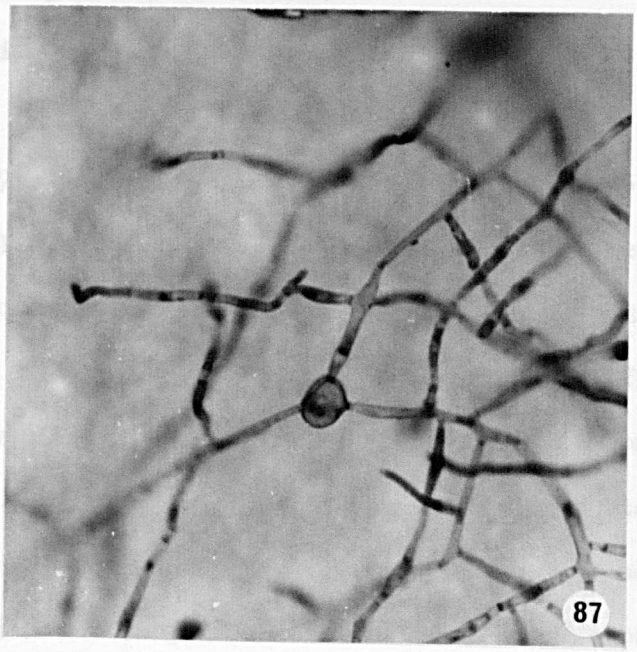
*Each figure is a mean of thirty observations

samples on two occasions between 1 and 6 May and stained colonies could be seen to originate from spores which were identified conclusively as ascospores (Plate 87). These ascospores remained integral and identifiable features of the colony. For comparison, a similar colony initiated by a conidium is shown in Plate 88 . These results, therefore, demonstrate quite clearly that cleistocarps, overwintered naturally, can produce ascospores which infect young hop leaves in the spring.

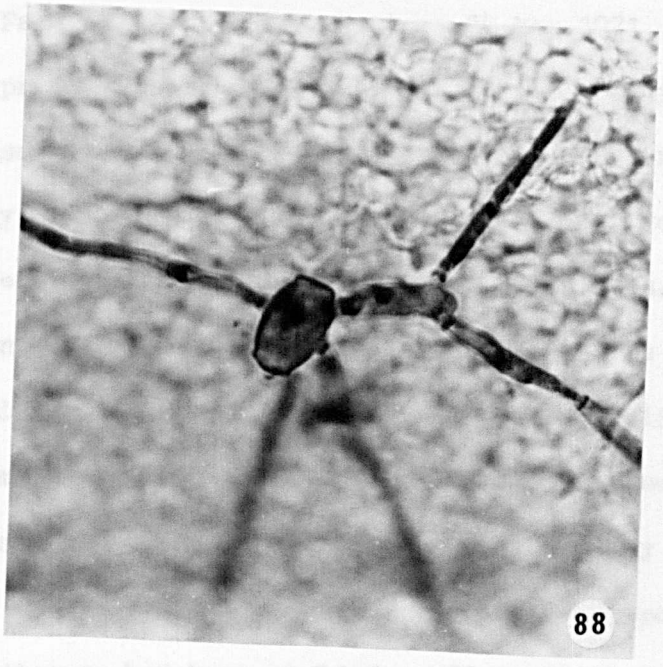
Positive identification of ascospores on the tapes of the Burkard spore trap was extremely difficult. In 1973 only one ascospore was recognised, which was caught during a dry period following a period of rainfall. This apparent failure to trap ascospores could either be due to their being dispersed principally by rainsplash, to low trajectory of the ascospore flights, or to their concentration in the air being below the threshold for the trap (approx. $14/m^3$).

Plate 87 A young colony on a leaf of cv. Northern Brewer from a plant in the field showing its ascospore origin (x 375).

Plate 88 A young colony on a leaf of cv. Northern Brewer initiated by a conidium (for comparison). (x 720).



87



88

(2) Overwintering in buds.

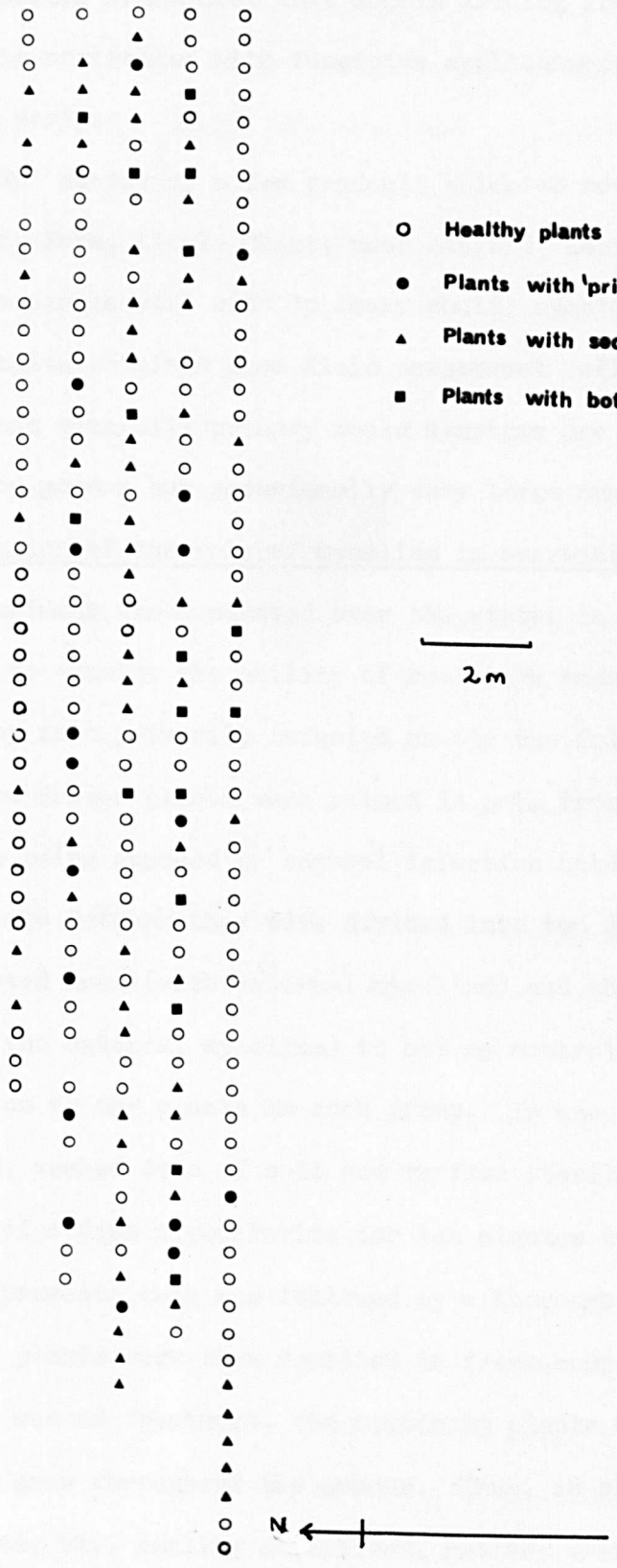
(a) The incidence of primary mould infection in commercial hop gardens.

In three non-cultivated farms in the Weald of Kent, surveys of the incidence of 'primary mould' were undertaken. Each farm had had a recent history of primary mould occurrence.

