# Powdery Mildew Infection of Susceptible and Resistant Hosts

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by

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ABSTRACT

The development of Erysiphe pisi on one susceptible and five resistant cultivars of pea in a growth cabinet at 22°C and a 16 h light period was monitored by light microscopy.

Plants of three ages (15, 22 and 33 days) were used and were inoculated near the beginning or end of the light period.

Germination and appressorium frequencies were significantly higher on the susceptible cultivar, during the first 8 h. Thereafter, germination frequencies remained lower on the resistant cultivars but appressorium numbers eventually became similar on all the cultivars. Frequencies of germination and appressoria were usually higher on 22-day than on 15-day plants. Haustoria were recorded on the resistant cultivars but their frequencies were low (due to failure at epidermal penetration). High proportions of haustoria formed were enclosed in abnormal extra-haustorial membranes. The colonies associated with these haustoria were small with few hyphae. The frequencies of haustoria and colonies and the total lengths of hyphae produced were greater after "morning"-inoculation on all the cultivars. Secondary appressoria were produced on all the resistant cultivars and secondary haustoria on only one of these. Sporulation occurred only on the susceptible and three resistant cultivars and was at a reduced level on the latter.

Attempts were made to induce endophytic mycelia of Sphaerotheca fuliginea in cucumber and of Erysiphe graminis f. sp. hordei in barley using heat and ice treatment of

leaves. No endophytic haustoria or mycelia were found but heat treatment of both hosts before inoculation increased the numbers of epidermal haustoria and conidia with elongating secondary hyphae.

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# SECTION 1

## Investigation of the development of *Erysiphe pisi* on susceptible and resistant pea cultivars

## A INTRODUCTION

The powdery mildew disease of Pisum sativum L. is caused by the Ascomycete fungus Erysiphe pisi, DC ex Saint Amans. This fungus, formerly known as Alphitomorpha communis (Walroth, 1819) was called Erysiphe communis (Wallr.) Fries by Fries (1829) and was later renamed Erysiphe martii by Leveille (1851). The name Erysiphe polygoni, adopted by Salmon (1900), was applied to fungi infecting species of Cruciferae, Papilionaceae, Ranunculaceae, Umbelliferae and Polygonaceae. It was later divided into fourteen species (Blumer, 1933), the clasification being based upon minor morphological characters of the cleistothecium. In Blumer's monograph the name Erysiphe polygoni was used only for forms occurring on Polygonaceae and the species infecting Pisum sativum was called Erysiphe pisi. In a further monograph on species of Erysiphaceae (Blumer, 1967) Erysiphe polygoni sensu Salmon was classified as a section named Linkomyces. This section consisted of Erysiphe ulmaniae, Erysiphe communis, Erysiphe heraclei and Erysiphe martii, each with three or four forms, this classification also being based on the characters of the cleistothecial appendages. Erysiphe pisi belongs to the sub-section Erysiphe ulmaniae.

Extensive investigations have been made, using a susceptible pea cultivar infected with Erysiphe pisi, of the ultrastructure of the haustorial complex (Gil, 1976, Gil and Gay, 1977) and of the physiology of the mature transport system (Gil, 1976; Gil and Gay, 1977; Manners, 1979; Manners and Gay, 1978 a and b; 1980; Spencer-Phillips

and Gay, 1980; 1981). However, little work has been done to characterize the developmental stages of Erysiphe pisi on pea (Smith, 1969). Furthermore, although other hosts resistant to mildew development have been described (Erysiphe graminis on cereals - Carver and Carr, 1977, 1978 & 1980, Cherewick, 1944; Corner, 1935; Edwards, 1975; Ellingboe, 1972; Graf-Marin, 1934; Hyde and Calhoun, 1975; Jorgensen and Mortensen, 1977; Lupton, 1956; Martin and Ellingboe, 1978; Masri and Ellingboe, 1966b; McCoy and Ellingboe, 1966; Slesinski and Ellingboe, 1969; 1970; 1971; Stanbridge et al, 1971; and Erysiphe polygoni on red clover - Smith, 1938, Stavely and Hanson, 1966b; Stavely et al, 1969) no detailed study has been made of mildew infection of resistant pea cultivars at the cellular or ultrastructural level. It is not known how host resistance is expressed nor the stage of the infection process at which it operates. Therefore, the purpose of this investigation was to make a comparative study of the effect of susceptible and resistant cultivars on the infection process by Erysiphe pisi. This necessitated a detailed investigation of the stages involved in the developmental sequence.

Previous work has shown resistance to be expressed at different stages in the infection process and the results of these investigations were used as a guide to the possible mechanisms of resistance which could operate in pea cultivars resistant to Erysiphe pisi. Powdery mildew appressoria may be incapable of effecting penetration of the cuticle or epidermal cell wall (Bushnell and Bergquist, 1975, Corner, 1935, Hyde and Colhoun, 1975, Lupton, 1956, Masri and

Ellingboe, 1966b). Papillae, which are localized appositions on the inner surfaces of plant walls, produced in response to the presence of or penetration by a pathogenic agent (Aist, 1976), may prevent the formation of haustoria by surrounding the penetration peg after it has breached the epidermal wall (Aist and Israel, 1977). Penetration of a preformed papilla may be incomplete (Aist and Israel, 1976, 1977, Bushnell and Bergquist, 1975, Carver and Carr, 1977, Edwards and Allen, 1970, Johnson et al, 1979, Sherwood and Vance, 1976, Vance and Sherwood, 1976) or, when the papilla is successfully penetrated, it may enlarge, or a secondary papilla may form to encase the penetration peg (Aist and Israel, 1976). Haustorial initials may be encased by a papilla in both powdery mildew (Aist and Israel, 1976, 1977, Neger, 1923) and rust (Bracker and Littlefield, 1973, Heath, 1971, Heath and Heath, 1971) infections.

If haustoria are initiated, the haustorial complex may fail to develop normally. A defective extra-haustorial membrane (Stavely et al, 1969), an haustorial body without fully developed lobes (Benada, 1970, Stavely et al, 1969) or a small haustorium (Carver and Carr, 1978a, Lupton, 1956, Mence and Hildebrandt, 1966, Stavely et al, 1969) may result. Degeneration of the haustorial complex may occur in a necrotic or non-necrotic host cell (Bushnell, 1972, Bushnell and Bergquist, 1975, Ellingboe, 1972, Heath, 1971, 1972, 1974, Heath and Heath, 1971, Hyde and Colhoun, 1975, Lupton, 1956, Masri and Ellingboe, 1966b, Stavely and Hanson, 1966b).

Necrosis of host cells may occur during (Maclean and Tommerup, 1979) or after (Maclean et al, 1974, Maclean and

Tommerup, 1979) penetration by downy mildews. Post-penetration necrosis has also been reported for powdery mildews (Bushnell, 1981, Edwards, 1975, Hyde and Colhoun, 1975, Johnson et al, 1979, Smith, 1938, Stavely and Hanson, 1966b) and rusts (Heath, 1971, 1972, 1974, 1981, Heath and Heath, 1971). Also, lignification of host cell walls may limit fungal growth in downy mildews (Ohguchi and Asada, 1975).

The genetic basis of host resistance to powdery mildews has also been investigated. It may be of the single gene type (Ellingboe, 1972, Lin and Edwards, 1974), where a host cultivar possesses a particular gene which controls the resistance to a particular race of pathogen (the "gene - for - gene" hypothesis - Flor, 1956), or of the adult - plant type (Graf-Marin, 1934, Shaner, 1973), which is not race specific. Little is known about the genetic basis of resistance of pea to Erysiphe pisi. Resistance has been shown to be controlled by a single recessive gene, $er_1$, in Peruvian cultivars (Harland, 1948) and in cultivar Stratagem (Pierce, 1948). Other investigations also showed resistance to be controlled by a single recessive genes (Cousin, 1965). Later work suggested that resistance, confined to the leaves of Peruvian cultivars, was controlled by a second recessive factor, $er_2$, (Heringa et al, 1969), and was related to tissue age (Cousin, 1965, Heringa et al, 1969).

In studying the basis of resistance of barley and wheat cultivars to Erysiphe graminis f.sp hordei and f. sp tritici, respectively, Ellingboe (1972) recorded the fungal development with genetically defined material, noting the stages in the infection process at which resistance genes

operated. In this investigation a similar approach was adopted, except that the genetic constitutions of the test cultivars of pea were unknown. A suitable model of the developmental sequence of a powdery mildew fungus, upon which to base the investigation of infection by Erysiphe pisi, was required. Most research into powdery mildew development has been done using Erysiphe graminis f sp hordei and f sp tritici on cultivars of barley and wheat, respectively, and these investigations provided a basis for the present work. The study of the development of Erysiphe pisi in this investigation, which involved a chronological and quantitative assessment of the developmental sequence on susceptible and resistant cultivars, was based on a characterization of the infection process into nine stages (Masri, 1965, Nair, 1962): (1) germination of conidia, (2) production of "club-shaped" appressorial initials, (3) maturation of appressoria, (4) penetration of the cuticle and epidermal cells, (5) formation of haustoria, (6) formation of secondary hyphal initials (7) elongation of secondary hyphae, (8) initiation of additional infections and (9) sporulation. This sequence was selected because it covered every possible stage at which resistance genes might operate and was chosen in preference to the criteria used in the investigation of Erysiphe pisi development on pea (Smith, 1969). Although the latter work provided detailed information about the production of secondary hyphae, the timing of the events was not monitored nor was any precise quantitative assessment made of proportions of conidia reaching various stages.

Investigations of development necessitate standardized environmental conditions because RH, light and temperature have been found to influence powdery mildew development on various hosts (Schnathorst, 1965, Yarwood et al, 1954, Yarwood, 1957). Most work has been done on the effect of environmental conditions on the development of Erysiphe graminis f sp hordei and f sp tritici on barley and wheat, respectively, (Carver and Williams, 1980, Cherewick, 1944, Hirata, 1967, Masri, 1965, Masri and Ellingboe, 1963, 1966a, Manners and Hossain, 1963, Nair, 1962, Nair and Ellingboe, 1962, 1965) and of Erysiphe polygoni on red clover (Brodie and Neuf eld, 1942, Stavely and Hanson, 1966a, Yarwood, 1932, 1936a and b). The RH and temperature, found to be optimal for development of Erysiphe polygoni in the above investigations, were selected for use in this study of Erysiphe pisi development because the two fungi are closely related and may be assumed to react similarly to environmental conditions. However, the effect of photoperiod on the development of Erysiphe pisi was investigated to find the light conditions providing optimal fungal growth because most available information for a powdery mildew had been obtained with Erysiphe graminis. There it was found that germination and initiation of appressoria were insensitive to light intensity (Nair and Ellingboe, 1962, 1965). Maturation of appressoria was optimal with low light intensities (25-50 ft-c) but was inhibited by high intensities (7200 ft-c) and darkness (Masri and Ellingboe, 1963, 1966a).

In summary, the purpose of this present investigation is to establish the stages in the developmental sequence of

*Erysiphe pisi* at which resistance might be expressed and hence attempt to identify the mechanism of resistance operating in the five test pea cultivars.

## B MATERIALS AND METHODS

### I HOST PLANTS AND INOCULUM

#### 1 Organisms and Sources

The cultivars of Pisum sativum L. provided by Mr. Brian Snoad of the John Innes Institute were as follows:-

Cv. BS10 - susceptible to infection by Erysiphe pisi

Cv. BS12
Cv. JI1047
Cv. JI1048
Cv. JI1049
Cv. JI1050
} - resistant to infection by Erysiphe pisi

The source of the pathogen, Erysiphe pisi, DC.Ex Saint Amans, was Plant Protection Ltd., Imperial Chemical Industries, Jealott's Hill.

#### 2 Cultivation of host plants

Seeds were germinated in the dark for 24 h in a propagation chamber comprising damp filter paper in a petri-dish. The seeds were then planted in vermiculite in 5 inch pots, two seeds per pot, and were maintained in a growth room or a growth cabinet (Fisons, Fitotron 60093/TTL) with a 16 h daylength at a mean temperature of 22°C and a relative humidity of 65%. Illumination was provided by a combination of fluorescent and tungsten lights amounting to 22 watts $m^{-2}$ at the base of the plants, and 37 watts $m^{-2}$ 45 cm above the base of the plant.

3 Maintenance of inoculum

Cultures of Erysiphe pisi were maintained on pea plants, cv. Onward, in a greenhouse with a 16 h daylength at an approximate temperature of 21°C and a relative humidity of 65 to 70%. Illumination was provided by a combination of natural, fluorescent and tungsten lights resulting in a light intensity of at least 25 watts $m^{-2}$.

4 Inoculation of host plants

4.1 Production of young conidia

An inoculum consisting of young mature conidia was obtained by blowing old conidia from infected leaves, and allowing the plants to continue growing in light for a further 6 h before the time specified for inoculation. This procedure was followed by Yarwood (1936), who showed that the fungus required 6 h of light for maturation of conidia before abscission.

4.2 Inoculation procedure

Plants were inoculated with young conidia by shaking infected leaves over them.

5 Time of inoculation

5.1 "Morning"-inoculation

This indicates inoculation at 0900 h of the plants growing under the following light régime: 16 h light period, 0900 h - 0100 h, and an 8 h dark period, 0100 h - 0900 h. The conidia were thus exposed to conditions of continuous light in the early stages of the infection process.

5.2 "Evening"-inoculation

This indicates inoculation at 0900 h of plants growing under the following light régime: 16 h light period, 1900 h - 1100 h, and on 8 h dark period, 1100 h - 1900 h. The conidia were thus exposed to a 2 h light period, followed by a period of darkness, in the early stages of the infection process.

## II PREPARATION FOR LIGHT MICROSCOPY

### 1 Stains and mounting media

1.1 Lactophenol

This mounting medium was prepared by mixing the following components : phenol, 100g; distilled water, 100ml; lactic acid, 100ml; glycerol, 100ml (25% aq). It was used in the preparation of stains and as a clearing agent.

1.2 Cotton blue (Aniline blue, ws)

A solution of cotton blue (0.1%, w/v) was prepared by dissolving 1g of water soluble aniline blue in 200 ml of distilled water. The solution was thoroughly mixed with 100ml of lactophenol which was prepared as described above (II 1.1). Samples were stained for 30-60 min in watch-glasses and staining was accelerated by warming gently over a flame. The excess stain was washed out using lactophenol and the samples were mounted in 50% glycerine (w/v) for observation.

1.3 Lactophenol methyl blue

A solution of methyl blue (0.06%, w/v) was prepared by dissolving 0.2g of the dye in lactophenol which was prepared as described above (II 1.1) (Hyde and Colhoun,

1975). The stain was stored in a dark bottle in a cool place and used in the same way as cotton blue (II 1.2).

1.4 Acid fuchsin

A solution of acid fuchsin (0.1%, w/v) was prepared by dissolving 0.1g of the dye in 100ml of lactophenol which was prepared as described above (II 1.1). The stain was used in the same way as cotton blue (II 1.2).

1.5 Potassium permanganate

A solution of potassium permanganate (0.2%, w/v) was prepared by dissolving 0.2g in 100ml of distilled water. Samples were stained for 30 min and, after excess stain was washed out with water, they were mounted in a drop of water for observation.

1.6 Alcian blue

A solutionof alcian blue (20%, w/v) was prepared by dissolving 20g in 100ml of distilled water. Samples were stained for 24 h and after excess stain was washed out with water they were mounted in a drop of water for observation.

1.7 Calcofluor White M2R New (American Cyanamid Co.)

The fluorescent brightener, calcofluor White M2R New, was used for observation of haustoria by staining infected epidermal strips. A solution of calcofluor (1%, w/v) was made up by dissolving 1g in 150ml of 0.1M phosphate buffer. Samples were floated in calcofluor in a petri dish for 10 min. The tissue was mounted on a glass slide in a drop of the buffer and was observed using an HBO-200 mercury vapour lamp in a Reichert Zetopan light microscope. E2 exciter (transmission range 280-410nm, peak transmission 360nm) and Sp3 barrier (transmission above

480nm) filters were used.

1.8 4-acetamido-4'-isothiocyanato-stilbene-2,2' disulphonic acid (SITS)

A 100μM solution of SITS stain was prepared in 0.05M phosphate buffer at pH 7. Epidermal strips were stained for 5 min in a petri dish and were mounted in a drop of buffer for observation. Observations were made using an HBO-50 mercury vapour lamp in Reichert-Jung light microscope. Rhodamine (exciter 546nm, peak transmission 560nm, and barrier O9 590, transmitting below 590nm) and FITC, Acridine Orange (exciter B912 and KV 418, peak transmission 500nm, and barrier O9515, transmitting below 515nm) filters were used.