Examination of 361 hop plants in a field of 8-9 year old Bramlings and 173 plants in a field of WGV at Bockingfold Farm, near Yalding, Kent on 29 April 1971, revealed only one plant with 'primary mould' symptoms in each of the two fields examined. A few leaves of the infected hills showed some apparently secondary infection with actively sporing discrete colonies. The fungus was apparently spreading from the primary foci in the extremely cold conditions prevailed during this period. Sections of the terminal buds of the primarily infected shoots revealed the presence of mycelium well within the bud scales and on rudimentary leaves as well.

Examination of 238 plants in a field of three-year old Northern Brewer at Gallant's Farm, East Farleigh, Kent on 14 May 1971, revealed many of the plants with 'primary mould' symptoms (Fig. 10). Thirty five (14.7%) of the plants possessed at least one primary mould shoot and 77 (32%) showed some secondary infection as pustules on expanding leaves. Both primary and secondary infections were recorded on 18 (7.6%) plants. In the previous growing season the hops in this particular garden were very severely affected by powdery mildew, despite fungicide sprays being applied at ten day intervals from 23 April to 7 August. When growth resumed in the spring of 1971, the first symptoms of primary mould were seen by the grower around the middle of April. To attempt to arrest the development of the disease at this time dinocap had been applied on 20 April then again on 6 May, but the level of primary infection recorded on 14 May was still very high.

Fig. 10 Part of a non-cultivated hop garden at Gallants Farm, East Farleigh, Kent, 14 May 1971, showing distribution of infected plants cv. Northern Brewer. 14.7% of plants contained one or more 'primary mould' shoots, 32.0% secondary infections of leaves and 7.6% both 'primary mould' and secondary infection.


Healthy plants
Plants with 'primary mould' symptoms
Plants with secondary colonies only
Plants with both symptoms
2 m
N

It appeared, therefore, that shoots arising from infected buds cannot effectively be controlled with fungicide applications, even if 'primary mould' was detected early.

A careful survey of a few randomly selected rows of cv. Northern Brewer at Chart Court Farm, Little Chart, near Ashford, Kent, on 21 April 1972, showed only a single hill with 'primary mould' symptoms.

The results obtained from field assessment reflected hop growers' experience that generally 'primary mould' symptoms are produced only in a few hills in a hop garden but occasionally very large numbers can occur.

(b) Examination of the role of mycelium in overwintered buds of pot plants.

An experiment was conducted over the winter in each of two years, 1971/72 and 1972/73, to examine the ability of rootstock buds to harbour the pathogen and to develop into primarily infected shoots the following year.

Northern Brewer plants were raised in pots from cuttings in sterile compost while being exposed to natural infection outdoors throughout the summer. In late October they were divided into two groups, one group with visibly infected buds (with external mycelium) and the other with apparently healthy buds (no external mycelium) to act as controls. Several treatments were imposed on to the plants in each group. In one treatment, the plants were depotted, washed free of soil and surface sterilized by dipping in a 10% solution of sodium hypochlorite for ten minutes to kill the superficial mycelium, if present; this was followed by a thorough washing in deionised water. These plants were then repotted in fresh compost in clean, plastic pots. In the second treatment, the remaining plants were left in the pots in which they grew throughout the summer. Thus, in all, there were four main treatments, viz. healthy sterilized, healthy untreated, infected sterilized and infected untreated. The plants within each group were now divided into two further sub-groups, one kept in the glasshouse and the other outdoors until March; half of the plants from each sub-group were then removed to grow in isolation in specially made boxes in an air-cooled

glasshouse. This prevented the possibility of any chance infection. The remaining plants whether in the glasshouse or outdoors were kept undisturbed and allowed to grow in these environments. Tables 31 and 32 show these treatments.

The results showed that some of the buds which showed external infection the previous autumn gave rise to primary infected shoots. Just prior to shoot development in March some buds were excised from the parent rootstock and the bud scales were teased apart under a binocular microscope (x50). None of them bore cleistocarps, thus dismissing the possibility of ascospores initiating this infection. Dissection of the infected buds revealed conidiophores bearing apparently normal conidia present on the tightly folded rudimentary leaves and their petioles within the buds. Later in March, when shoot growth had commenced the internal mycelium rapidly infected all the outer tissues of the buds, so that, when the leaves emerged, they were already covered with mycelium. Primary infected shoots identical to those found naturally in the field (Plate 2) were finally produced from these pot plants. Various stages of infected shoot development in the spring are shown in Plates 89-91.

Shoot infection arose earlier in the glasshouse isolation boxes or in the open glasshouse than outdoors. It is likely that the outdoor climatic conditions delayed growth of the infected buds.

It was also observed that the tips of those shoots in the glasshouse isolation boxes which were apparently infected heavily during spring, tended to grow away from the infection, resulting in healthy apical growth. It was uncommon to find this occurring on plants growing in natural conditions. Surface sterilization of buds seemed to have little effect on the development of infected shoots.

Table 31. Percentage above-soil buds developing into infected ('primary mould') shoots in April, 1972, after various treatments

| Plants in late October | Treatment of rootstocks (October) | Overwintering site | Regime for shoot development (March) | |
|---|---|---|---|---|
| | | | Same as overwintering site | Isolation boxes in glasshouse |
| with some infected buds | untreated | outdoors | 10.0 | 15.8 |
| | | glasshouse | 13.6* | 19.1 |
| | surface sterilized | outdoors | 22.7 | 13.3 |
| | | glasshouse | 10.0 | 39.1 |
| with apparently healthy buds | untreated | outdoors | 0 | 0 |
| | | glasshouse | 0 | 0 |
| | surface sterilized | outdoors | 0 | 0 |
| | | glasshouse | 4.2 | 2.9 |

*4.6% of these buds bore cleistocarps

Table 32. Percentage above-soil buds developing into infected ('primary mould') shoots in April, 1973, after various treatments

| Plants in late October | Treatment of rootstocks (October) | Overwintering site | Regime for shoot development (March) | |
|---|---|---|---|---|
| | | | Same as overwintering site | Isolation boxes in glasshouse |
| with some infected buds | untreated | outdoors | 29.3 | 5.0 |
| | | glasshouse | 14.3 | 11.5 |
| | surface sterilized | outdoors | 20.8 | 18.2 |
| | | glasshouse | 14.3 | 9.0 |
| with apparently healthy buds | untreated | outdoors | 0 | 0 |
| | | glasshouse | 0 | 0 |
| | surface sterilized | outdoors | 0 | 0 |
| | | glasshouse | 0 | 0 |

Plate 89 'Primary mould' on a young shoot (on the right) after bud break. A healthy shoot is on the left.

Plate 90 A later stage of the 'primary mould' symptom. The fungus has continued to colonise the expanding shoot system, smothering young leaves and upper internodal region of the stem with heavily sporing mycelium.


89


90

Plate 91 Still later stage of the 'primary mould' symptom. The entire shoot is smothered with the fungus which is drastically affecting growth.


91

Some buds that were apparently healthy in the autumn of 1971 produced infected shoots in the spring. Almost certainly, these buds were infected with mycelium only within the bud. Not all buds which showed infection in the autumn produced infected shoots in spring, some died.

It was interesting to note that some of the dormant buds of infected plants kept in the glasshouse, when examined with a hand lens (x10) showed the presence of cleistocarps mostly confined to the outer bud scales. Laboratory examination of the cleistocarp contents revealed varying degrees of maturity of their contents.

(c) Production of infected shoots by plants in isolation.

In order to be satisfied that mycelium within buds could initiate 'primary mould' symptoms in spring, buds of plants kept outdoors during the winter were allowed both to overwinter and to produce shoots under quarantine conditions to prevent any chance infection.

Ten plants of cv. Northern Brewer, possessing buds which were heavily infected externally in October 1972 were allowed to stand outdoors until spring, 1973 on plastic trays covered by muslin-covered perspex cylinders. The rootstocks were then depotted, soil removed by washing under a tap, and then they were surface-sterilized by dipping in a 10% solution of sodium hyphchlorite for ten minutes. The pot roots were drained, thoroughly washed with deionised water and then each was repotted in sterile compost in clean plastic pots and kept on plastic trays containing clean quartz sand. These plants were covered with fresh perspex cylinders. Five plants were incubated in the growthroom (18°C) for two weeks and the remaining five plants were kept outdoors for three weeks when the buds produced shoots.

Only one infected shoot was produced in each treatment, but this was sufficient to confirm that mycelium within buds during the winter could initiate primary mildew symptoms in the spring.

(d) The time of infection of buds.

The studies carried out so far indicated that the mycelium of S. humuli could perennate successfully in the buds and initiate infection the following spring. Information was now sought on the time of year when buds become infected and its influence on symptom production the following year. The opportunity also allowed the time of cleistocarp formation on buds to be examined.

The experimental method employed involved exposing batches of plants to natural infection at different times during the summer, 1972. Plants of the cv. Northern Brewer and seedling 11/66/28 were raised from cuttings in sterile compost in plastic pots. They were allowed to grow in a glasshouse and were kept virtually free of disease by occasionally spraying the plants with dinocap. Fungicide application stopped one week before introducing the plants to outdoor conditions.

The first batch of thirty plants cv. Northern Brewer were exposed from 16 June throughout the summer to natural infection outdoors, thereafter three more batches of fifty plants each were exposed on 1 July, 15 July and 1 August for the remaining summer period. For each batch of Northern Brewer plants eight control plants raised and grown under similar conditions were placed outdoors about 10m away from the treatment plants. A weekly application of dinocap was used in an attempt to keep the control plants free of primary mildew infection. In addition, on 1 July, twenty plants of 11/66/28 were exposed along with eight control plants. The number of infected and healthy buds situated above the soil level in the pots were recorded on 25 October after all the leaves were shed. In addition, a record of the number of buds bearing cleistocarps was also taken.

The results (Table 33) show that infection of buds could take place on plants exposed from any stage. Plants exposed on 1 August, which had

Table 33. Effect of time of exposing plants to natural inoculum on dormant bud infection of Northern Brewer, 1972

| period of exposure | cv./seedling | % buds infected with external mycelium | % buds apparently healthy | % buds bearing cleistocarps |
|---|---|---|---|---|
| 16 June - 25 Oct | Northern Brewer | 67.8 | 32.2 | 0 |
| | Northern Brewer(control) | 31.5 | 68.5 | 0 |
| 1 July - 25 Oct | Northern Brewer | 81.9 | 18.1 | 0 |
| | Northern Brewer(control) | 45.3 | 54.7 | 0 |
| | 11/66/28 | 2.2 | 97.8 | 0 |
| | 11/66/28(control) | 0 | 100.0 | 0 |
| 15 July - 25 Oct | Northern Brewer | 88.2 | 11.8 | 2.2 |
| | Northern Brewer(control) | 66.7 | 33.3 | 2.6 |
| 1 August - 25 Oct | Northern Brewer | 92.2 | 7.8 | 17.5 |
| | Northern Brewer(control) | 49.0 | 51.0 | 0 |

the shortest period of exposure to infection, showed the greatest bud infection and the number of buds infected decreased with increasing exposure time. There were only a few small buds formed on the plants exposed on 16 June. The foliage of these plants became heavily infected possibly weakening their capacity to form rootstocks. This was reflected in the low number of buds which eventually differentiated.

A slight amount of leaf infection developed on 11/66/28 and very little bud infection resulted because of its inherent resistance.

Some buds of control plants also became infected despite weekly fungicide application during the growing period. It is possible that many of these buds were infected following cessation of the fungicide programme, that the fungicide applied had a poor effect or, most likely, that bud infection was caused by inoculum originating from neighbouring heavily infected plants.

Cleistocarps were found only on buds of the two batches of plants exposed on 15 July and 1 August most occurring on the last batch exposed.

(e) The effect of time of bud infection on 'primary mould' the following spring.

After recording external bud infection in October, 1972, the plants of each of the four batches in the previous experiment were divided into two groups: those with some infected buds and others with only apparently healthy buds. The same treatments as in previous experiments were then imposed on to each group; i.e. surface sterlisation of rootstocks vs. untreated, overwintering in a glasshouse vs. outdoors. The following spring, buds of all plants were allowed to develop shoots in the same environment in which they had been kept during the winter. At the outset all buds bearing cleistocarps externally were removed to leave only those infected buds with mycelium. The incidence of 'primary mould' shoots was recorded on 30 April when a selection of infected buds at an early stage of shoot development

were dissected to examine for cleistocarps. None were found indicating that all primary infection arose from mycelium.

Irrespective of the period of exposure to infection the previous summer, a proportion of the buds bearing mycelium in the autumn produced infected shoots the following spring. The plants exposed last, from 1 August, yielded most infected shoots. Otherwise the results of the various treatments followed the same pattern as before and the detailed results are, therefore, not presented.

(f) The susceptibility to infection of dormant buds of various hop cultivars.

The foliage of twenty plants of each of the cvs./seedlings was inoculated with the Glasshouse isolate on 20 June, 1972. Infection was allowed to develop in a glasshouse and the plants were then placed outdoors on 20 July. Further development of infection was allowed to proceed throughout the summer and the numbers of apparently healthy buds, and of buds with external mycelium and of those with cleistocarps were assessed on 25 October after the leaves had fallen.

The results (Table 34) show, as expected, that the cvs./seedlings which were normally susceptible to leaf infection showed a high proportion of dormant bud infection in the autumn. Although a few buds of 11/66/28 also were slightly infected, the other resistant cultivars remained free of any infection.

(g) Effect of temperature on the survival of mycelium in dormant buds.

The previous experiments carried out during 1970/71 and 1971/72 suggested that temperature during the overwintering period might be a factor in the survival of mycelium within buds. This was examined in an experiment in 1972 in which plants with infected buds were overwintered in different localities, representative of different temperatures.

Table 34. Incidence of bud infection on several cultivars and seedlings in the autumn, 1972

| cv./seedling | % buds infected with external mycelium | % buds apparently healthy | % buds bearing cleistocarps |
|---|---|---|---|
| Northern Brewer | 56.2 | 43.8 | 5.0 |
| 14/66/50 | 55.4 | 44.6 | 3.6 |
| 11/66/28 | 1.2 | 98.8 | 0 |
| Wye Challenger | 0 | 100.0 | 0 |
| Wye Target | 0 | 100.0 | 0 |

Fifty plants of cv. Northern Brewer which showed visible bud infection after exposure to natural infection throughout the summer 1972 were removed from their pots on 27 October and were divided into five equal groups. Five similar groups of strap cuts, cv. Northern Brewer, obtained from beneath the soil ridges of a hop garden served as controls. The numbers of apparently healthy and visibly infected buds on the plants were counted before subjecting them to the following treatments:

| | |
|---|---|
| Unheated glasshouse | Approximate temperature 6± 3°C |
| Outdoors | 4± 5°C |
| Incubator | 8± 2°C |
| Refrigerator | 4± 1°C |
| Deepfreeze | -4°C |

The plants to be overwintered in the unheated glasshouse and outdoors were repotted in fresh sterile compost in clean plastic pots, while those to be kept in the incubator, refrigerator and deepfreeze were placed separately in sealed polythene bags. On 23 April these plants were repotted in fresh compost in clean plastic pots. All plants were then placed in isolation boxes in the air-cooled glasshouse and shoots allowed to develop.