1.9 Aniline blue

A solution of water soluble aniline blue (0.05%, w/v) was made up by dissolving 0.05g in 100ml of 0.05M phosphate buffer at pH 8.5. Epidermal strips were stained for 30 min in a glass petri dish and mounted in buffer for observation using an HBO-200 mercury vapour lamp and a Reichert Zetopan light microscope. E3 exciter (transmission range 325-480nm, peak transmission 412nm) and Sp3 barrier (transmission above 480nm) filters were used.

1. 10 Neutral red

A solution of neutral red (0.1% w/v) was prepared by dissolving 0.1g in 100ml of water. Epidermal strips were stained for 20 min, the excess stain was washed out and the strips were mounted in water for observation.

2 Clearing agents

2.1 Chloral hydrate

A solution of chloral hydrate (250% w/v) was prepared by dissolving 5g in 2ml of distilled water. Samples were treated for 24 h.

2.2 Lactic acid : chloroform : methanol

This clearing agent was prepared by mixing the components 1 : 1 : 1 by volume. Samples were treated for 24 h.

2.3 Formaldehyde : glacial acetic acid : ethanol

This clearing agent was prepared by mixing the components 1 : 9 : 9 by volume. Samples were treated for 30 min.

2.4 Methanol

Samples were placed in the test tubes containing methanol and were heated in a boiling water bath for 10 to 15 min. The test tubes were then cooled to room temperature.

2.5 Lactophenol

Samples were treated for 24 h in lactophenol prepared as described above (II 1.2).

2.6 Lactophenol methyl blue

Leaf samples were covered with the stain, prepared as described above (II 1.3), in test tubes and were heated in a boiling water bath for 1 min. After cooling the excess stain was removed with lactophenol.

2.7 Methanol - lactophenol cotton blue

This clearing agent was made up by mixing the components in the proportions 2:1 by volume. Samples were covered with the stain in test tubes and heated in a boiling water bath for 1 min. After cooling the excess stain was

removed with lactophenol.

2.8 Alcoholic lactophenol cotton blue (Ref. Shipton and Brown, 1962).

Samples were heated in alcoholic lactophenol cotton blue, made up by mixing 95% alcohol with lactophenol cotton blue (II 1.2) in the proportions 2:1 by volume. After heating, the samples were left to sink and were then re-heated for 30 sec. The tissue was left for 48 h at 25°C after which it was removed and rinsed in water. Further clearing was achieved by leaving the samples in chloral hydrate, made up as described previously (II 2.1) for 30 to 35 min.

3 Adhesive preparations used for observation of conidia and fungal structures after detachment from leaf surfaces.

3.1 Sellotape strips

Leaves were cut off inoculated plants and transferred to a flat surface with the inoculated adaxial surface facing upwards. A strip of sellotape was gently pressed onto the leaf and was then peeled away leaving the conidia. The strip was stuck onto a glass slide and stained for 15 min by adding a few drops of stain. Excess stain was drained off using a narrow piece of filter paper.

3.2 Necoloidine films (BDH Chemicals Ltd.)

Leaves were cut off inoculated plants and transferred to a flat surface with the inoculated adaxial surface facing upwards. A drop of Necoloidine was placed on the leaf and quickly spread over the entire surface. The film

was allowed to dry for 1 min when it was peeled off and stained. Care was taken not to leave the film on the leaf for periods longer than 1 min as the edges tended to curl making its removal difficult. After staining for 5 min and removing the excess stain with lactophenol, made up as described above (II 1.1), the film was mounted on a glass slide in lactophenol. A coverslip was placed over the film and the edges were sealed with clear varnish.

4 Other methods of examining epidermal infections.

4.1 Epidermal strips

Epidermal strips were removed from turgid leaves and stems using fine forceps. Larger epidermal strips were obtained by stripping from the centreof a vein.

4.2 Excised epidermis

Areas of infected epidermis were exposed by cutting away the uninoculated abaxial epidermis and the underlying mesophyll. The leaf was sampled by punching out a disc with a cork borer (13mm diameter) from a turgid leaf. The disc was placed with the uninfected abaxial surface facing upwards onto a modified hand microtome. The modification comprised a disc of sintered polythene, attached to a millipore filter support grid, attached, in turn, to a cylindical brass cylinder inserted into the microtome. The cylinder was connected to a vacuum pump and a flask in the connection collected water (for light microscopy) or buffer (for electron microscopy) which was continuously applied to the leaf disc during the cutting process. This enabled it to be held flat and firmly attached to the polythene sinter

and also lubricated the knife. A safety razor, with the safety guard removed to fully expose the edge of the blade, was used to cut away the uninfected leaf tissues.

The cutting was attempted in two ways to find the best way of removing the uninoculated leaf tissue and avoiding damage of the adaxial epidermis. In the first cutting technique the platform bearing the leaf disc was set at a certain height and the angle of the blade to the leaf, beginning at 45°, was altered to accomodate thinning of the disc. Cuts were made in short smooth strokes in the direction of the main vein when one was present. The second cutting technique involved the incorporation of a metal support in the modified safety razor. The support rested directly on the microtome platform and enabled the blade to be kept at a constant angle to the leaf surface. When areas of the adaxial epidermis became visible cutting was finished and the leaf disc was removed from the suction apparatus. In order to remove extraneous debris it was washed in distilled water or buffer.

## 5 Quantitative assessment of colonies

### 5.1 Total length of hyphae

Measurements were made from micrographs of colonies stained on sellotape strips (II 3.1) or Necoloidine films (II 3.2). The images from the negative were projected onto a matrix digitizor linked to a Hewlett Packard calculator which had been programmed to record measured lengths. Measurements of the mycelium produced per conidium were made by moving the sensor along individual

hyphae and the values were printed by the apparatus. Individual lengths were added together to give the mycelium produced per conidium. The data were finally expressed in mm after making the necessary corrections for the magnifications made during microphotography and projection on to the digitizor matrix.

## III STATISTICAL ANALYSIS

The statistical test used in this work was Student's t test for comparing means of small samples where $n_1$ or $n_2$ (sample size) is less than 30 (Bailey, 1968).

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s \frac{1}{n_1} + \frac{1}{n_2}}$$

where the covariance, $S^2$ =

$$\frac{1}{n_1 + n_2 - 2} \{ \Sigma_1 \frac{x^2 - (\Sigma_1 x)^2}{n_1} + \Sigma_2 \, x^2 \frac{(\Sigma_2 x)^2}{n_2} \}$$

Degrees of freedom = $n_1 + n_2 - 2$

Mean value = $\bar{x}$

## IV PREPARATION FOR ELECTRON MICROSCOPY

### 1 Scanning electron microscopy

Leaf samples were prepared for scanning electron microscopy by air-drying. Critical point drying was not carried out because the lipidic solvents used in this technique would have modified the cuticular waxes possibly altering their appearance. The leaves were placed in a dessicator containing silica gel and small weights were placed on

their perimeters to prevent curling of the edges during drying. The leaves were left to dry for 48 h at room temperature. Small pieces, approximately 3 $mm^2$, were then cut from each leaf using a razor blade, selecting areas both close to and distant from the leaf midrib. These pieces were mounted, adaxial epidermis uppermost, on metal stubs using Durofix adhesive; a little Silverdag (Agar Aids) was placed on the edges of the samples to improve electrical conductivity during microscopy. The stubs bearing the leaf pieces were coated with gold for 4 min using a Sputter coater (Polaron Ltd.). They were then examined using a Philips 500 scanning electron microscopy.

2 Transmission electron microscopy

2.1 Preparation of whole leaf pieces

Infected leaves were fixed overnight in glutaraldehyde (2.5%, v/v) in cacodylate buffer (pH 7.0, 0.1M) after cutting into pieces, approximately 2 $mm^2$, under the glutaraldehyde mixture. They were then washed three times in buffer and post-fixed in osmium tetroxide (1%, w/v) for 3 h. The samples were dehydrated in an alcohol series and then prepared for embedding by successive treatment with absolute alcohol: epoxy propane (1:1 by volume) for 30 min, twice with epoxy propane for 15 min and finally with epoxy propane: Epon overnight. After three resin changes the samples were polymerized in Epon (1A : 1B) (Luft, 1961) for two days at 60°C. Thin transverse sections were cut with a LKB Ultratome 1 and a diamond knife and stained with aqueous uranyl acetate (2%, w/v) for 15 min at 60°C

and lead (Reynolds, 1963) for 10 min at room temperature. The preparations were examined with AE1 EM6B and Philips 301 G electron microscopes.

2.2 Preparation of excised epidermes

The adaxial uninfected epidermis was stripped from infected leaves under a glutaraldehyde (2.5% v/v) : formaldehyde (4%, v/v) mixture in cacodylate buffer (pH 7.0, 0.1M) and leaf discs, 13 mm in diameter, were punched out, also under fixative. The leaf discs were fixed in glutaraldehyde (2.5%, v/v) for 3 h at room temperature. After fixation the adaxial epidermis was exposed by the excision method (BII 4.2), washing constantly with buffer during cutting. The excised samples were fixed overnight in glutaraldehyde (2.5%, v/v) containing alcian blue (20%, w/v) (BII 1.6) after cutting into small pieces, approximately 5 $mm^2$, leaving some mesophyll around the exposed area to support the epidermal tissue. After fixation the samples were washed three times with buffer and post-fixed with osmium tetroxide (1%, w/v) for $1\frac{1}{4}$ h. The epidermes were prepared for embedding as described above (BIV 2.1). The samples were embedded in Epon (1A : 1B) (Luft, 1961) in shallow plastic petri dishes precoated with three layers to carbon to facilitate removal of the resin discs after polymerization. A very thin layer of resin was used and the samples were weighed down with 100 mesh copper grids to minimize movement of the epidermis during polymerization. The resin was heated for 20 min at $60^{o}C$ before putting in the samples, to allow for movement of the Epon, which may have caused undulations in the epidermes. After poly-

merization for 2 days longitudinal sections were cut, stained and examined, as described above (BIV 2.1).

2.3 Preparation of epidermes exposed by grinding

Infected leaves were cut into pieces, approximately 7 mm x 2 mm, and prepared for embedding as described above (BIV 2.1). They were embedded in rubber flat moulds in Epon (1A : 1B) (Luft, 1961). After polymerization the specimen blocks were removed and the abaxial epidermis and part of the mesophyll were ground away using silicon carbide paper (Wetodry, Awt, 220 mesh; Minnesota Mining and Manufacturing - 3M, St. Paul, MN 55 101). The blocks were mounted in immersion oil on a microscope slide. The top of the block was covered with immersion oil and a coverslip. The oil eliminated most of the optical interference due to the scratches caused by grinding and allowed the location of haustoria. After location of haustoria the oil was removed with detergent in preparation for trimming and sectioning, which was carried out as described above (BIV 2.1). This technique is based on one developed by Zeyen and Bushnell (1981).

# C EXPERIMENTAL PROCEDURES AND RESULTS

## I EVALUATION OF CLEARING AND STAINING METHODS

### 1 Introduction

The purpose of these investigations was to select stains, to be used in future experiments, which enabled the most efficient observation and enumeration of conidia with associated structures and haustorial complexes of Erysiphe pisi on leaves of Pisum sativum. The fungal structures were examined with and without staining on uncleared leaves, on leaves cleared by several different methods and on stripped and excised epidermes.

### 2 Clearing of leaves

#### 2.1 Experimental

Infected leaves from cultivar BS10 were cleared, as described above, in chloral hydrate (B11 2.1), lactic acid : chloroform : methanol (B11 2.2), formaldehyde : glacial acetic acid : ethanol (B11 2.3), methanol (B11 2.4), lactophenol (B11 2.5), lactophenol methyl blue (B11 2.6), methanol : lactophenol cotton blue (B11 2.7) and alcoholic lactophenol cotton blue (B11 2.8). The leaf samples were than examined by bright field microscopy and note was made of the efficiency of the clearing methods, of the ease of observing conidia on the leaf surface, of haustorial complexes in the adaxial and abaxial epidermes and of the epidermal cell contents.

#### 2.2 Results

The most efficient clearing agent was chloral

hydrate as it removed all the chlorophyll from the mesophyll cells whereas some remained after treatment for 24 h with lactophenol : chloroform : methanol. Treatment with boiling methanol also resulted in well cleared leaves but some conidia on the leaf surface may possibly have been removed by turbulence during heating in methanol. Clearing in formaldehyde : acetic acid and in lactophenol did not remove all the chlorophyll in the mesophyll. The combination of cotton blue with a clearing agent was moderately successful with all three clearing mixtures but staining was too dense in parts of the leaf, obscuring the haustoria, and not all the conidia were stained. Loss of some ungerminated conidia may have occurred during heating.

### 2.3 Conclusions

Chloral hydrate was selected as the most efficient clearing agent for subsequent experiments in preference to methanol because, as noted above, the latter removed some conidia and was too time consuming when samples from different cultivars had to be treated one at a time.

## 3 Staining of conidia with germ tubes and appressoria on infected leaves

### 3.1 Experimental

Infected leaves from cultivar BS10 were cleared in chloral hydrate (B11 2.0) and then stained in lactophenol cotton blue (B11 1.2), lactophenol methyl blue (B11 1.3) and acid fuchsin in lactophenol (B11 1.4). Uncleared leaves were similarly stained. The conidia were then observed using bright field microscopy.

### 3.2 Results

Staining of conidia on uncleared leaves resulted in very dense staining of the mesophyll, even after removal of excess stain, and it obscured some of the conidia. This occurred to a lesser extent on cleared leaves. All three stains resulted in good staining of the conidia. A disadvantage of using acid fuchsin in lactophenol was that much of the stain was washed out during removal of the excess stain. Lactophenol methyl blue, on the other hand, was not washed out enough from the mesophyll and resulted in a very dark preparation.

### 3.3 Conclusions

Lactophenol cotton blue was selected for staining conidia with associated structures in subsequent experiments. The results showed that neither cleared nor uncleared leaves were suitable for detailed observations of the structures because of the obscuring effect of the background staining. It was decided to try using adhesive preparations for observing conidia and associated structures after detachment from leaf surfaces.

## 4 Adhesive preparation methods for observation of conidia and associated structures after detachment from leaf surfaces

### 4.1 Experimental

The surface fungal structures on infected leaves of cultivar BS10 were detached with sellotape strips (B11 3.1) and Necoloidine films (B11 3.2) and then stained in lactophenol cotton blue (B11 1.2). The preparations were then

observed using bright field microscopy.

4.2 Results

Both adhesive preparations were suitable for monitoring conidia because the washing subsequent to staining removed all excess stain from the artificial films, facilitating observations and enumerations. Checking of the leaf samples ensured that all the structures were removed from the leaf surface.

4.3 Conclusions

It was decided that both adhesive preparations could be used in subsequent experiments. However, sellotape strips were not suitable when the preparations were to be kept for times longer than 7 days, because the stain faded and the conidia became shrunken and sometimes distorted.

## 5 Staining of haustorial complexes in cleared and uncleared leaves

5.1 Experimental

Infected leaves from cultivar BS10 were cleared in chloral hydrate (B11 2.1) and stained in acid fuchsin in lactophenol (B11 1.4), potassium permanganate (B11 1.5) and alcian blue (B11 1.6). Also, leaves from which surface fungal structures had been removed were similarly cleared and stained. The haustoria were then observed using bright field microscopy.

5.2 Results

None of the preparations was satisfactory because the staining of the mesophyll made identification of haustoria and examination, in any detail, extremely difficult. The

presence of conidia on the leaf surface obscured haustoria in the epidermis. Alcian blue stained the extra-haustorial membrane but caused the darkest background staining. Despite background staining, haustoria were well stained with acid fuchsin and potassium permanganate, and both stains showed the neck bands clearly.

### 5.3 Conclusions

The use of cleared leaves for observation of haustoria was not adopted and it was decided to try using leaf samples from which the mesophyll had been removed.

## 6 Staining of haustorial complexes on stripped and excised epidermes

### 6.1

Epidermal preparations were made by stripping (Bll 4.1) and excision (Bll 4.2) using turgid infected leaves of cultivar BS10. These samples were stained in acid fuchsin in lactophenol (Bll 1.4), potassium permanganate (Bll 1.5) and alcian blue (Bll 1.6) and haustorial complexes observed using bright field microscopy.

### 6.2 Results

Haustorial complexes could be seen very clearly in epidermal strips whereas with the excised epidermes debris and sections of mesophyll obscured some epidermal cells even after washing the samples. The time for preparation of epidermal strips was shorter than for excising the epidermes, and almost all the epidermis could be removed from the leaf; this was not possible with the cutting technique as only leaf discs could be used and all the

epidermis in these was not always exposed. The stains were all satisfactory for staining the extra-haustorial membrane, and acid fuchsin in lactophenol and potassium permanganate showed the neck bands clearly.