Only the plants kept during the winter outdoors or in the glasshouse produced 'primary mould' symptoms (Table 35). While only a small proportion of the buds of plants kept in the refrigerator and outdoors were dead in the spring, all the buds in the plants kept in the deepfreeze were dead.

(h) Anatomy of infected and healthy buds.

The field surveys showed that only a very few buds are usually infected in a hop garden and it was considered that enormous numbers of field buds would have to be examined to guarantee an infected bud. Buds from potted plants were therefore used for this study.

Table 35. Effect of temperature on survival of mycelium in dormant buds

| Site of overwintering | Date of assessment | Plants with some visibly infected buds | | | Control plants | |
|---|---|---|---|---|---|---|
| | | % healthy | % infected | % dead buds | % healthy | % dead buds |
| Glasshouse | Buds 27 October | 42.1 | 57.9 | - | 100.0 | - |
| | Shoots 19 March | 47.4 | 52.6 | 0 | 100.0 | 0 |
| Outdoors | Buds 27 October | 4.5 | 95.5 | - | 100.0 | - |
| | Shoots 19 March | 83.7 | 16.3 | 26.9 | 100.0 | 19.4 |
| Incubator | Buds 27 October | 19.3 | 80.7 | - | 100.0 | - |
| | Shoots 23 March | - | - | - | - | - |
| Refrigerator | Buds 27 October | 24.5 | 75.5 | - | 100.0 | - |
| | Shoots 10 May | 100.0 | 0 | 43.4 | 100.0 | - |
| Deepfreeze | Buds 27 October | 42.9 | 57.1 | - | 100.0 | 46.2 |
| | Shoots 10 May | 0 | 0 | 100.0 | 0 | 100.0 |

Plants of cv. Northern Brewer were raised from cuttings in sterile compost in plastic pots. These plants were grown in a glasshouse and then exposed to natural infection outdoors during the summer, 1971. Examination of these plants on 28 October showed that a large proportion of the buds which were above the soil surface had apparently become infected with powdery mildew. The infection appeared as a white superficial mycelium covering the outer bud scales (Plate 92). A large number of buds showing severe infection, and some which were apparently healthy, were then excised from the plants for examination of internal structures. These were fixed in formalin-acetic-alcohol under vacuum, embedded in paraffin wax, sectioned at 10 µm thickness and finally stained with phenol-acetic-aniline blue. Buds taken from plants overwintered in the glasshouse and outdoors were also used for sectioning.

Median longitudinal sections through infected buds collected in autumn showed mycelium on the surface of the outer bud scales, sometimes with conidiophores bearing conidia (Plate 93 & 94). Mycelium was also within the bud, between the spaces made by the loosely arranged bud scales (Plate 95 & 96). The spaces between the scales could range from three to twenty times the hyphal diameter (Plate 96). Some buds were heavily infected with mycelium concentrated at the apices of the inner bud scales (Plate 97). For comparison, a section through a healthy bud, also in the autumn, is illustrated in Plate 98.

The mycelium between the bud scales appeared to be separated from the scale surface, in most instances, presumably during dehydration and sectioning. Haustoria were present in the cells of the inner bud scales (Plate 99).

During November/December the outer scales ensheathing the buds became suberised and brown in colour (Plate 100). The established fungus which had sporulated freely in the autumn on the outer scales had disappeared. However, the mycelium within the bud scales sometimes persisted throughout the winter.

Plate 92 Appearance of infected dormant buds of pot plants of cv. Northern Brewer in October after being exposed to natural infection during the summer.


92

Plate 93 A median longitudinal section through an infected dormant bud of a pot plant cv. Northern Brewer in the autumn showing the pathogen on the outside of outer bud scales (arrowed). (x 20).

Plate 94 An enlarged view of a portion of the above bud to show sporing mycelium on the surface of the outer bud scales, haustoria (arrowed) within the host cells and hyphal fragments between the bud scales. (x 165).


93

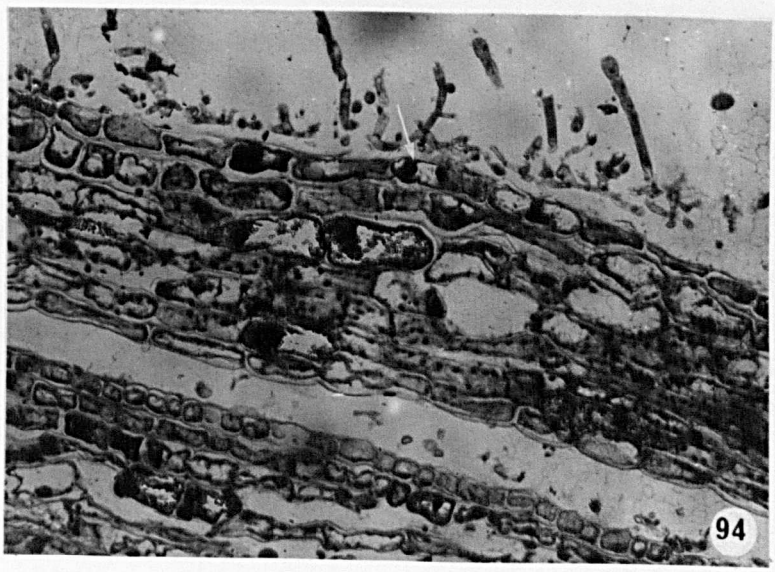

94

Plate 95 A median logitudinal section through an infected dormant bud of a pot plant cv.Northern Brewer, showing mycelium between bud scales (arrowed). Note the loose arrangement of overlapping bud scales at the apex and the accumulation of mycelium at the apex. (x 18).

Plate 96 An enlarged view of a portion of the above bud to show the mycelium between the inner bud scales (arrowed). Note hyphal dimensions in relation to the inter-scale spaces. (x 120).


95

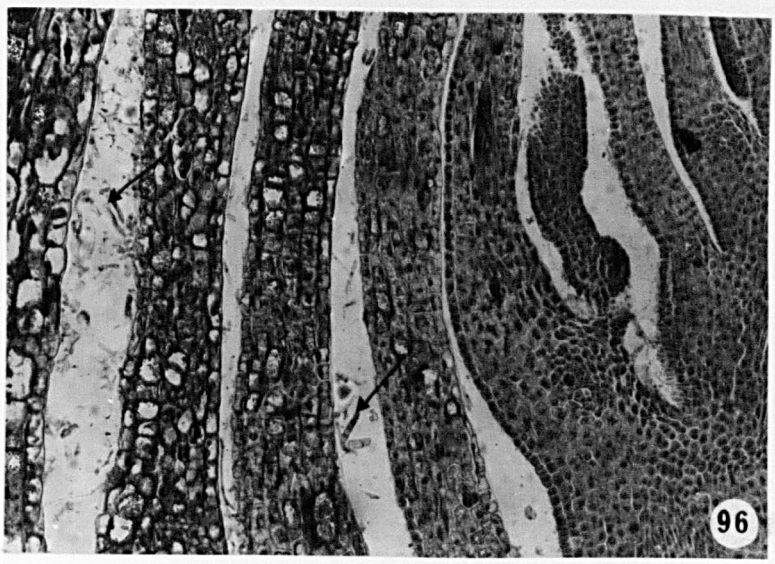
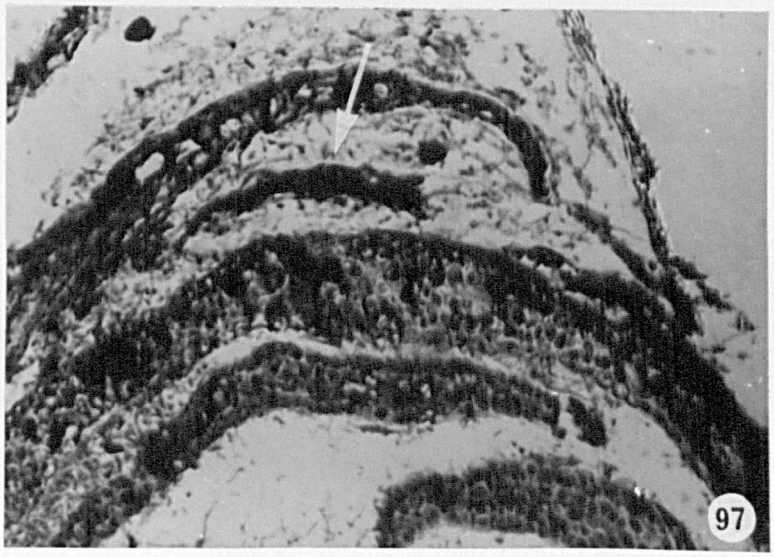

96

Plate 97 Median longitudinal section through the apical region of an infected bud of a pot plant cv. Northern Brewer. Note the concentration of mycelium at the apex of the bud (arrowed). (x 65).

Plate 98 Median longitudinal section through a healthy bud of a pot plant cv. Northern Brewer. (x 40).


97

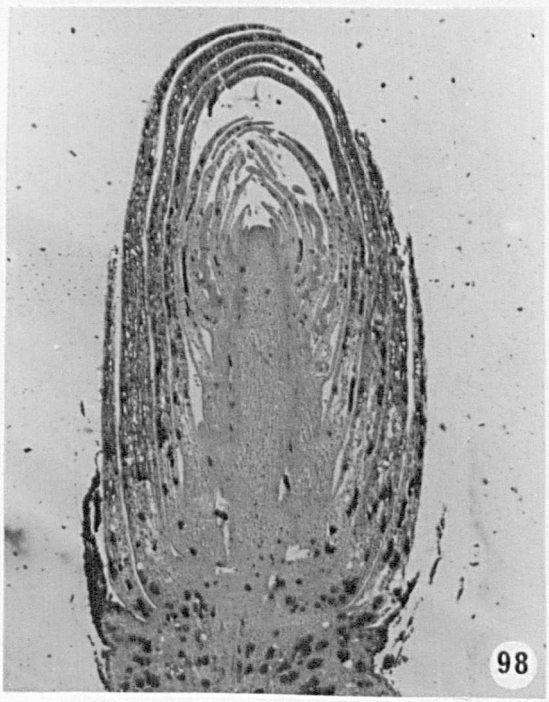
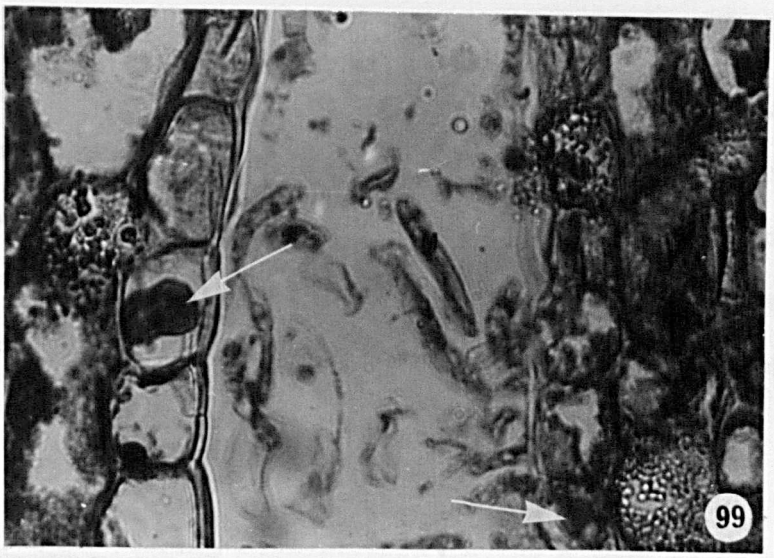
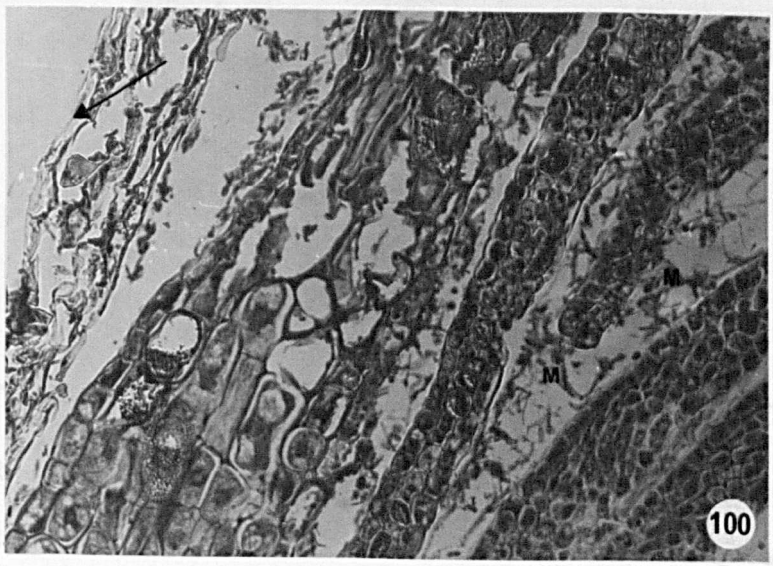

98

Plate 99 Haustoria (arrowed) established in cells of the inner bud scales and mycelium (M) between the scales. There was evidence of some haustoria degenerating. (x 600).

Plate 100 Median longitudinal section of an infected bud of a pot plant cv. Northern Brewer, seen in the spring after overwintering outdoors, to show suberised tissues (arrowed) on the outer scales, mycelium (M) can be seen between the scales. (x 155).


99


M
M
100

(i) Observations on the formation of cleistocarps on buds of pot plants and their histology.

In 1972, hop plants of the cvs./seedlings Northern Brewer, 14/66/50, Wye Challenger and Wye Target were raised from cuttings. Sixteen plants of each were inoculated with 24h old inoculum of the Glasshouse isolate on 22 June. The plants were kept in the glasshouse and a month later were brought outdoors where they were kept until the late summer when the dormant rootstock buds were examined. Large numbers of the dormant buds of only the susceptible cvs./seedlings, 14/66/50 and Northern Brewer, bore cleistocarps (Table 36).

Table 36. Dormant buds bearing cleistocarps in the autumn of four cultivars/seedlings

| cv./seedling | Total number of buds | Total number of buds with cleistocarps | % Buds with cleistocarps |
|---|---|---|---|
| 14/66/50 | 16 | 13 | 81.3 |
| Northern Brewer | 16 | 10 | 62.5 |
| Wye Challenger | 12 | 0 | 0 |
| Wye Target | 14 | 0 | 0 |

Some of the buds bearing cleistocarps were removed from the plants, cv. Northern Brewer, on 27 October and others on 15 March. They were fixed, stored in formalin-acetic-alcohol and later sectioned and prepared for examination.

In the autumn, cleistocarps occurred in abundance on dormant buds (Plate 101). Sometimes cleistocarps were confined to the outer surfaces of the outermost scales (Plate 102); otherwise they were found well inside the bud, between the inner bud scales (Plate 103). The cleistocarps of various stages of maturity were seen in buds sampled on 15 March (Plates 104-106). Most cleistocarps were small and immature and ascospores were not well differentiated.