### 6.3 Conclusions

The use of epidermal strips was selected for observation of haustorial complexes in subsequent experiments. Acid fuchsin was selected from the three stains tested because it did not wash out of the haustorial complexes as much as potassium permanganate. It was preferable to alcian blue because it stained the neck bands.

# II PRELIMINARY INVESTIGATIONS OF GERMINATION OF CONIDIA AND FORMATION OF APPRESSORIA BY ERYSIPHE PISI

## 1 Introduction

The purpose of this investigation was to discover the effect of time of inoculation with respect to the photocycle on germination of conidia and formation of appressoria on susceptible and resistant cultivars. These experiments were carried out because data for pea mildew were not available and it was necessary for designing subsequent experiments. The investigation consisted of two parts, the first being concerned with the earlier stages (up to 24 h) of the primary infection process, the second covering the period between 24 h and 72 h after inoculation; this was to see if any trends emerging in the earlier stages persisted for a longer period.

## 2 Experimental

Two batches of the six test cultivars, using 22-day old plants, were inoculated at 0900 h and incubated in growth rooms with photoperiods which were of the same duration i.e. 16 h light, 8 h dark, but not synchronous. In one, illumination continued for 6 h after inoculation and was followed by an 8 h dark period - "morning"- inoculation (BI 5.1). In the other - "evening"- inoculation (BI 5.2) the plants were illuminated for 2 h and this was followed by the 8 h dark and 16 h light periods. Samples of fungal structures were prepared for examination using sello-tape strips (Bll 3.1) with five replicates in each case, and were stained in lactophenol cotton blue (Bll 1.2). Samples

were taken 2, 4, 6 and 24 h after inoculation and counts were made of conidia producing a germ tube only and those producing mature appressoria. The data, expressed as a percentage of total and germinated conidia are recorded in Tables 1 and 2, respectively. The mean percentages of conidia germinating and of germinated conidia producing appressoria were compared using Student's t test (BII 1) and the results are also recorded in the Tables. The experiment was repeated taking samples after 24, 48 and 72 h. The data, expressed as above, and results of statistical analysis of germination frequencies and of the frequency of appressoria are recorded in Tables 3 and 4.

## 3 Results

### 3.1 Effect of time of inoculation on germination of conidia and formation of appressoria during the early stages of primary infection (up to 24 h after inoculation).

The time of inoculation with respect to the photocycle affected germination on different cultivars differently (Table 1) (Fig. 1). Significant variation (usually $p = 0.001$) occurred in the first 2 h but this could not be attributed to the photocycle since both "morning"- and "evening"-inoculated plants were incubated in the light throughout this period. By 24 h, significantly higher (usually $p = 0.001$) germination frequencies occurred under one or other of the treatments; larger proportions of conidia germinated after "morning"- inoculation of BS10, BS12, JI1047 and JI1049 and "evening"- inoculation of JI1048 and JI1050. At the intermediate times, germination on the last

two cultivars was consistently higher but with the others there were some variations. The proportion of conidia germinating after 24 h varied from 33.8% (JI1049, "evening"-) to 85.8% (BS10, "morning"-).

Appressorium formation was first observed after 4 h on all cultivars except BS10, BS12 and JI1048 after "morning"-inoculation, and on all except cultivars BS12 after "evening"-inoculation (Table 1) (Fig. 2). The delays in formation of appressoria are correlated with delays in germination which occurred on these cultivars. In order to distinguish between the dependance of appresorium formation on time of inoculation and germination, the frequency of appressorium formation is expressed as a proportion of germinated conidia (Table 2). This shows that formation of appresoria was significantly more frequent after "evening"- inoculation on cultivars BS10, JI1047, JI1048 and JI1050 after 4 and 6 h, on cultivar JI1049 after 6 h and on all cultivars except JI1047 after 24 h.

Thus, frequencies of appressorium formation and germination were correlated only for cultivars JI1047, JI1048 and JI1050. The results indicate that appressorium formation is influenced by the photocycle during first 24 h of the primary infection process. Variations in germination, however, cannot be solely attributed to the photocycle.

3.2 Effect of time of inoculation on germination of conidia and formation of appresoria during the later period of primary infection (24 to 72 h after inoculation).

The time of inoculation with respect to the photocycle

affected germination of conidia on different cultivars differently up to 48 h after inoculation (Table 3) (Fig. 1). At 24 h, significantly higher germination ($p = 0.01$) occurred in the "morning"- inoculated batch on cultivars BS10, JI1048 and JI1050 and in the "evening"- inoculated plants of JI1049 but on cultivars BS12 and JI1047 the difference between "morning"- and "evening"- inoculation was insignificant. By 48 h the difference remained insignificant on cultivars BS12 and JI1047 and became so on JI1049; and "morning"- inoculation of JI1050 still gave higher germination. However, higher frequencies of conidial germination were recorded on BS10 and JI1048 after "evening"- inoculation but by 72 h none of the cultivars showed a significant difference ($p = 0.05$) resulting from time of inoculation.

The frequency of appressorium formation was significantly higher on cultivars BS10, JI1049 and JI1050 in the "evening"- inoculated batch after 24 h, higher on JI1048 in the "morning"- inoculated batch but on BS12 and JI1047 the effect of inoculation time was insignificant (Table 4) (Fig. 2). At 24 h the proportions of appressoria remained higher on "evening"- inoculated JI1049 and "morning"- inoculated JI1048, and were now significantly higher on JI1047 in the "evening"- inoculated batch. At this sampling time with BS10, BS12 and JI1050 the effect of inoculation time was insignificant. By 72 h there was no significant difference in the frequency of appresorium formation between "morning"- and "evening"- inoculated batches on any of the cultivars except JI1047 where it was

higher on the "morning"- inoculated plants.

Frequencies of germination and appressorium formation were correlated for all the cultivars at 24 and 72 h but only for BS12 and JI1048 at 48 h. Variations in germination can be attributed to a number of factors possibly including photocycle, although the last cause cannot definitely apply to all the cultivars. The results indicate that formation of appressoria is affected on some cultivars up to 24 h after inoculation after which the effect of inoculation time gradually becomes insignificant.

Table 1 Germination of conidia and formation of appressoria on the oldest leaves of plants 22 days after sowing following "morning"- and "evening"-inoculation. Samples were taken 2, 4, 6 and 24 h after inoculation. The results are expressed as a % of total conidia and probability values (p) obtained from Student's t test indicate the significance of inoculation time on germination.

| CULTIVAR | Sampling time (h) | GERMINATION (total) "Morning"-inoculation | GERMINATION (total) "Evening"-inoculation | P | GERM TUBE ONLY "Morning"-inoculation | GERM TUBE ONLY "Evening" inoculation | MATURE APPRESSORIUM "Morning"-inoculation | MATURE APPRESSORIUM "Evening"-inoculation |
|---|---|---|---|---|---|---|---|---|
| BS10 | 2 | 0 | 15.2 | 0.001 | 0 | 15.2 | 0 | 0 |
| | 4 | 13.7 | 36.3 | 0.001 | 13.7 | 17.6 | 0 | 18.7 |
| | 6 | 62.7 | 56.4 | 0.05 | 39.8 | 23.4 | 22.9 | 33.0 |
| | 24 | 85.8 | 70.4 | 0.001 | 30.8 | 11.7 | 55.0 | 58.7 |
| BS12 | 2 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| | 4 | 20.5 | 0 | 0.001 | 20.5 | 0 | 0 | 0 |
| | 6 | 22.8 | 27.2 | 0.3 | 17.3 | 22.3 | 5.3 | 4.9 |
| | 24 | 66.7 | 58.7 | 0.001 | 38.0 | 12.9 | 28.7 | 45.8 |
| JI1047 | 2 | 0 | 0 | | 0 | 0 | 0 | 0 |
| | 4 | 39.0 | 40.8 | 0.3 | 34.0 | 26.4 | 5.0 | 14.4 |
| | 6 | 45.0 | 39.1 | 0.001 | 33.8 | 13.3 | 11.2 | 25.8 |
| | 24 | 53.6 | 48.0 | 0.01 | 11.6 | 9.6 | 42.0 | 38.4 |
| JI1048 | 2 | 0 | 12.1 | 0.001 | 0 | 12.1 | 0 | 0 |
| | 4 | 10.7 | 18.4 | 0.01 | 10.7 | 16.8 | 0 | 1.6 |
| | 6 | 32.0 | 34.2 | 0.05 | 21.8 | 6.6 | 10.2 | 27.6 |
| | 24 | 46.2 | 74.5 | 0.001 | 18.1 | 1.3 | 28.1 | 71.9 |
| JI1049 | 2 | 22.8 | 0 | 0.001 | 22.8 | 0 | 0 | 0 |
| | 4 | 29.2 | 32.2 | 0.1 | 23.6 | 25.6 | 5.6 | 6.6 |
| | 6 | 42.5 | 43.6 | 0.4 | 30.7 | 24.0 | 11.8 | 19.6 |
| | 24 | 44.4 | 33.8 | 0.001 | 11.1 | 4.6 | 33.3 | 29.2 |
| JI1050 | 2 | 11.7 | 15.2 | 0.02 | 11.7 | 15.2 | 0 | 0 |
| | 4 | 29.4 | 34.1 | 0.01 | 19.3 | 18.2 | 10.1 | 15.9 |
| | 6 | 34.5 | 50.0 | 0.001 | 19.5 | 11.7 | 14.9 | 38.3 |
| | 24 | 36.2 | 46.4 | 0.001 | 17.4 | 9.8 | 18.8 | 36.3 |

Fig. 1 Comparisons of germination frequencies on six 22-day cultivars of pea following "morning"- and "evening"- inoculation.

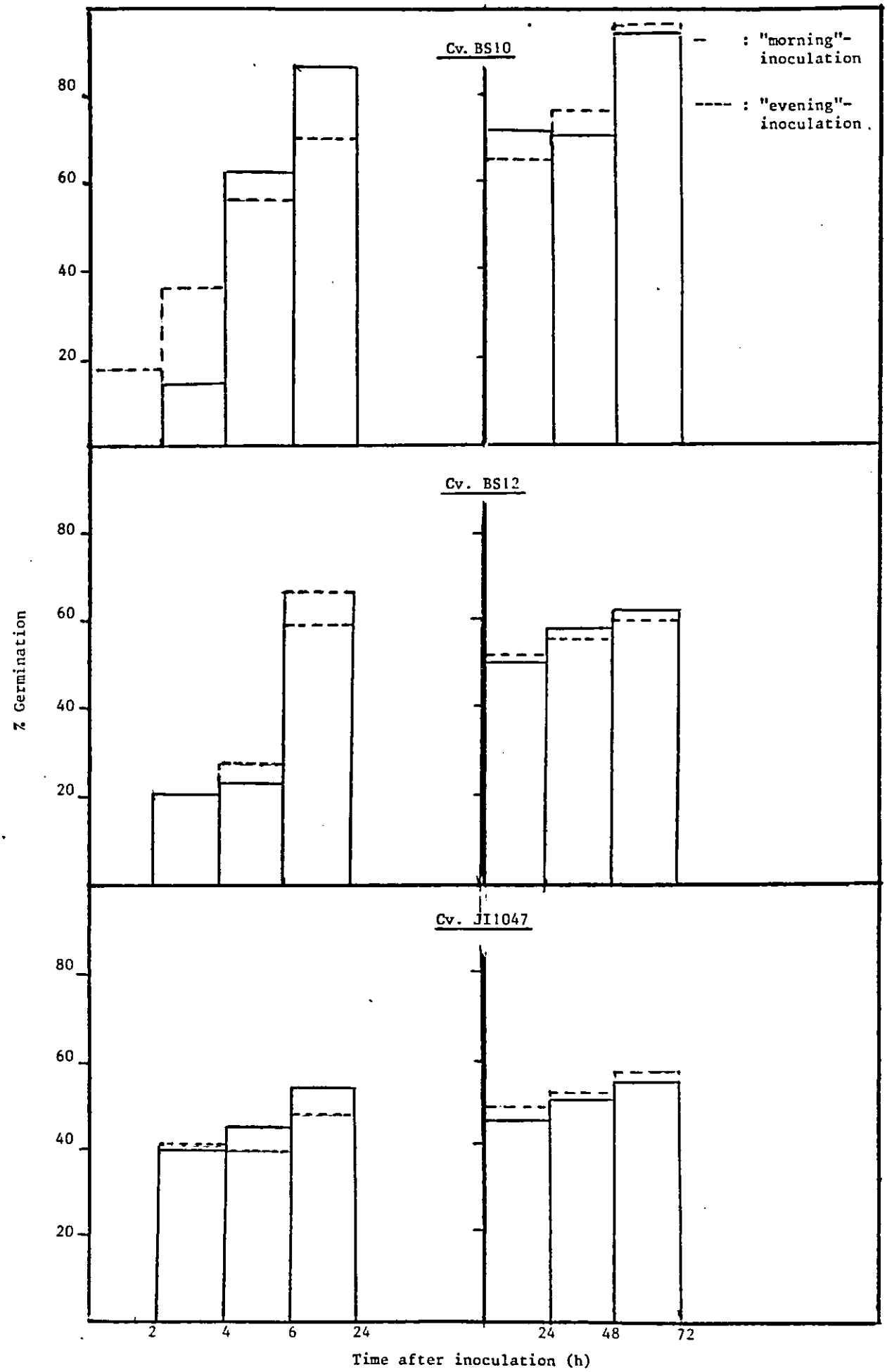


Fig. 1 (Continued)

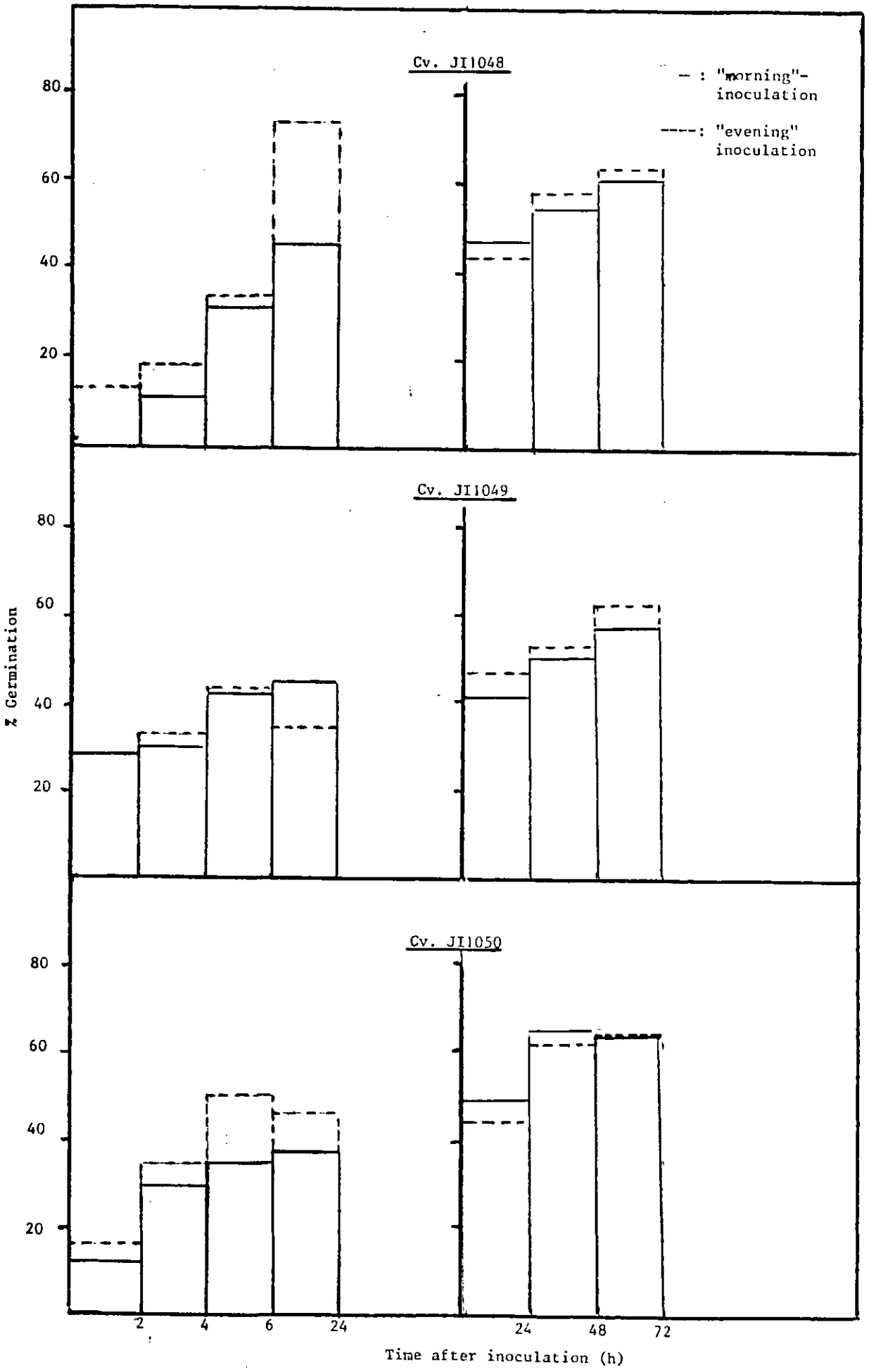


Table 2 Germination of conidia and formation of appressoria on the oldest leaves of plants 22 days after sowing following "morning"- and "evening"- inoculation. Samples were taken 2, 4, 6 and 24 h after inoculation. The results are expressed as a% of germinated conidia and probability values (p) obtained from Student's t test indicate the significance of inoculation time on appressorium formation.