Plate 101 Infected dormant buds of a pot plant cv. Northern Brower in the autumn showing dense cleistocarps (arrowed).


101

Plate 102 Median longitudinal section of an infected bud in autumn to show cleistocarps (arrowed) only external to the outer bud scales. Note the closely enveloped bud scales at the apex. (x 20).

Plate 103 Median longitudinal section through an infected bud in autumn showing cleistocarps both external to and well within the bud scales. Note the loosely arranged bud scales at the apex. (x 25).


103

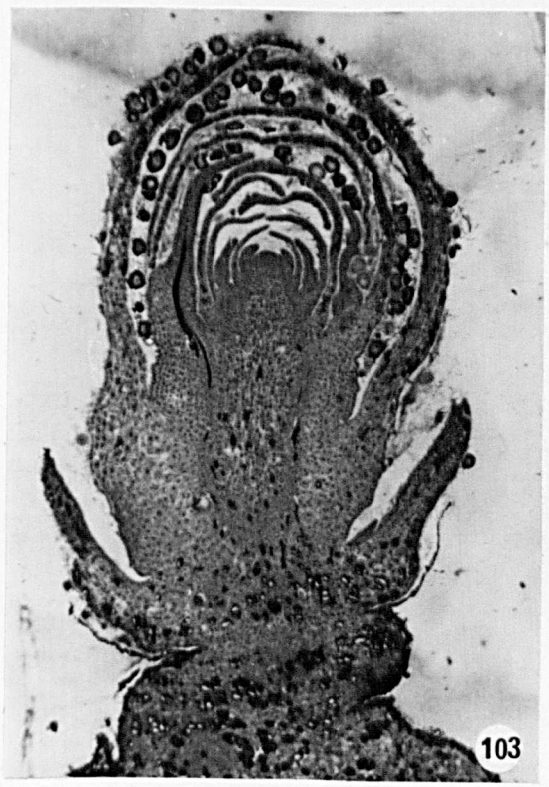

103

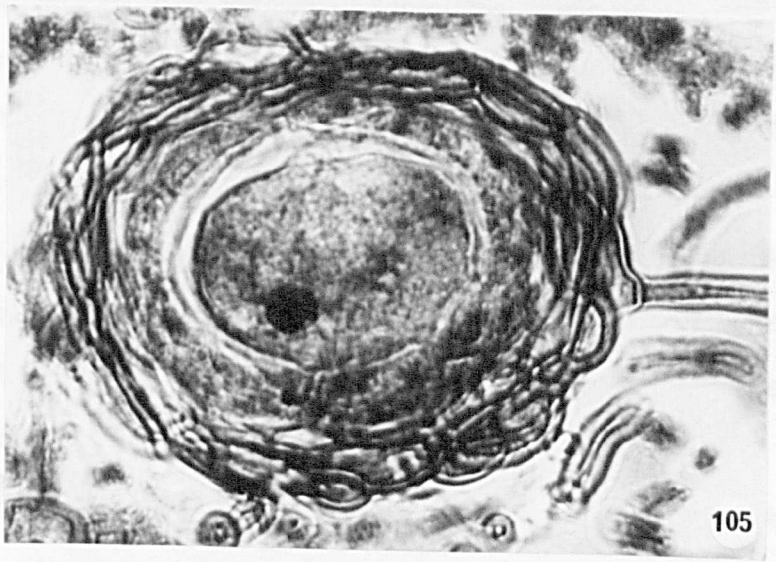
Plate 104 Enlarged view of cleistocarps embedded in dense mycelium occupying the area inbetween the scales of a bud sampled in autumn. (x 115).

Plate 105 Section through an immature cleistocarp within a bud showing thick cleistocarp wall surrounding undifferentiated cytoplasm of the ascus in which the nucleus can be seen. (x 105).

Plate 106 Section through a cleistocarp within a bud showing an advanced stage of ascospore differentiation. (x 900).



104



105



106

3. Overwintering on alternate hosts

(a) Inoculation of a number of different plant species with S. humuli

Seeds of thirty eight species of hop garden weeds, most of which have been recorded by Salmon (1900) as hosts of a fungus morphologically identical with S. humuli, were obtained from the Weed Research Organisation or from Thompson & Morgan (Ipswich) Ltd.

They were sown in seed compost either on 1 June 1972 or on 4 April 1973. Plants of the cultivated and Japanese hops and strawberry (Fragaria chiloensis) were raised in compost using strap cuts and runners, respectively. All plants were grown in the disease-free atmosphere of the air-cooled glasshouse. Disks, 13mm diameter, or pieces, 13mm long, were cut from leaves as soon as they were large enough. They were placed on moist foam in a Perspex compartment box and inoculated with 24h old inoculum using the settling towers described earlier. The leaf material was then incubated (18°C) for 7 days after which they were decolourized, stained and mounted in Euparal.

The fungus failed to produce sproring colonies on all the weed species examined and on strawberry, though on most of these conidia germinated and produced a germ tube. Only H. lupulus and H. japonicus were actively parasitised.

Leaves of the following seven weed species infected with a powdery mildew fungus were collected from the Silks hop garden, Wye, on 12 October 1970: Capsella bursa pastoris, Heracleum sphondylium, Lamium parpureum, Plantago sp., Polygonum aviculare, Senecio vulgaris, Taraxacum officinale. The leaves incubated for two days in a moist chamber and then five 13mm hop leaf discs, cv. Northern Brewer, were inoculated with conidia from each of the weeds. The leaf disks were examined after an incubation period of 7 days in the growthroom, but they failed to support sporulating colonies.

List of weed species tested as hosts of *S. humuli*

Agropyron repens
Anthemis nobilis
Arabis alpina
Atriplex patula
Calendula officinalis
Capsella bursa pastoris
Centaurea cyanus
Chenopodium album
Cirsium arvense
Convolulus arvensis
Crepis rubra
Dimorphotheca aurantiaca
Fumaria officinalis
Gallium aparine
Geranium dissectum
Heracleum sphondylium
Impatiens balsamina
Ipomea hybrida
Lamium purpureum
Lanaria vulgaris
Linaria alpina
Morina longifolia
Plantago major
Poa annua
Polygonum persicaria
Potentilla reptans
Ranunculus repens
Rosa rubrifolia
Scabiosa caucasica
Senecio vulgaris
Spiraea ulmaria
Stellaria media
Taraxacum officinale
Tragopogon pratensis
Urtica urens

## DISCUSSION

Cleistocarps of S. humuli on hop debris lying on the surface of a hop garden can survive the conditions prevailing in England during the winter to discharge viable and infective ascospores the following spring. This coincides with the seasonal production of the first leaves on hop plants in the field. In the experiments on ascospore release, however, there was no indication that the ascospores released by cleistocarps were infective although they showed a limited amount of germ tube growth on glass slides and on leaves. This failure to demonstrate ascospore infection is certainly not due to an insufficiency of mature cleistocarps, since ascospores were trapped on glass slides in April and May in three years' tests. It is more likely due to the wrong conditions being provided for ascospore infection, since nothing is known about this aspect of hop mildew biology. Wheeler, Cook, Fagan, Chandarasrivongs, Khan and Patra Achavasmit (1973) showed that ascospores of E. cichoracearum were very nonspecific in their requirements and could germinate within a wide range of temperature, 3-30°C, and relative humidity, 32-100%. A more detailed investigation of ascospore behaviour on hop leaves is required but made difficult by the irregularity with which cleistocarps of S. humuli are formed and dehisce.

Successful overwintering of cleistocarps has been demonstrated conclusively for only four other powdery mildews, E. graminis on wheat (Salmon, 1903 ), E. cichoracearum on Arctium lappa (Cook & Wheeler, 1967) and S. mors-uvae on blackcurrant (Jordan, 1966, 1967; Merriman & Wheeler, 1968), and on gooseberry (Jackson, 1971). Other workers have obtained cleistocarp dehiscence and infection with ascospores but not with cleistocarps produced naturally or outdoors (e.g. Salmon, 1903 , 1904a, 1905 and Powers & Moseman, 1957). Salmon (1907) collected hop leaves bearing cleistocarps in January and found

that, after they had been kept dry for 3-4 months in the laboratory, they would dehisce if placed in water. Blodgett (1915) found that cleistocarps of hops on leaves and cones, when collected from the field in April, were ready to dehisce immediately. He also found that some cleistocarps, taken from infected glasshouse plants in mid-November and placed outdoors, released ascospores in February under laboratory conditions. Dehiscence took place more rapidly when the cleistocarps left outdoors until April and some were found to dehisce even in late May. In the present investigation, however, naturally-overwintered cleistocarps never dehisced and released ascospores before April. From this time cleistocarps dehisced both under natural conditions in the field and in the laboratory after they had been kept during the winter in various temperature regimes. It was also found that, under laboratory conditions, cleistocarp dehiscence and ascospore release both increased with increasing temperature up to 18°C when maximum response was obtained. A further increase of temperature to 24°C resulted in a decline in cleistocarp dehiscence. Blodgett (1915) assumed that ascospores were released readily from the infected hop leaves in April due to warmer weather conditions. It is possible that temperature may be a principal factor controlling the discharge of ascospores from cleistocarps in the field. Cutter & Wheeler (1968) were of a similar opinion concerning behaviour of cleistocarps of E. cichoracearum and Smith (1971) suggested that cleistocarps of Uncinula salicis on Willow and Microsphaera penicillata on Aspen were preserved intact until the following spring due to the low temperatures of the extreme conditions in Canada. The failure of cleistocarps of S. humuli to dehisce before April in the present experiments strongly suggests that a dormancy period has to elapse before they are capable of releasing ascospores. Similar observations for air-dried cleistocarps of E. cichoracearum kept at constant temperature or outdoors made Wheeler et al (1973) suggest a dormancy period

was required during which further maturation of the ascus and ascospores occurred.

The present studies showed that cleistocarp samples kept air-dried in different temperature regimes liberated ascospores after April upon wetting, indicating that free water is necessary for dehiscence. The ability of ascospores of E. cichoracearum to germinate in free water has been demonstrated by Wheeler et al (1973) and similar conditions may be needed by S. humuli ascospores. If cleistocarps dehisce only after a period of sudden wetness, which could be either by rain or dew, then the ascospores released would then find ideal conditions for germination and infection.

A chemical degradation by enzymes secreted by micro-organisms may bring about a weakening of the cleistocarp wall. Jackson (1972) showed that about 35-40% of the mature cell wall preparations of cleistocarps and secondary mycelium of S. mors-uvae consisted of chitin and glucan. He also demonstrated the association of certain bacteria and actinomycetes with overwintered cleistocarps. However, he failed to observe a significant increase in hydrolysis of mature walls when he used bacterial chitinase and fungal β-(1-3) glycanase.

The finding of cleistocarps within the digestive tracts of earthworms suggested that soil fauna could reduce the amount of cleistocarps available for spring infection. Jackson (1972) often found a psocid, Ectopsoccis briggsi among cleistocarps of S. mors-uvae. Collembola sp. were found in abundance among the samples of infected hops being overwintered outdoors although there was no evidence that these insects had fed on cleistocarps. They are known to feed on mycelium and spores of other fungi (Jackson, 1972). Jackson gave some circumstantial evidence for an association of actinomycetes with cleistocarp degeneration also he found Cephalosporium spp. on the walls of cleistocarps from the field.

The exact role of soil fauna and flora in the persistence of cleistocarps in a hop garden and their contribution to dehiscence is not clear and much work needs to be undertaken to obtain more detailed information.

When cleistocarps of S. humuli were kept dry at 18°C during the winter a number of them dehisced when wetted. Histo chemical studies of cleistocarps of S. mors-uvae by Jackson (1972) revealed the presence of abundant glycogen in the ascus at ascospore differentiation. It is possible that these reserve food materials were converted to a form of reducing sugar during the interim period before they dehisced in the spring. If such a process occurs, then the rapid intake of water by the ascus might result in changes of osmotic potential; when sufficient pressure to overcome the strength of cleistocarp wall was built up dehiscence could occur. Less dehiscence of S. humuli cleistocarps overwintered outdoors occurred than of those kept dry at 18°C during the winter probably due to various processes involving microorganisms and physical factors, such as weathering.

The proportion of mature and nearly mature cleistocarps in the samples examined during the winter reached a peak twice, in November and in March/April. This twin peak is not easy to explain. The first peak was probably due to completion of the process of differentiation after the cleistocarps were no longer associated with living host tissue, at hop picking. The second peak indicated that some cleistocarps, which had remained immature throughout the winter, had now matured. There was a concomitant decline in the proportions of some immature cleistocarps in the samples at this time. On the other hand some degeneration of the contents of hitherto mature cleistocarps was also indicated in April/May. It is interesting that a similar double peak in seasonal cleistocarp maturity was recorded by Price (1970) in S. pannosa who suggested a two phase differentiation process was occurring.

Cleistocarps collected in hop samples in October during three years experiments exhibit varying degrees of maturity. A large proportion were immature, and remained so even after the overwintering period, due mainly to their late formation in infected hop tissue. Several factors have been suggested for late cleistocarp formation, for example, low vitality of host plants (Dash, 1912), host variety (Laibach, 1930), onset of host senescence (Schnathorst, 1959), low night temperatures (Itoi, Nakayama & Kubomura, 1962) and sugar content of tissue (Jackson 1972). Smith (1970) suggested that lateness or irregularity in cleistocarp formation in E. polygoni in the field was due to the absence of necessary mating types and it is possible that S. humuli is heterothallic, though there is no evidence for this as yet. Heterothallism has been proved for only a relatively small number of powdery mildews; E. cichoracearum on sunflower (Yarwood, 1935) and lettuce (Schnathorst, 1959), E. graminis (Hiura & Tomoda, 1959) and wheat (Powers & Moseman, 1957), S. fuliginea on Taraxacum officinale (Homma, 1937) and E. polygoni for several hosts including pea and lupins (Smith, 1970). Homothallism has been demonstrated of E. graminis (Cherewick, 1944), S. fuliginea on T. officinale (Homma, 1933).

The time in a growing season when cleistocarps form may be an important factor influencing their capacity to release ascospores in spring. Salmon (1914a) speculated that only cleistocarps formed relatively early (in July and August) survived the winter. Although on hops no critical assessments were done, general observations indicated that cleistocarps formed early had a better chance to overwinter successfully. This was probably because ascospores had had time to differentiate completely by the time infected tissue was detached from the parent plant.

Powdery mildew colonies on test plants in the field were first visible towards the latter part of April 1972 and 1973. Microscopic examination of stained leaves showed that young colonies, invisible to the naked eye, were initiated by ascospores.

The appearance of these colonies before the first spring lesions were found on leaves of permanent plants throughout the hop garden gave convincing supporting evidence that the colonies were initiated by ascospores released from overwintered cleistocarps.