| CULTIVAR | Sampling time (h) | GERM TUBE ONLY | | MATURE APPRESORIUM | | |
|---|---|---|---|---|---|---|
| | | "Morning"-inoculation | "Evening"-inoculation | "Morning"-inoculation | "Evening"-inoculation | P |
| BS10 | 2 | 0 | 100 | 0 | 0 | |
| | 4 | 100 | 48.4 | 0 | 51.6 | 0.001 |
| | 6 | 61.6 | 41.8 | 38.4 | 58.2 | 0.001 |
| | 24 | 34.5 | 16.8 | 65.5 | 83.2 | 0.001 |
| BS12 | 2 | 0 | 0 | 0 | 0 | |
| | 4 | 100 | 0 | 0 | 0 | |
| | 6 | 76.8 | 82.8 | 23.2 | 17.2 | 0.3 |
| | 24 | 57.1 | 21.9 | 42.9 | 78.1 | 0.001 |
| JI1047 | 2 | 0 | 0 | 0 | 0 | |
| | 4 | 86.8 | 64.1 | 13.2 | 35.9 | 0.001 |
| | 6 | 75.0 | 33.5 | 25.0 | 66.5 | 0.001 |
| | 24 | 21.5 | 20.1 | 78.5 | 79.9 | 0.6 |
| JI1048 | 2 | 0 | 100 | 0 | 0 | |
| | 4 | 100 | 91.1 | 0 | 8.9 | 0.01 |
| | 6 | 68.6 | 19.5 | 31.4 | 80.5 | 0.001 |
| | 24 | 39.2 | 1.7 | 60.8 | 98.3 | 0.001 |
| JI1049 | 2 | 100 | 0 | 0 | 0 | |
| | 4 | 81.4 | 80.1 | 18.6 | 19.9 | 0.8 |
| | 6 | 76.1 | 54.9 | 23.9 | 45.1 | 0.01 |
| | 24 | 25.1 | 14.1 | 74.9 | 85.9 | 0.001 |
| JI1050 | 2 | 100 | 100 | 0 | 0 | |
| | 4 | 64.9 | 53.2 | 35.1 | 46.8 | 0.02 |
| | 6 | 58.3 | 23.4 | 41.7 | 76.6 | 0.001 |
| | 24 | 47.8 | 21.2 | 52.2 | 78.8 | 0.01 |

Fig. 2 Comparisons of frequencies of mature appressorium formation (expressed as a % of germinated conidia) on six 22-day cultivars of pea following "morning"- and "evening"- inoculation.

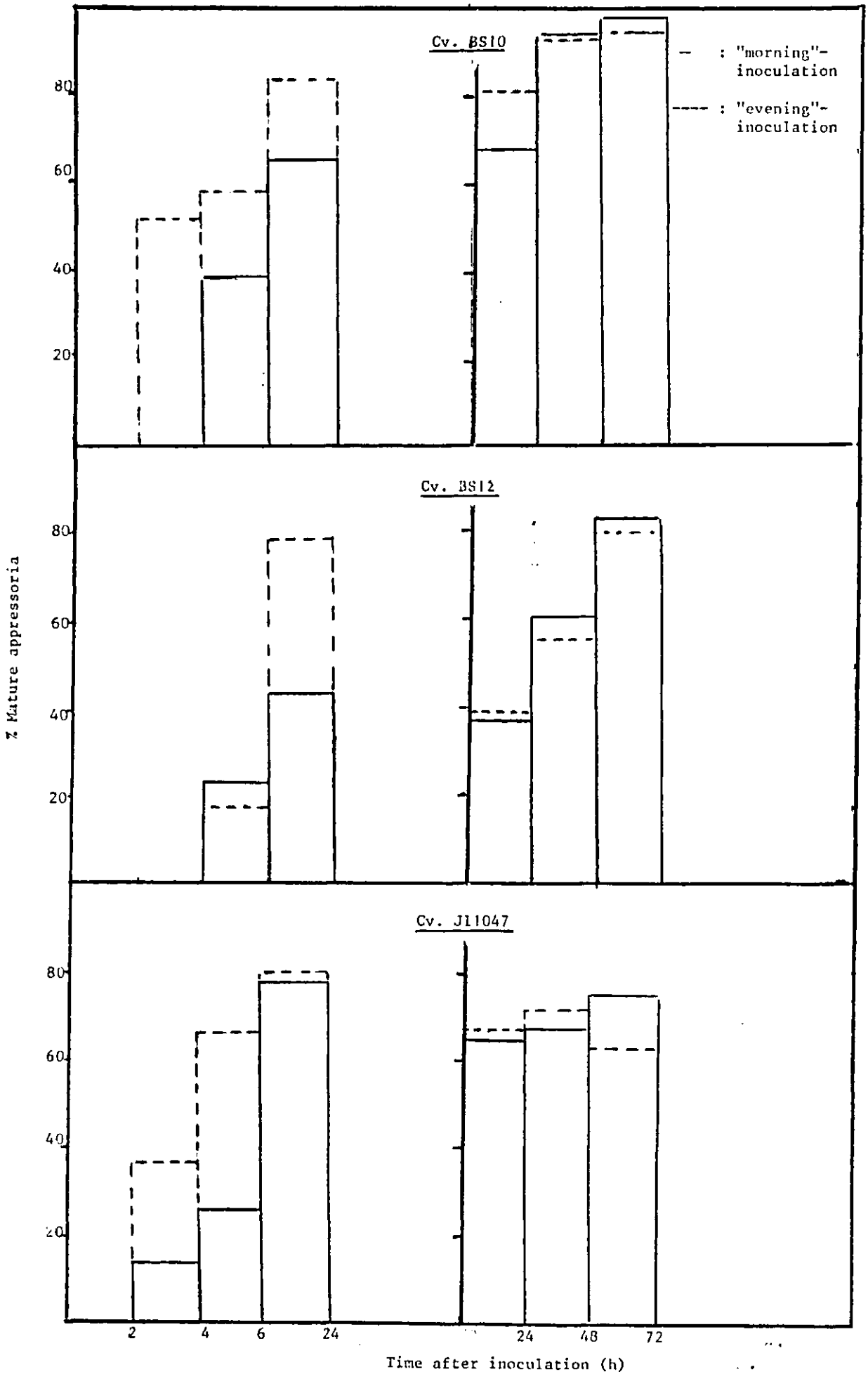


Fig. 2 (Continued)

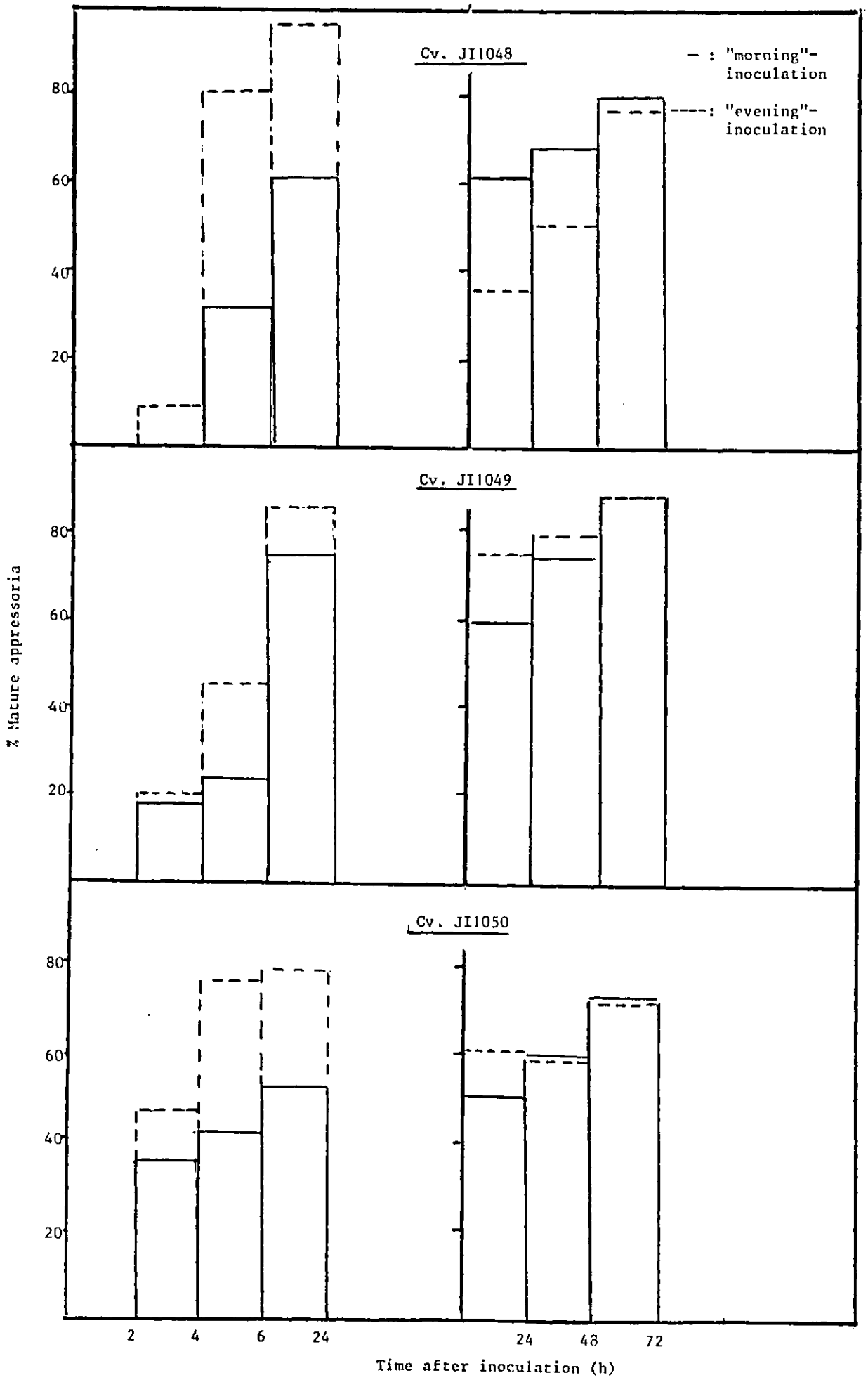


Table 3 Germination of conidia and formation of appressoria on the oldest leaves of plants 22 days after sowing following "morning"- and "evening"- inoculation. Samples were taken 24, 48 and 72 h after inoculation. The results are experssed as a % of total conidia and probability values (p), obtained from Student's t test, indicate the significance of inoculation time on germination.

| CULTIVAR | Sampling time (h) | GERMINATION (total) 'Morning'- inoculation | GERMINATION (total) "Evening"- inoculation | P | GERM TUBE ONLY 'Morning'- inoculation | GERM TUBE ONLY "Evening"- inoculation | MATURE APPRESSORIUM "Morning"- inoculation | MATURE APPRESSORIUM "Evening"- inoculation |
|---|---|---|---|---|---|---|---|---|
| BS10 | 24 | 71.1 | 64.3 | 0.01 | 24.1 | 20.1 | 48.0 | 52.0 |
| | 48 | 70.4 | 76.2 | 0.01 | 5.0 | 0.2 | 65.4 | 70.2 |
| | 72 | 94.2 | 96.2 | 0.05 | 4.4 | 4.1 | 89.9 | 90.1 |
| BS12 | 24 | 49.7 | 52.1 | 0.05 | 31.1 | 29.6 | 18.6 | 20.1 |
| | 48 | 58.9 | 55.8 | 0.05 | 23.3 | 28.0 | 35.6 | 30.9 |
| | 72 | 62.6 | 60.4 | 0.05 | 10.7 | 13.8 | 51.9 | 48.8 |
| JI1047 | 24 | 46.3 | 49.1 | 0.05 | 16.6 | 13.5 | 29.7 | 32.8 |
| | 48 | 49.9 | 52.1 | 0.05 | 16.7 | 12.8 | 33.2 | 37.1 |
| | 72 | 55.5 | 56.8 | 0.05 | 14.0 | 19.5 | 41.5 | 36.0 |
| JI1048 | 24 | 47.0 | 43.1 | 0.01 | 18.2 | 31.8 | 28.8 | 15.2 |
| | 48 | 54.5 | 58.2 | 0.01 | 17.5 | 28.8 | 37.0 | 29.4 |
| | 72 | 60.4 | 62.1 | 0.05 | 12.2 | 15.0 | 48.2 | 47.1 |
| JI1049 | 24 | 40.9 | 47.3 | 0.01 | 16.5 | 11.6 | 24.4 | 35.7 |
| | 48 | 50.0 | 52.1 | 0.05 | 13.1 | 15.0 | 36.9 | 31.1 |
| | 72 | 57.1 | 61.8 | 0.05 | 7.0 | 7.6 | 50.1 | 54.2 |
| JI1050 | 24 | 48.5 | 44.2 | 0.01 | 24.4 | 17.6 | 24.1 | 26.6 |
| | 48 | 66.0 | 62.0 | 0.01 | 27.1 | 25.9 | 38.9 | 36.1 |
| | 72 | 65.1 | 65.0 | 0.05 | 17.5 | 18.1 | 47.6 | 46.9 |

Table 4 Germination of conidia and formation of appressoria on the oldest leaves of plants 22 days after sowing following "morning"- and "evening"- inoculation. Samples were taken 24, 48 and 72 h after inoculation. The results all expressed as a % of germinated conidia and probability values (p) obtained from Student's t test indicate the significance of inoculation time on appresorium formation.

| CULTIVAR | Sampling time (h) | GERM TUBE ONLY | | MATURE APPRESSORIUM | | |
|---|---|---|---|---|---|---|
| | | 'Morning'-inoculation | "Evening"-inoculation | 'Morning'-inoculation | 'Evening'-inoculation | P |
| BS10 | 24 | 33.5 | 19.2 | 66.5 | 80.8 | 0.001 |
| | 48 | 7.1 | 7.9 | 92.9 | 92.1 | 0.05 |
| | 72 | 4.7 | 6.3 | 95.3 | 93.7 | 0.05 |
| BS12 | 24 | 62.6 | 61.4 | 37.4 | 38.6 | 0.05 |
| | 48 | 39.6 | 44.6 | 60.4 | 55.4 | 0.05 |
| | 72 | 17.1 | 19.2 | 82.9 | 80.8 | 0.05 |
| JI1047 | 24 | 35.9 | 33.2 | 64.1 | 66.8 | 0.05 |
| | 48 | 33.5 | 28.8 | 66.5 | 71.2 | 0.001 |
| | 72 | 25.3 | 36.6 | 74.7 | 63.4 | 0.001 |
| JI1048 | 24 | 38.7 | 64.7 | 61.3 | 35.3 | 0.001 |
| | 48 | 32.1 | 49.5 | 67.9 | 50.5 | 0.001 |
| | 72 | 21.1 | 24.2 | 79.8 | 75.8 | 0.05 |
| JI1049 | 24 | 40.3 | 24.5 | 59.7 | 75.5 | 0.001 |
| | 48 | 26.2 | 40.3 | 73.8 | 79.7 | 0.01 |
| | 72 | 12.3 | 12.3 | 87.7 | 87.7 | 0.05 |
| JI1050 | 24 | 50.3 | 39.8 | 49.7 | 60.2 | 0.001 |
| | 48 | 41.0 | 41.8 | 59.0 | 58.2 | 0.05 |
| | 72 | 26.9 | 27.8 | 73.1 | 72.2 | 0.05 |

# III DEVELOPMENT OF ERYSIPHE PISI ON SUSCEPTIBLE AND RESISTANT CULTIVARS

## a Germination of conidia and formation of appressoria

### 1 Effect of cultivar and age of cultivar

#### 1.1 Introduction

The time of inoculation did not affect the germination of conidia uniformly with different host cultivars (CII 3.1, 3.2). Formation of appressoria, however, was constantly more frequent after "evening"- inoculation on all the cultivars in the first 24 h of the primary infection process. This inoculation time was consequently selected for this experiment which investigated the effect of cultivar and age of cultivar on germination of conidia and formation of appressoria.