Ascospores were not detected with a Hirst trap except on a single occasion when structures resembling ascospores in size and shape were detected. As already indicated, failure to trap ascospores is most likely because their concentration in the air never reached the threshold concentration for detection by the trap. The possibility cannot be excluded, however, that ascospores may be trapped but undergo a process of degeneration on the trapping surface. Salmon (1907) and Blodgett (1915) found that ascospores were trapped on slides 1.5cm and 5mm respectively above the dehiscing cleistocarps. A low dispersal trajectory may be a further reason for the failure to catch ascospores in the suction trap.

The occurrence of ascospore-initiated disease relative to weather conditions could be studied by regularly sampling leaves of plants exposed to a cleistocarp source over the period of natural cleistocarp dehiscence, rather like Preece (1965) did with American gooseberry mildew. This could be an alternative method of ascospore trapping by artificial surfaces. Information on the dates of first discharge of ascospore release from cleistocarps, the progressive increase in spore output, and the eventual exhaustion of ascospore supply is of great value to the grower in his disease control programme. However, the latter aspect may be difficult to determine with leaf sampling techniques as conidia released from colonies initiated by ascospores may in turn initiate secondary colonies which eventually coalesce.

Cleistocarps were present mainly on cones or leaf debris but could also be lodged between the scales of dormant buds. If these cleistocarps have become mature it is likely that a certain proportion could be a source of infection for developing buds. In this work, however, there was no evidence of this.

Since the secondary development of powdery mildew is likely to be related to the amount of primary mildew in spring, the logical approach to disease control would be to reduce the amount of initial inoculum. Several cultural methods to do this are already recommended to growers such as burning bines with severely infected cones, before cleistocarps become distributed widely over the hop garden floor. The effects of poor field sanitation in this respect were clearly demonstrated in one of the field surveys carried out in this investigation.

Further field experiments carried out in the present investigation showed that mycelium of S. humuli could perennate successfully in dormant buds of hops and cause infection of young shoots in spring. By inducing surface-sterilized buds to burst under conditions which precluded chance infection, and then dissecting buds showing early symptoms, it was possible to trace shoot infection to perennating mycelium, established during the previous season.

For successful perennation the fungus must invade the buds during the summer and, once within the buds, the hyphae must remain dormant until the following spring, when host activity is renewed the fungus spreads to cover the emerging leaves forming primary infected shoots. Overwintering as mycelium in dormant buds is known for the rose powdery mildew fungus S. pannosa var. rosae (Yarwood, 1939; 1944), and the gooseberry mildew fungus, S. mors-uvae (Merriman & Wheeler, 1968).

Entry of mycelium into a bud is likely to occur by its spread from an established infection of the outer bud scales or by conidia lodging between the bud scales. It was demonstrated that bud infection could occur at any time during the summer but more so towards the latter part. Although a very large number of buds of pot plants were observed to be infected on their outer scales only a small proportion of these yielded infected shoots probably because

buds were infected only superficially, without mycelium actually getting into the bud. In these circumstances the mycelium is unlikely to survive the winter period not only because of being exposed directly to severe conditions, but also because the outer bud scales become suberised. Some buds apparently infected in the autumn died during the winter. The fungus probably damages the apical meristem weakening the buds often leading to their death. Frost is probably the most lethal factor likely to kill buds made sensitive to adverse conditions by the presence of the pathogen.

It was interesting to note that none of the infected buds of pot roots kept in the refrigerator over the winter produced infected shoots in the spring. Indeed both infected and healthy buds were killed. Death of infected buds was the major cause of low survival of apple mildew in buds after a cold winter but freezing of the fungus itself may further lower the level of subsequent infection (Covey, 1969). Similarly, Preece (1965) observed that severe winter conditions killed S. mors-uvae in gooseberry buds. Although the results of experiments in the present investigation provide evidence that low temperature conditions were harmful to both infected and healthy buds, further work has to be undertaken especially to determine the effect of the fluctuating temperatures in outdoor conditions on the survival of mycelium within buds.

When growth of shoots commenced in the spring the hyphae lying within the bud scales rapidly infected all the outer tissues of the buds so that when the leaves emerged they were already covered with fungal mycelium. These new shoots emerging from mildewed dormant buds appear to be the main primary source of inoculum in some non-cultivated gardens even though there is usually a low frequency of these shoots in a hop garden. It was noted that secondary spread by conidia arising from 'primary mould' shoots began as early as April. The conidia of S. pannosa germinated and produced sporulating

colonies at 3°C and were able to withstand fairly long periods at 0°C in a moisture-saturated atmosphere (Price, 1970). Similar results have been obtained by other workers with different powdery mildews. e.g. E. cichoracearum (Hashioka, 1937; Deslandes, 1954; Schnathorst, 1960) and S. macularis (Peries, 1962; Jhooty and McKeen, 1965). Hermansen (1966) also reported that conidia of E. graminis remained viable after storage at -12°C to -18°C for nine months. It appears that conidia are able to withstand these low temperatures provided desiccation does not occur. This could be because most of their water is in a bound state (Somers & Horsfall, 1966).

Cross inoculation tests on weed hosts and other plants showed that S. humuli derived from hops is capable of infecting only the cultivated hop and its close relative, the Japanese hop. It may be concluded that weeds do not play any role in harbouring the hop powdery mildew fungus over the winter. However, until inoculation experiments with all the host plants which are known to be infected by a morphologically identical fungus, as listed by Salmon (1900) are done, one can not be certain that S. humuli be specific to the hop.

## SUMMARY

1. In the field overwintered cleistocarps released ascospores to initiate infection in the spring, though under laboratory conditions ascospores germinated but did not infect leaves of a susceptible cultivar.

2. Natural dehiscence of overwintered cleistocarps occurred in the laboratory from April to May. During the same period, naturally dehisced cleistocarps were found among samples of cleistocarps overwintered in the field. These events coincided with the time the first leaves on young hop shoots in the field were produced.

3. Cleistocarps kept in various constant environments or in the field over the winter were capable of releasing ascospores only after April, suggesting they require a dormancy period.

4. Most cleistocarps dehisced and most ascospores were released towards the end of April. There was then a sharp decline in May. Ascospore germination on glass slides followed a similar pattern.

5. Cleistocarps were of a wide range of maturity from collection, in October, and through the winter. There was some suggestion of a further maturation of cleistocarps after their collection in November and again in March, though some degeneration of the contents of mature cleistocarps was also indicated.

6. Free water appeared to be necessary for cleistocarp dehiscence. The greatest number of ascospore was released during the first 24h after wetting.

7. Cleistocarp dehiscence and ascospore release were relatively low at 4° and at 24°C, and greatest at 18°C. In otherwise similar conditions cleistocarp dehiscence was also greater at 18° than in variable temperatures outdoors.

8. Soil fauna, especially earthworms, may remove some cleistocarps from a hop garden during the winter.

9. The pathogen can perennate within dormant rootstock buds and then initiate primary infection of developing shoots ('primary mould') the following spring.

10. On plants exposed to natural infection in the summer, the buds could be infected at any time once they were differentiated. The following spring buds infected late the previous summer gave rise to most infected shoots.

11. For successful survival, the mycelium had to be completely enveloped by the bud scales; myoelium on the surface of buds perished during the winter, probably because of suberisation of the outer bud scales.

12. Some buds which appeared infected in the autumn either perished during the winter or grew away healthy in the spring.

13. Surveys of the incidence of 'primary mould' in hop gardens showed it to be uncommon, generally, though it could occasionally be widespread within a garden.

14. The fungus appeared to be incapable of infecting a wide range of common weeds of a hop garden and therefore is unlikely to overwinter in this way.

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