#### 1.2 Experimental

Germination of conidia and formation of appressoria were studied on six cultivars of Pisum sativum and the results obtained on the oldest leaves of 15-, 22- and 33-day old plants were compared. After "evening"- inoculation (BI 5,2) the plants were inoculated in a growth room with 16 h light and 8 h dark periods. Samples were taken 2, 4, 6 and 24 h after inoculation. The fungal structures, prepared for observation on sellotape strips (BII 3.1), with five replicates in each case, were stained in lacto-phenol cotton blue (BII 1.2). Counts made of conidia producing a germ tube only and those producing mature appressoria were expressed as a percentage of total and germinated conidia and are recorded in Tables 5 and 6,

respectively. Results of the tests of significance of mean germination and formation of appressoria on susceptible and resistant cultivars, obtained from Student's t test (BII 1), are given in Tables 7 and 8, respectively, and those from comparisons of different age groups in Tables 9 and 10, respectively.

1.3 Results

1.3.1 Effect of susceptible and resistant cultivar

1.3.1.1 15-day old plants

Germination was recorded after 2 h on cultivars BS10 and BS12 whereas a further 2 h delay occurred on the other cultivars (Table 5) (Fig. 3). Germination was significantly higher on BS10 than on all the resistant cultivars except BS12 throughout the experimental period (Table 7). On cultivar BS12 germination was significantly lower than on BS10 at 2 and 4 h but the difference was insignificant at 6 and 24 h.

Appressoria were formed by 4 h on cultivars JI1049 and JI1050, by 6 h on cultivars BS10, BS12 and JI1048, and between 6 and 24 h after inoculation on cultivar JI1047 (Table 6) (Fig. 4). There was no significant difference between the frequencies of appressorium formation on cultivars BS10, BS12 and JI1048 at 6 h (Table 8) but the frequency was significantly higher on BS10 at 24 h. It was significantly higher on cultivar BS10 than on JI1047 throughout the experimental period but showed no significant difference with cultivars BS10, JI1049 and JI1050 at 6 and 24 h and with JI1050 at 4 h.

1.3.1.2 22-day old plants

Germination was recorded at 2 h on cultivars BS10, JI1048 and JI1050, a further 2 h delay occurred on JI1047 and JI1049 and one of 4 h on BS12 (Table 5) (Fig. 3). Significance tests (Table 7) showed the germination frequency to be significantly higher on cultivar BS10 than on BS12 and JI1049 throughout the experimental period. It was significantly lower on cultivar JI1047 at 2, 6 and 24 h, on JI1048 at 4 and 6 h and on JI1050 at 6 and 24 h than on BS10.

Appressoria were produced by 4 h on all cultivars except BS12 where a further 2 h delay occurred (Table 6) (Fig. 4). The frequency of appressorium formation was significantly higher on cultivar BS10 than on BS12 throughout the experimental period (Table 8). Greater variations, however, were seen in comparisons between BS10 and the other resistant cultivars. The frequencies of appressorium formation were significantly lower on cultivars JI1047, JI1048 and JI1049 than on BS10 at 4 h but the difference was not significant on JI1050. At 6 h the proportions of conidia producing appressoria were higher on cultivars JI1048 and JI1050 than on BS10, lower on JI1049 and the difference was insignificant on JI1047. There was no significant difference between cultivars BS10, JI1047, JI1049 and JI1050 at 24 h but the frequency of appressorium formation was significantly higher on JI1048 than on any of the other cultivars.

1.3.1.3 33-day old plants

Germination was first recorded at 2 h on cultivars JI1048 and JI1049, a further delay of 2 h occurred on BS10, JI1047 and JI1050 and of 4 h on BS12 (Table 5) (Fig. 3). Significance tests showed the frequency of germination to be higher on cultivar BS10 at 24 h than on all the other cultivars except JI1050 where the difference was insignificant (Table 7). Germination frequencies were significantly higher on cultivars JI1048 and JI1049 than on BS10 at 2 and 4 h, also at 6 h on JI1049 and at 4 and 6 h on JI1050. Germination was more frequent on cultivar BS10 than on BS12 at 4 h. At all other sampling times the differences were insignificant.

Appressorium formation was recorded at 4 h on all cultivars except BS12 where a further 2 h delay occurred (Table 6) (Fig. 4). Significance tests showed the frequency of appressorium formation to be higher on BS10 than on all the resistant cultivars at 24 h (Table 8). It was also significantly higher on cultivars BS12, JI1048 and JI1049 at 4 and 6 h but the difference between BS10, JI1047 and JI1050 was insignificant at these sampling times.

1.3.2 Effect of host plant age

1.3.2.1 Comparison of 15- and 22-day old plants

Germination occurred within 2 h on 15-day old plants of cultivars BS10 and BS12 and on 22-day old plants of BS10, JI1048 and JI1050 (Table 5) (Fig. 5). On 22-day plants of BS12 germination was not recorded until 6 h. Results of significance tests (Table 9) showed that

germination frequencies were higher on 15-day plants of BS10 and BS12 at 2 and 4 h and also at 6 h on the latter. At 24 h, however, frequencies on these cultivars were higher on 22-day plants. Higher values were also recorded on 22-day plants of JI1048 at 2, 6 and 24 h. Similarly significantly greater proportions of conidia germinated on 22-day plants of cultivar JI1047 at 2 and 4 h, of JI1049 at 4 and 6 h of JI1050 at 2, 4 and 6 h. The differences in germination frequencies on the two age groups were insignificant by 24 h on these cultivars.

Formation of appressoria on 15-day plants was first recorded at 4 h on cultivar JI1049 and JI1050 (Table 6) (Fig. 6). A further 2 h-delay occurred on cultivars BS10, BS12 and JI1048 and on JI1047 no appressoria were produced until 24 h. In the 22-day group formation of appresoria was first observed at 4 h on all cultivars except BS12. The frequencies of appressorium formation were higher on 22-day plants throughout the experimental period on all cultivars except at 24 h on JI1050, where the difference was insignificant (Table 10).

1.3.2.2 Comparison of 15-day old plants

Germination on 33-day old plants of cultivars of BS10, JI1047 and JI1050 was not recorded until 4 h and only after 6 h on BS12 (Table 5) (Fig. 5). It was recorded above (CII 1.3.2.1) that germination occurred within 2 h on 15-day old plants of BS10 and BS12 and results of significance tests showed frequencies to be higher on 15-day plants throughout the experimental period on these cultivars (Table 9). On cultivar JI1047 the frequency of

germination was significantly higher on 33-day old plants at 4 h and on JI1048 at 2, 4 and 6 h but by 24 h it had become significantly higher on 15-day plants of both cultivars. Proportions of germinating conidia were significantly higher on 33-day old plants of cultivar JI1049 throughout the experimental period but only at 2 and 4 h on JI1050, the difference becoming insignificant at 24 h.

Appressoria were first observed at 4 h on 33-day old plants of all cultivars except BS12 where a further 2 h-delay occurred (Table 6) (Fig. 6). Significance tests showed that numbers of appressoria were higher on all 33-day cultivars throughout the experimental period except JI1049 and JI1050 where the difference was insignificant by 24 h (Table 10).

1.3.2.3. Comparison of 22-day and 33-day old plants

Significance tests showed germination frequencies to be higher on 22-day old plants of cultivars BS10 and JI1047 throughout the experimental period but only at 24 h on BS12 (Table 9) (Fig. 5). On 33-day plants of cultivar JI1048 and 22-day plants of JI1050 the frequencies of germination were significantly higher at 2 and 4 h. The reverse occurred at 6 and 24 h on these cultivars. Germination was more frequent on 33-day plants of cultivar JI1049 at 2 h but the difference was insignificant at 4 and 6 h. By 24 h, however, germination again became significantly higher on 33-day plants.

Comparison of the frequencies of appressorium formation in the two age groups showed greater variations

Table 5 Germination of conidia and formation of appressoria on the oldest leaves of plants 15, 22 and 33 days after sowing. Samples were taken 2, 4, 6 and 24 h after inoculation. The results are expressed as % of total conidia.

| CULTIVAR | Sampling time (h) | GERMINATION (Total) Age of plants (days) | | | GERM TUBE ONLY Age of plants (days) | | | MATURE APPRESSORIUM Age of plants (days) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 15 | 22 | 33 | 15 | 22 | 33 | 15 | 22 | 33 |
| BS10 | 2 | 34.7 | 15.2 | 0 | 34.7 | 15.2 | 0 | 0 | 0 | 0 |
| | 4 | 40.0 | 36.3 | 20.9 | 40.0 | 17.6 | 8.5 | 0 | 18.7 | 12.4 |
| | 6 | 45.0 | 56.4 | 30.4 | 38.5 | 23.4 | 11.6 | 6.5 | 33.0 | 18.8 |
| | 24 | 68.7 | 70.4 | 60.3 | 15.5 | 11.7 | 7.9 | 53.2 | 58.7 | 52.4 |
| BS12 | 2 | 25.0 | 0 | 0 | 25.0 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 34.8 | 0 | 0 | 34.8 | 0 | 0 | 0 | 0 | 0 |
| | 6 | 45.2 | 27.2 | 31.4 | 39.5 | 22.3 | 24.0 | 5.7 | 4.9 | 7.4 |
| | 24 | 45.8 | 58.7 | 31.9 | 13.6 | 12.9 | 10.2 | 32.2 | 45.8 | 21.7 |
| JI1047 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 7.2 | 46.8 | 20.6 | 7.2 | 26.4 | 10.0 | 0 | 20.4 | 10.6 |
| | 6 | 34.8 | 39.1 | 32.8 | 34.8 | 13.3 | 13.4 | 0 | 25.8 | 19.8 |
| | 24 | 46.6 | 48.0 | 38.0 | 28.3 | 9.6 | 10.8 | 18.3 | 38.4 | 27.2 |
| JI1048 | 2 | 0 | 12.1 | 28.7 | 0 | 12.1 | 28.7 | 0 | 0 | 0 |
| | 4 | 18.2 | 18.4 | 28.1 | 18.2 | 16.8 | 25.9 | 0 | 1.6 | 2.2 |
| | 6 | 18.4 | 34.2 | 27.3 | 16.6 | 6.6 | 12.7 | 1.8 | 27.6 | 14.6 |
| | 24 | 51.7 | 74.5 | 39.3 | 21.1 | 1.3 | 12.1 | 27.6 | 71.9 | 27.2 |
| JI1049 | 2 | 0 | 0 | 18.1 | 0 | 0 | 18.1 | 0 | 0 | 0 |
| | 4 | 21.6 | 32.2 | 32.3 | 20.5 | 25.6 | 25.6 | 1.1 | 6.6 | 6.7 |
| | 6 | 24.2 | 43.6 | 43.5 | 19.4 | 24.0 | 24.1 | 4.8 | 19.6 | 19.4 |
| | 24 | 36.9 | 33.8 | 49.3 | 10.8 | 4.6 | 16.0 | 26.1 | 29.2 | 33.3 |
| JI1050 | 2 | 0 | 15.2 | 0 | 0 | 15.2 | 0 | 0 | 0 | 0 |
| | 4 | 26.7 | 34.1 | 31.2 | 24.7 | 18.2 | 9.0 | 2.0 | 15.9 | 22.2 |
| | 6 | 18.3 | 50.0 | 55.4 | 16.8 | 11.7 | 19.2 | 1.5 | 38.3 | 36.2 |
| | 24 | 57.2 | 46.4 | 59.4 | 16.0 | 9.8 | 11.8 | 41.2 | 36.6 | 47.6 |

Fig. 3 Comparisons of germination frequencies on susceptible and resistant cultivars of pea following "evening"- inoculation.

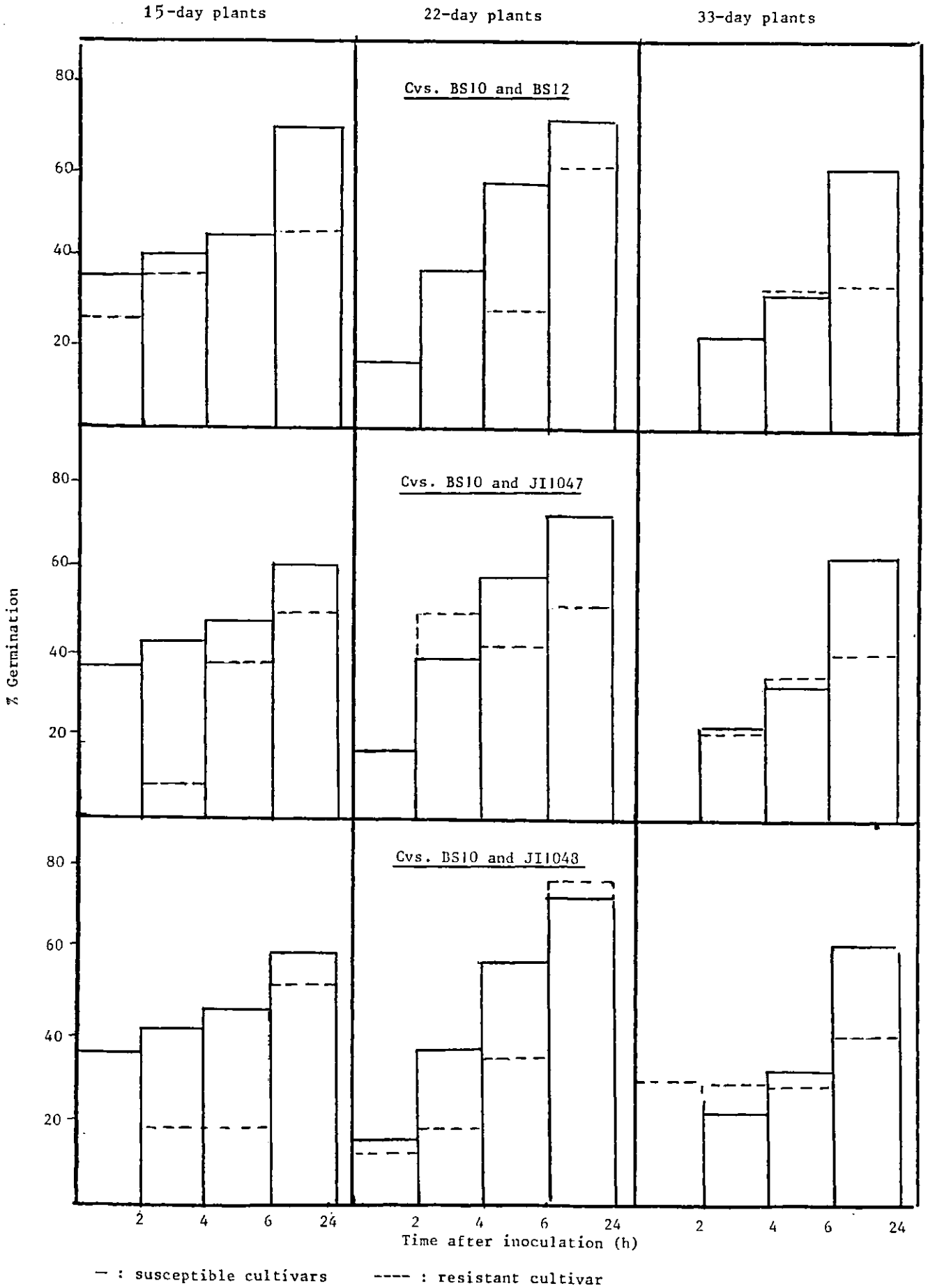


Fig. 3 (Continued)

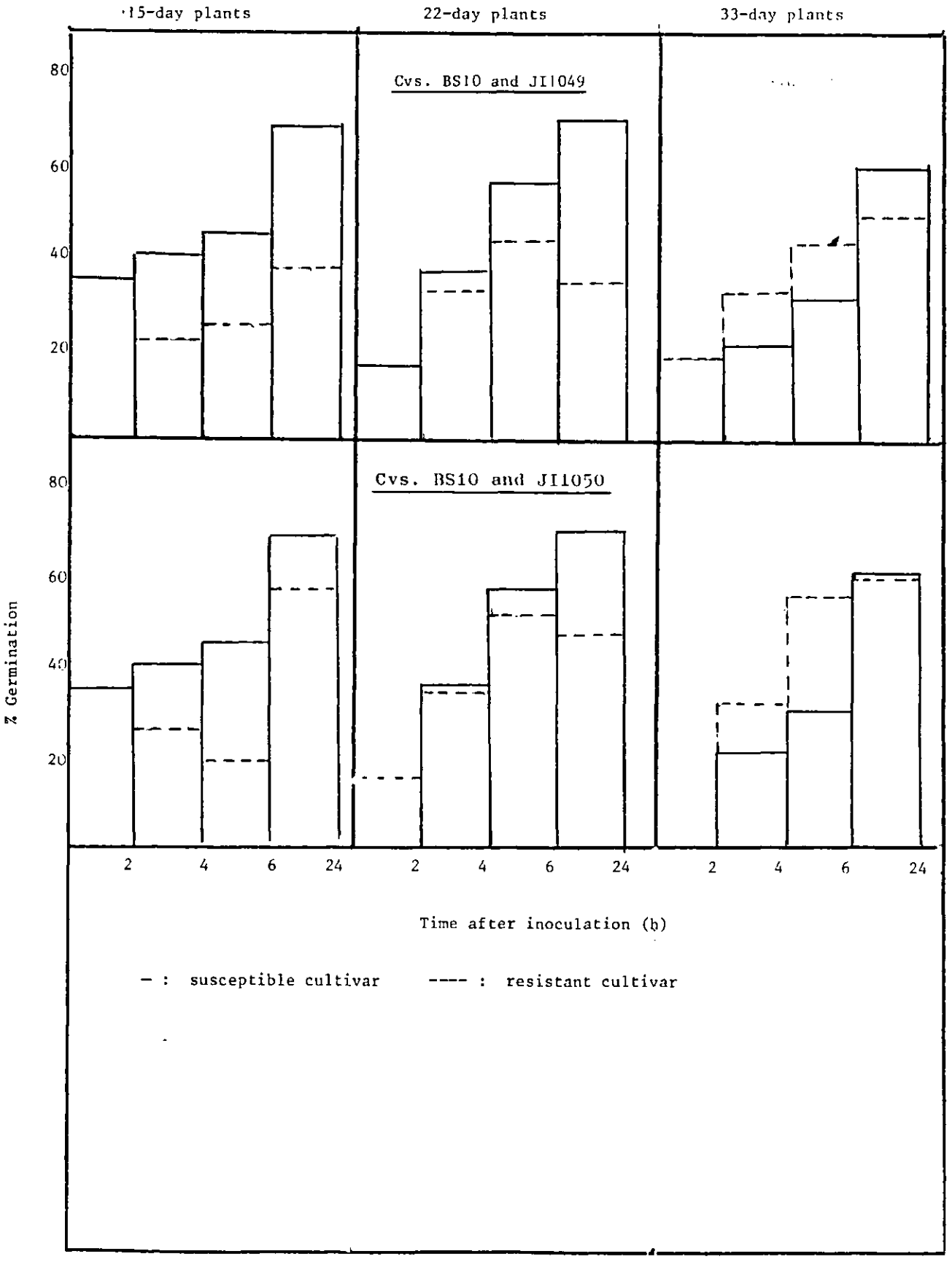


Table 6 Germination of conidia and formation of appressoria on the oldest leaves of plants 15, 22 and 33 days after sowing following "evening"-inouclation. Samples were taken 2, 4, 6 and 24 h after inoculation. The results are expressed as % of germinated conidia.

| CULTIVAR | Sampling time (h) | GERM TUBE ONLY Age of plants (days) | | | MATURE APPRESSORIUM Age of plants (days) | | |
|---|---|---|---|---|---|---|---|
| | | 15 | 22 | 33 | 15 | 22 | 33 |
| BS10 | 2 | 100 | 100 | 0 | 0 | 0 | 0 |
| | 4 | 100 | 48.4 | 40.4 | 0 | 51.6 | 59.6 |
| | 6 | 87.0 | 41.8 | 38.3 | 13.0 | 58.2 | 61.7 |
| | 24 | 22.6 | 16.8 | 13.1 | 77.4 | 83.2 | 86.9 |
| BS12 | 2 | 100 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 100 | 0 | 0 | 0 | 0 | 0 |
| | 6 | 87.8 | 82.8 | 76.9 | 12.2 | 17.2 | 23.1 |
| | 24 | 29.7 | 21.9 | 32.3 | 70.3 | 78.1 | 67.7 |
| JI1047 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 100 | 64.7 | 48.9 | 0 | 35.9 | 51.1 |
| | 6 | 100 | 34.1 | 41.0 | 0 | 66.5 | 59.0 |
| | 24 | 61.0 | 20.1 | 28.3 | 39.0 | 79.9 | 71.7 |
| JI1048 | 2 | 0 | 100 | 100 | 0 | 0 | 0 |
| | 4 | 100 | 91.3 | 92.5 | 0 | 8.7 | 7.5 |
| | 6 | 90.0 | 19.5 | 44.6 | 10.0 | 80.5 | 55.4 |
| | 24 | 46.3 | 1.7 | 30.8 | 53.7 | 98.3 | 69.2 |
| JI1049 | 2 | 0 | 0 | 100 | 0 | 0 | 0 |
| | 4 | 94.9 | 80.1 | 79.5 | 5.1 | 19.9 | 20.5 |
| | 6 | 79.7 | 54.9 | 55.5 | 20.3 | 45.1 | 44.5 |
| | 24 | 29.3 | 14.1 | 32.6 | 70.7 | 85.9 | 67.4 |
| JI1050 | 2 | 0 | 100 | 0 | 0 | 0 | 0 |
| | 4 | 97.9 | 53.2 | 28.9 | 2.1 | 46.8 | 71.1 |
| | 6 | 93.0 | 23.4 | 34.8 | 7.0 | 76.6 | 65.2 |
| | 24 | 28.7 | 21.2 | 20.0 | 71.3 | 78.8 | 80.0 |

Table 7 Statistical analysis of data presented in Table 5 to compare the effect of cultivar on germination of conidia on the oldest leaves of plants 15, 22 and 33 days after sowing following "evening"-inoculation. Samples were taken 2, 4, 6and 24 h after inoculation. The results are expressed as % of total conidia and probability values (p) obtained from Student's t test.

| Sampling time (h) | 15 day plants | | | 22 day plants | | | 33 day plants | | |
|---|---|---|---|---|---|---|---|---|---|
| | BS10 | BS12 | P | BS10 | BS12 | P | BS10 | BS12 | P |
| 2 | 34.7 | 25.0 | 0.001 | 15.2 | 0 | 0.001 | 0 | 0 | |
| 4 | 40.0 | 34.8 | 0.01 | 36.3 | 0 | 0.001 | 20.9 | 0 | 0.001 |
| 6 | 45.0 | 45.2 | 1.0 | 56.4 | 27.2 | 0.001 | 30.4 | 31.4 | 0.5 |
| 24 | 68.7 | 45.8 | 0.4 | 70.4 | 58.7 | 0.001 | 60.3 | 31.9 | 0.001 |
| | BS10 | JI1047 | P | BS10 | JI1047 | P | BS10 | JI1047 | P |
| 2 | 34.7 | 0 | 0.001 | 15.2 | 0 | 0.001 | 0 | 0 | |
| 4 | 40.0 | 7.2 | 0.001 | 36.3 | 46.8 | 0.05 | 20.9 | 20.6 | 0.7 |
| 6 | 45.0 | 34.8 | 0.001 | 56.4 | 39.1 | 0.001 | 30.4 | 32.8 | 0.2 |
| 24 | 68.7 | 46.6 | 0.001 | 70.4 | 48.0 | 0.001 | 60.3 | 38.0 | 0.001 |
| | BS10 | JI1048 | P | BS10 | JI1048 | P | BS10 | JI1048 | P |
| 2 | 34.7 | 0 | 0.001 | 15.2 | 12.1 | 0.2 | 0 | 28.7 | 0.001 |
| 4 | 40.0 | 18.2 | 0.001 | 36.3 | 18.4 | 0.001 | 20.9 | 28.1 | 0.001 |
| 6 | 45.0 | 18.4 | 0.001 | 56.4 | 34.2 | 0.001 | 30.4 | 27.3 | 0.05 |
| 24 | 68.7 | 51.7 | 0.001 | 70.4 | 74.5 | 0.3 | 60.3 | 39.3 | 0.001 |
| | BS10 | JI1049 | P | BS10 | JI1049 | P | BS10 | JI1049 | P |
| 2 | 34.7 | 0 | 0.001 | 15.2 | 0 | 0.001 | 0 | 18.1 | 0.001 |
| 4 | 40.0 | 21.6 | 0.001 | 36.3 | 32.2 | 0.001 | 20.9 | 32.2 | 0.001 |
| 6 | 45.0 | 24.2 | 0.001 | 56.4 | 43.6 | 0.001 | 30.4 | 43.5 | 0.001 |
| 24 | 68.7 | 36.9 | 0.001 | 70.4 | 33.8 | 0.001 | 60.3 | 49.3 | 0.001 |
| | BS10 | JI1050 | P | BS10 | JI1050 | P | BS10 | JI1050 | P |
| 2 | 34.7 | 0 | 0.001 | 15.2 | 15.2 | | 0 | 0 | |
| 4 | 40.0 | 26.7 | 0.001 | 36.3 | 34.1 | 0.2 | 20.9 | 31.2 | 0.001 |
| 6 | 45.0 | 18.3 | 0.001 | 56.4 | 50.0 | 0.01 | 30.4 | 55.4 | 0.001 |
| 24 | 68.7 | 57.2 | 0.001 | 70.4 | 46.4 | 0.001 | 60.3 | 59.4 | 0.6 |

Table 8 Statistical analysis of data presented in Table 6 to compare the effect of cultivar on formation of appressoria on the oldest leaves of plants 15, 22 and 33 days after sowing following "evening"-inoculation. Samples were taken 2, 4, 6 and 24 h after inoculation. The results are expressed as % of germinated conidia and probability values obtained from Student's t test.

| Sampling time (h) | 15 day plants | | | 22 day plants | | | 33 day plants | | |
|---|---|---|---|---|---|---|---|---|---|
| | BS10 | BS12 | P | BS10 | BS12 | P | BS10 | BS12 | P |
| 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| 4 | 0 | 0 | | 51.6 | 0 | 0.001 | 59.6 | 0 | 0.001 |
| 6 | 13.0 | 12.2 | 0.8 | 58.2 | 17.2 | 0.001 | 61.7 | 23.1 | 0.001 |
| 24 | 77.4 | 70.3 | 0.01 | 83.2 | 78.1 | 0.01 | 86.9 | 67.7 | 0.001 |
| | BS10 | JI1047 | P | BS10 | JI1047 | P | BS10 | JI1047 | P |
| 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| 4 | 0 | 0 | | 51.6 | 35.9 | 0.001 | 59.6 | 51.1 | 0.1 |
| 6 | 13.0 | 0 | 0.001 | 58.2 | 66.5 | 0.05 | 61.7 | 59.0 | 0.2 |
| 24 | 77.4 | 39.0 | 0.001 | 83.2 | 79.9 | 0.2 | 86.9 | 71.7 | 0.001 |
| | BS10 | JI1048 | P | BS10 | JI1048 | P | BS10 | JI1048 | P |
| 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| 4 | 0 | 0 | | 51.6 | 8.9 | 0.001 | 59.6 | 7.5 | 0.001 |
| 6 | 13.0 | 10.0 | 0.4 | 58.2 | 80.5 | 0.001 | 61.7 | 55.4 | 0.001 |
| 24 | 77.4 | 53.7 | 0.001 | 83.2 | 98.3 | 0.001 | 86.9 | 69.2 | 0.001 |
| | BS10 | JI1049 | P | BS10 | JI1049 | P | BS10 | JI1049 | P |
| 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| 4 | 0 | 5.1 | 0.01 | 51.6 | 19.9 | 0.001 | 59.6 | 20.5 | 0.001 |
| 6 | 13.0 | 20.3 | 0.2 | 58.2 | 45.1 | 0.01 | 61.7 | 44.5 | 0.001 |
| 24 | 77.7 | 70.7 | 0.1 | 83.2 | 85.9 | 0.05 | 86.9 | 67.4 | 0.001 |
| | BS10 | JI1050 | P | BS10 | JI1050 | P | BS10 | JI1050 | P |
| 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| 4 | 0 | 2.1 | 0.1 | 51.6 | 46.8 | 0.2 | 59.6 | 71.1 | 0.05 |
| 6 | 13.0 | 7.0 | 0.1 | 58.2 | 76.6 | 0.001 | 61.7 | 65.2 | 0.3 |
| 24 | 77.7 | 71.3 | 0.05 | 83.2 | 78.8 | 0.1 | 86.9 | 80.0 | 0.001 |

Fig. 4 Comparisons of frequencies of mature appressorium formation (expressed as a % of germinated conidia) on susceptible and resistant cultivars of pea following "evening"- inoculation.

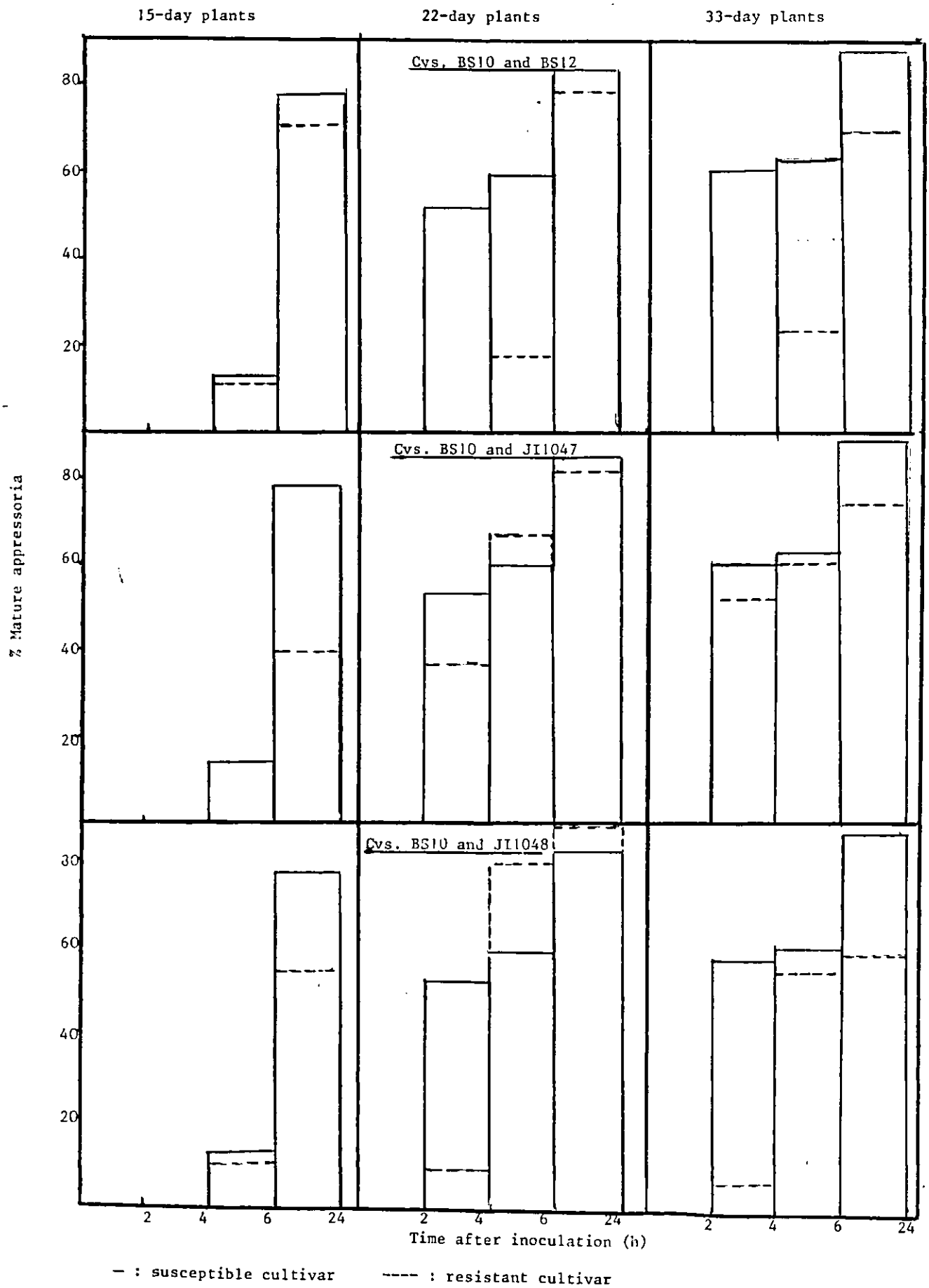


Fig. 4 (Continued)

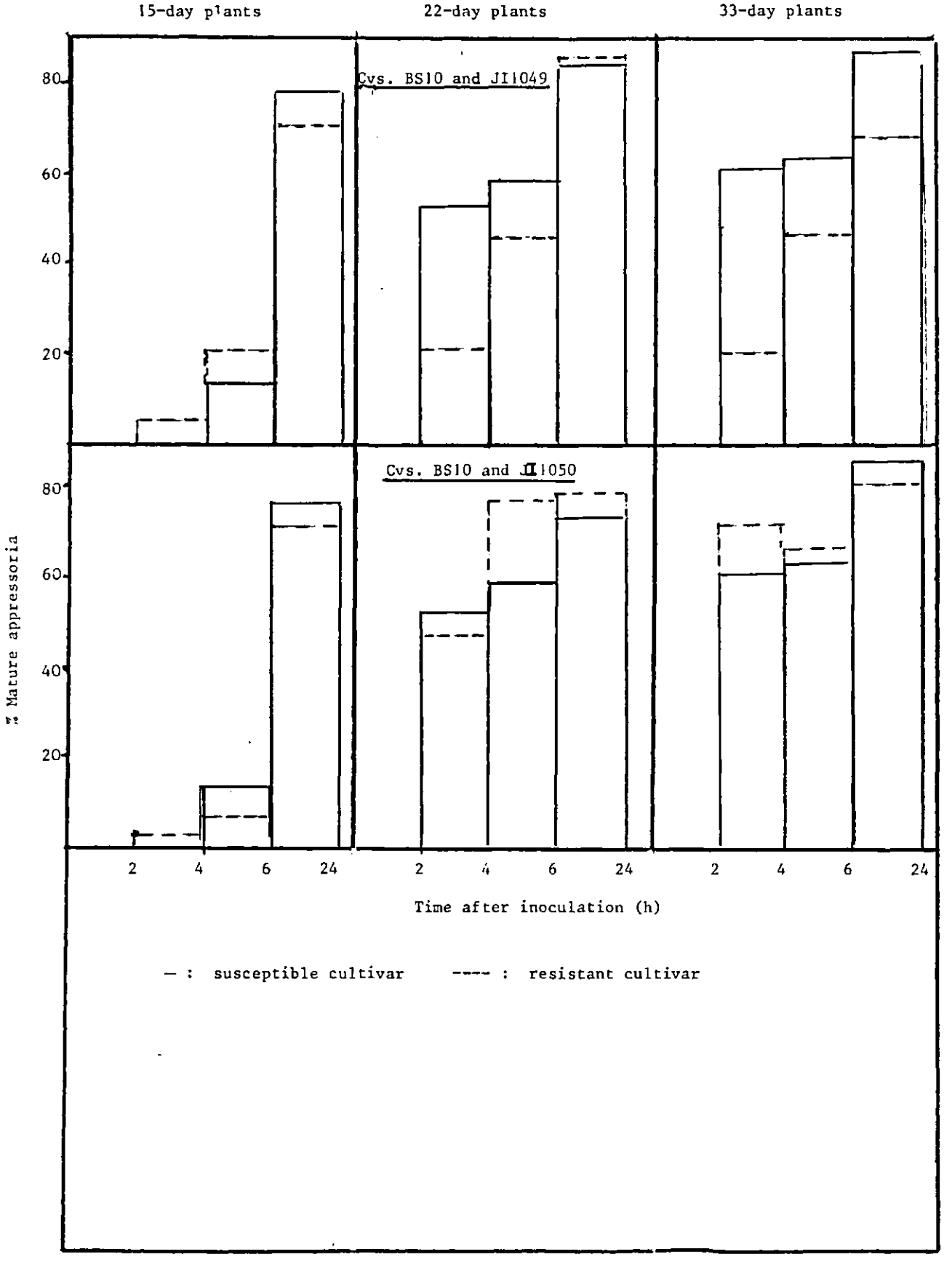


Table 9 Statistical analysis of data presented in Table 5 to compare the effect of host plant age on germination of conidia following "evening"-inoculation. Samples were taken 2, 4, 6 and 24 h after inoculation. The results are expressed as a % of total conidia and probability values (p) obtained from Student's t test.

| CULTIVAR | Sampling time (h) | GERMINATION (total) Age of Plants | | | GERMINATION (total) Age of Plants | | | GERMINATION (total) Age of Plants | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 15 days | 22 days | P | 15 days | 22 days | P | 15 days | 22 days | P |
| BS10 | 2 | 34.7 | 15.2 | 0.001 | 34.7 | 0 | 0.001 | 15.2 | 0 | 0.001 |
| | 4 | 40.0 | 36.3 | 0.05 | 40.0 | 20.9 | 0.001 | 36.3 | 20.9 | 0.001 |
| | 6 | 45.0 | 56.4 | 0.001 | 45.0 | 30.4 | 0.001 | 56.4 | 30.4 | 0.001 |
| | 24 | 68.7 | 70.4 | 0.6 | 68.7 | 60.3 | 0.01 | 70.4 | 60.3 | 0.02 |
| BS12 | 2 | 25.0 | 0 | 0.001 | 25.0 | 0 | 0.001 | 0 | 0 | - |
| | 4 | 34.8 | 0 | 0.001 | 34.8 | 0 | 0.001 | 0 | 0 | - |
| | 6 | 45.2 | 27.2 | 0.001 | 45.1 | 31.4 | 0.001 | 27.2 | 31.4 | 0.05 |
| | 24 | 45.8 | 58.7 | 0.001 | 45.8 | 31.9 | 0.001 | 58.7 | 31.9 | 0.001 |
| JI1047 | 2 | 0 | 0 | - | 0 | 0 | - | 0 | 0 | - |
| | 4 | 7.2 | 46.8 | 0.001 | 7.2 | 20.6 | 0.001 | 46.8 | 20.6 | 0.001 |
| | 6 | 34.8 | 39.1 | 0.01 | 34.8 | 32.8 | 0.4 | 39.1 | 32.8 | 0.001 |
| | 24 | 46.6 | 48.0 | 0.2 | 46.6 | 38.0 | 0.001 | 48.0 | 38.0 | 0.001 |
| JI1048 | 2 | 0 | 12.1 | 0.001 | 0 | 28.7 | 0.001 | 12.1 | 28.7 | 0.001 |
| | 4 | 18.2 | 18.4 | 0.9 | 18.2 | 28.1 | 0.001 | 18.4 | 28.1 | 0.001 |
| | 6 | 18.4 | 34.2 | 0.001 | 18.4 | 27.3 | 0.001 | 34.2 | 27.3 | 0.001 |
| | 24 | 51.7 | 74.5 | 0.001 | 51.7 | 39.3 | 0.001 | 74.5 | 39.3 | 0.001 |
| JI1049 | 2 | 0 | 0 | - | 0 | 18.1 | 0.001 | 0 | 18.1 | 0.001 |
| | 4 | 21.6 | 32.2 | 0.001 | 21.6 | 32.3 | 0.001 | 32.2 | 32.3 | 0.9 |
| | 6 | 24.2 | 43.6 | 0.001 | 24.2 | 43.5 | 0.001 | 43.6 | 43.5 | 0.9 |
| | 24 | 36.9 | 33.8 | 0.2 | 36.9 | 49.3 | 0.001 | 33.8 | 49.3 | 0.001 |
| JI1050 | 2 | 0 | 15.2 | 0.001 | 0 | 0 | | 15.2 | 0 | 0.001 |
| | 4 | 26.7 | 34.1 | 0.001 | 26.7 | 31.2 | 0.01 | 34.1 | 31.2 | 0.02 |
| | 6 | 18.3 | 50.0 | 0.001 | 18.3 | 55.4 | 0.001 | 50.0 | 55.4 | 0.01 |
| | 24 | 57.2 | 46.4 | 0.001 | 57.2 | 59.4 | 0.3 | 46.4 | 59.4 | 0.001 |

Fig. 5 Comparisons of germination frequencies on 15-, 22- and 33- day cultivars of pea following "evening"- inoculation.

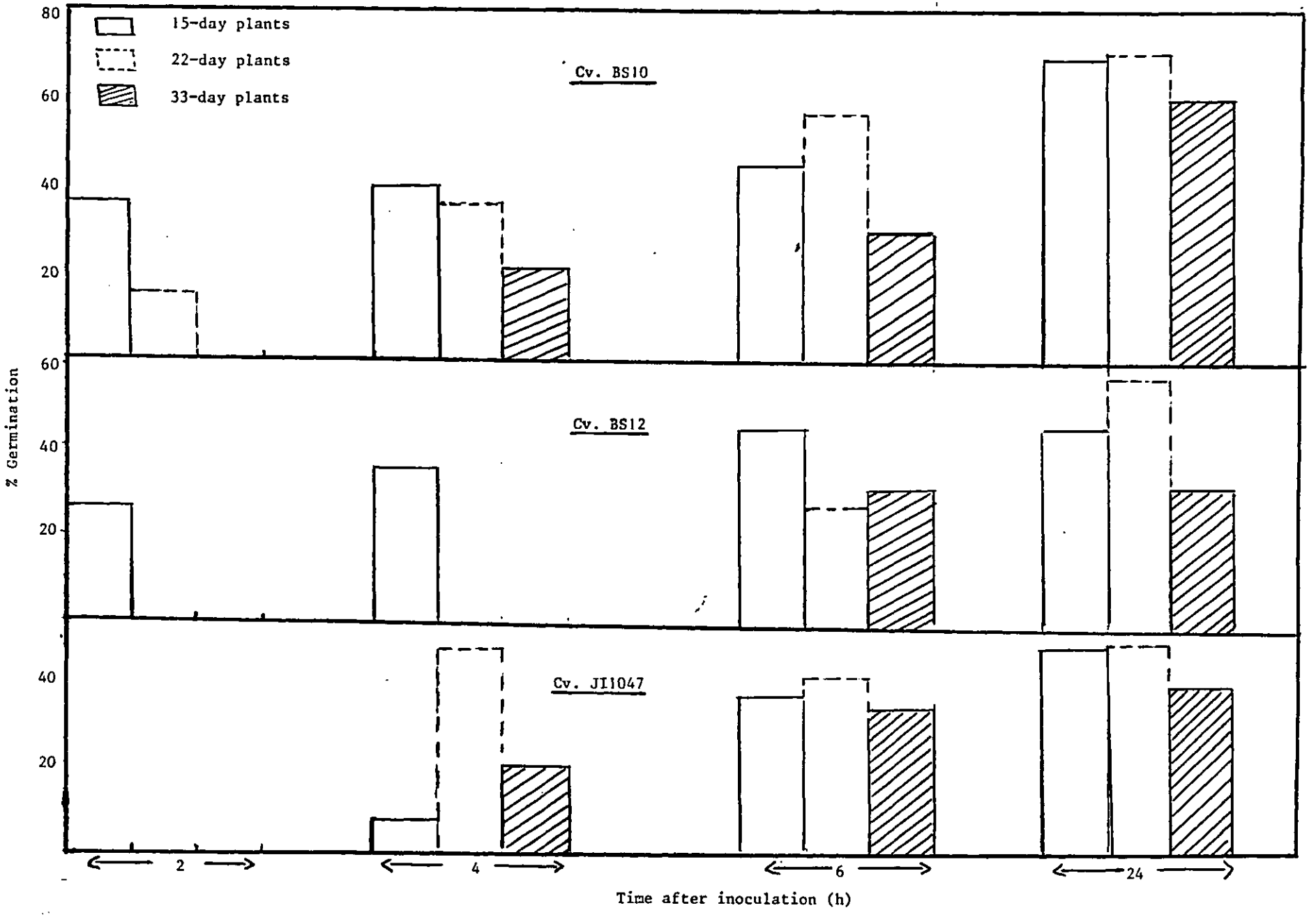


Fig. 3 (continued)

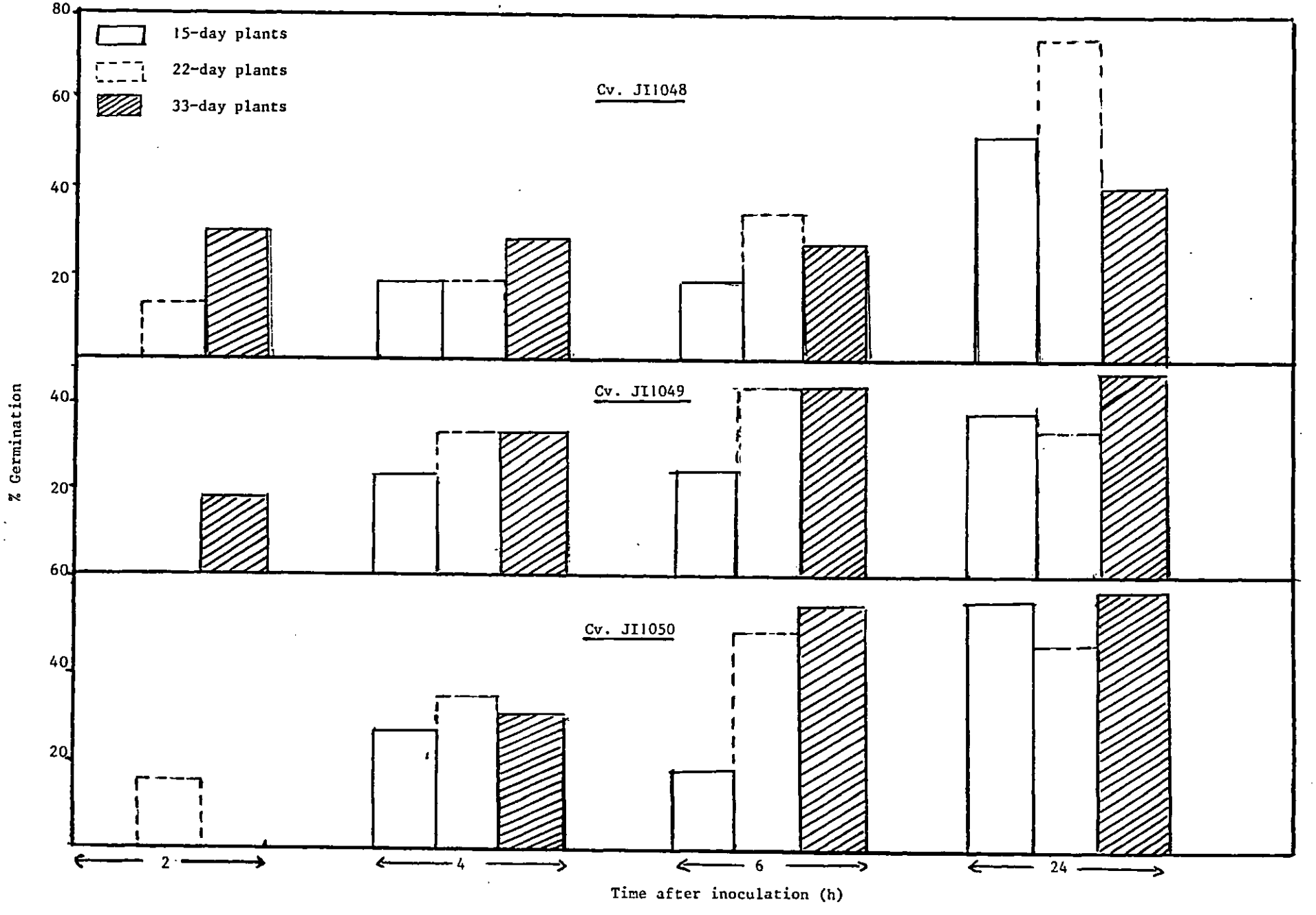


Table 10 Statistical analysis of data presented in Table 6 to compare the effect of host plant age on formation of appressoria following "evening"-inoculation. Samples were taken 2, 4, 6 and 24 h after inoculation. The results are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| CULTIVAR | Sampling time (h) | MATURE APPRESSORIUM Age of Plants 15 days | 22 days | P | MATURE APPRESSORIUM Age of Plants 15 days | 33 days | P | MATURE APPRESSORIUM Age of Plants 22 days | 33 days | P |
|---|---|---|---|---|---|---|---|---|---|---|
| BS10 | 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | 4 | 0 | 51.6 | 0.001 | 0 | 59.6 | 0.001 | 51.6 | 59.6 | 0.2 |
| | 6 | 13.0 | 58.2 | 0.001 | 13.0 | 61.7 | 0.001 | 58.2 | 61.7 | 0.3 |
| | 24 | 77.4 | 83.2 | 0.01 | 77.4 | 86.9 | 0.001 | 83.2 | 86.9 | 0.05 |
| BS12 | 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | 4 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | 6 | 0 | 77.2 | 0.01 | 0 | 23.1 | 0.001 | 17.2 | 23.1 | 0.3 |
| | 24 | 70.3 | 78.1 | 0.001 | 70.3 | 67.7 | 0.3 | 78.1 | 67.7 | 0.001 |
| JI1047 | 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | 4 | 0 | 35.9 | 0.001 | 0 | 51.1 | 0.001 | 35.9 | 51.1 | 0.001 |
| | 6 | 0 | 66.5 | 0.001 | 0 | 59.0 | 0.001 | 66.5 | 59.0 | 0.01 |
| | 24 | 39.0 | 79.9 | 0.001 | 39.0 | 71.7 | 0.001 | 79.9 | 71.7 | 0.01 |
| JI1048 | 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | 4 | 0 | 8.9 | 0.01 | 0 | 7.5 | 0.001 | 8.9 | 7.5 | 0.6 |
| | 6 | 10.0 | 80.5 | 0.001 | 10.0 | 55.4 | 0.001 | 80.5 | 55.4 | 0.001 |
| | 24 | 53.7 | 98.3 | 0.001 | 53.7 | 69.2 | 0.001 | 98.3 | 69.2 | 0.001 |
| JI1049 | 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | 4 | 5.1 | 19.9 | 0.01 | 5.1 | 20.5 | 0.001 | 19.9 | 20.5 | 0.9 |
| | 6 | 20.3 | 45.1 | 0.01 | 20.3 | 44.5 | 0.001 | 45.1 | 44.5 | 0.9 |
| | 24 | 70.7 | 85.9 | 0.01 | 70.7 | 67.4 | 0.5 | 85.9 | 67.4 | 0.001 |
| JI1050 | 2 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | 4 | 2.1 | 46.8 | 0.001 | 2.1 | 71.1 | 0.001 | 46.8 | 71.1 | 0.02 |
| | 6 | 7.0 | 76.6 | 0.001 | 7.0 | 65.2 | 0.001 | 76.6 | 65.2 | 0.01 |
| | 24 | 71.3 | 78.8 | 0.05 | 71.3 | 80.0 | 0.02 | 78.8 | 80.0 | 0.6 |

Fig. 6 Comparisons of frequencies of mature appressorium formation (expressed as a % of germinated conidia) on 15-, 22- and 33-day cultivars of pea following "evening"- inoculation.

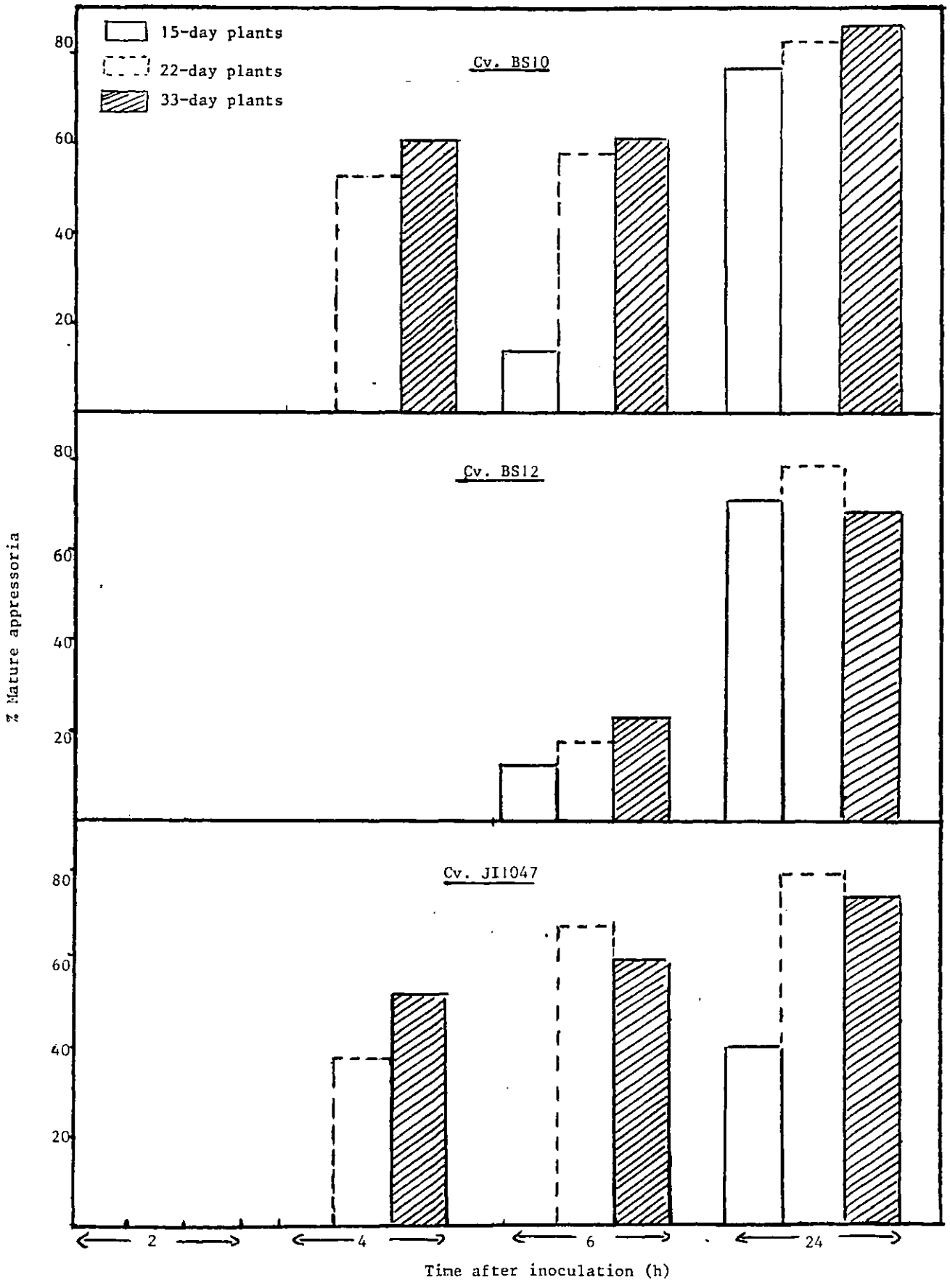
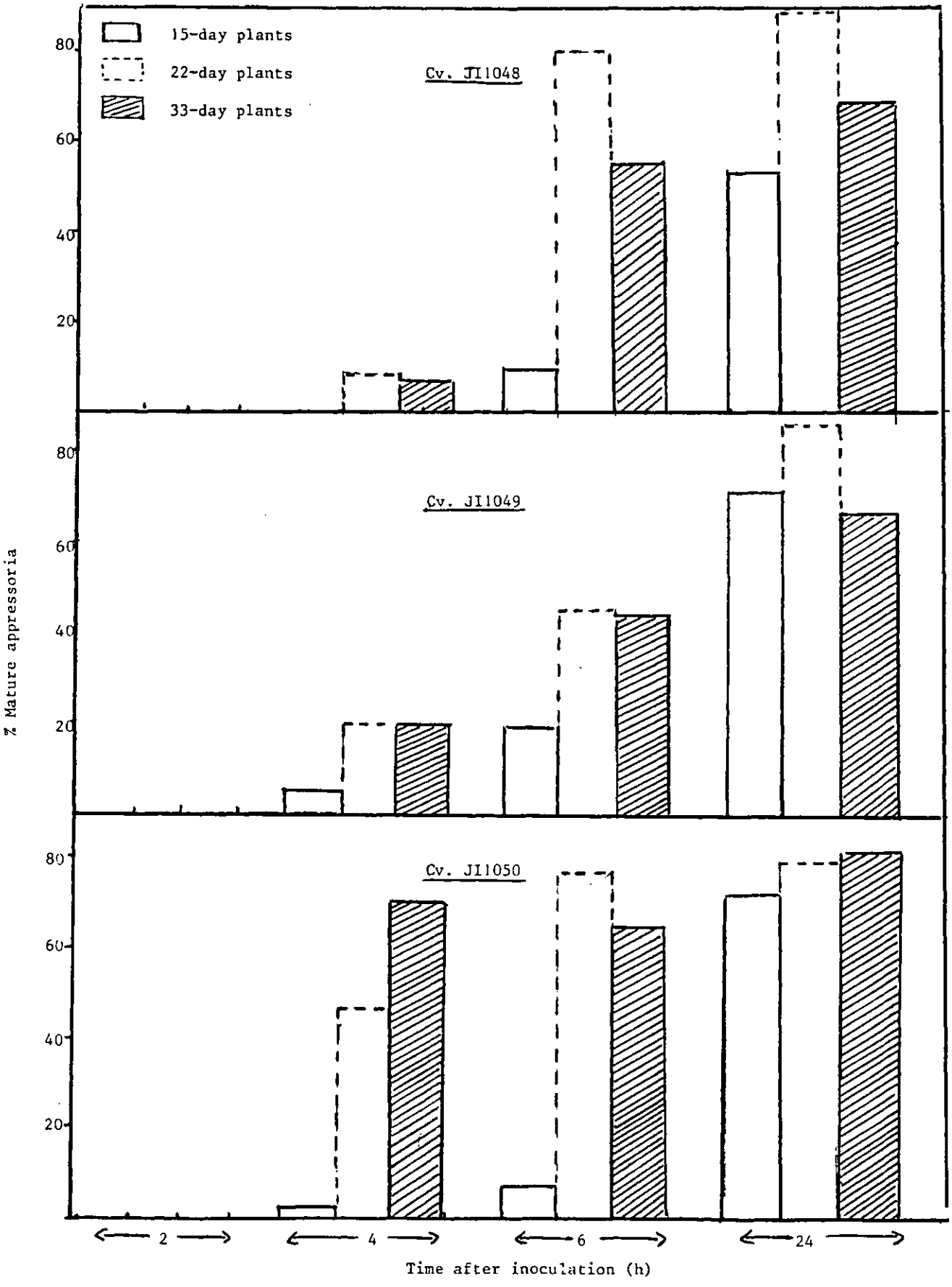


Fig. 6 (Continued)



than in the other comparisons (CII 1.3.2.1 and 1.3.2.2) (Fig. 6). There was no significant difference between numbers of appressoria on 22- and 33-day old plants of cultivar BS10 throughout the experimental period and not until 24 h on BS12 and JI1049 when they were higher on 22-day plants (Table 10). The frequency of appressoria was significantly higher on 33-day cultivar JI1047 at 4 h but after wards the difference became less significant. On cultivar JI1048 the frequency of appressoria on 22-day plants of cultivar JI1048 became significantly higher by 4 h. On cultivar JI1050 the frequency of appressoria was significantly higher on 33-day plants at 4 and 6 h but the difference became insignificant by 24 h.

## 2 Detailed investigation of germination of conidia and formation of appressoria in the early stages (0-8 h) of the primary infection process

### 2.1 Introduction

The investigation of the effect of cultivar on germination of conidia and formation of appressoria (CII 1.3.1.2.3) showed these developmental stages to be affected differently by different cultivars. The experiment was repeated, introducing a more detailed categorization of developmental stages. Results from the investigation of the effect of host plant age on fungal development (CIIIa 1.3.2 ) showed that germination and appressorium formation frequencies on cultivar BS10 were significantly higher on 22-day old plants. This age group was selected for subsequent experiments because optimal development of Erysiphe pisi on the susceptible cultivar was

necessary in order to validate comparisons with development on resistant cultivars.

2.2 Experimental

After "evening"- inoculation (BI 5.2), 22-day old plants of the six test cultivars were incubated in a Fison's Fitotron, 600G3/THTL, with 16 h light and 8 h dark periods. Samples were taken 1, 2, 3, 4, 6 and 8 h after inoculation, fungal structures were prepared for observation on Necoloidine films (BII 3.2 ) with four replicates in each case, and stained in lactophenol cotton blue (BII 1.2 ). Four categories of conidia were recognised. Conidia producing a germ tube only either with a germ tube shorter than the width of the conidium, termed a "short" germ tube (a), or a germ tube whose length was at least equal to the width of the conidium, termed a "normal" germ tube (b), conidia producing appressorial initials (c) or with mature appressoria (d) were recorded. At least 100 conidia in each sample were counted and the data are expressed as a percentage of total and germinated conidia, recorded in table 11 and 12, respectively. Results of tests of significance of germination, formation of initial and mature appressoria on susceptible and resistant cultivars are given in tables 13, 14 and 15.

2.3 Results

Production of "short"-germ tubes was first recorded at 1 h on all cultivars except JI1049 where a further delay of 1 h occurred (Table 9). "Normal"-germ tubes were also first seen at 1 h on cultivars JI1048 and JI1050, at 2 h on BS10, BS12 and JI1049, and at 3 h on JI1047 (Fig. 7).

Significance tests showed that the germination frequency was higher on cultivar BS10 than on BS12, JI1047, JI1048 and JI1050 throughout the experimental period (Table 13), with the exception of the 2 h sample on BS12 and the 3 h sample on JI1047. On cultivar JI1049, however, germintion frequencies were significantly higher than on BS10 at 2, 3 and 4 h, the difference becoming insignificant by 6 h.

Production of appressorial initials was first recorded at 3 h (Table 9) on all cultivars except BS12 and JI1048 where they were seen at 4 h. Significance tests showed that at 3 h the numbers of appressorial-initials (Table 14) were higher on cultivar BS10 than on BS12 and JI1048 because of the 1 h delay on the last two cultivars. The difference became insignificant thereafter. There were no significant differences in the frequencies on cultivars BS10, JI1047 and JI1050 throughout the experimental period, except at 4 h on JI1047 and at 6 h on JI1050 where the numbers of appressorial initials were higher. The proportions of conidia producing appressorial initials were significantly higher on cultivar JI1049 than on BS10 throughout the experimental period.

Mature appressoria were first seen at 3 h on cultivar JI1049 (Table 11) (Fig. 8), at 4 h on BS10, JI1047, JI1048 and JI1050, and at 6 h on BS12. Significance tests showed that the frequency of mature appressorium formation was higher on cultivar BS10 than on all the resistant cultivars at 4 and 6 h (Table 15), and also at 8 h on BS12, JI1049 and JI1050.

The results show that germination frequency was

greater on cultivar BS10 than on all the resistant cultivars except JI1049. Variations in the frequency of conidia producing appressorial-initials occurred on different cultivars but in general, except for the comparison with JI1049, the difference in frequencies on BS10 and the resistant cultivars was insignificant. More appressoria reached maturity on cultivar BS10 than on all the resistant cultivars during the first 4 h, the difference in frequencies on BS10, JI1047 and JI1048 becoming insignificant by 8 h.

3 Detailed investigation of germination of conidia and formation of appressoria during the total period 22-240 h after inoculation.

3.1 Introduction

Results from the detailed investigation of fungal development in the early stages of infection (CIIIa 2.3 ) indicated that frequencies of germination and of formation of mature appressoria were, in general, significantly higher on cultivar BS10 than on the resistant cultivars. The experiment was extended to discover if the trends emerging in the earlier stages of the primary infection process persisted for a longer period.

3.2 Experimental

After "evening"- inoculation (BI 5.2) 22-day old plants of the six test cultivars were incubated in a Fisons Fitotron, 600G3/THTL, with 16 h light and 8 h dark periods. Samples were taken 22, 44, 68, 92, 164 and 240 h after inoculation. Fungal structures were prepared for observation on Necoloidine films (BII 3.2 ), with four replicates in each case, and stained in lactophenol cotton

blue (BII 1.2 ). Numbers of conidia producing a germ tube only, initial and mature appressoria were recorded, counting at least 100 conidia in each sample. The data, expressed as a percentage of total and germinated conidia, are shown in tables 16 and 17, respectively. Results of tests of significance of mean germination and formation of initial and mature appressoria on susceptible and resistant cultivars are recorded in Tables 18, 19 and 20.

3.3 Results

Germination frequencies were significantly higher (usually $p = 0.001$) on BS10 than on the resistant cultivars at all sampling times after 22 h (Table 18) (Fig. 9). Before this they were significantly higher on all the resistant cultivars except JI1049. Conidia continued to germinate after 22 h on cultivar BS10 whereas little, if any, increase in germination frequencies occurred on the resistant cultivars. Maximum germination frequencies were achieved at 22 h on cultivars BS12 (63%), JI1047 (59%), JI1048 (65%) and JI1049 (55%) and by 92 h on BS10 (92%) and JI1050 (59%).

Greater proportions of conidia remained at the appressorial initial stage on resistant cultivars than on BS10 (Table 17). Significance tests showed that the frequencies of appresorial-initial formation were higher on all resistant cultivars at 22, 44 and 68 h (Table 19). They were also higher on cultivars JI1048 and JI1050 at 92 h, on BS12 and JI1050 at 164 h and on JI1047 and JI1049 at 240 h. Maximum frequencies of mature appressorium formation were reached on cultivars BS10 (98%) at 68 h, on JI1047 (93%) at BS12 (95%), JI1048 (93%) and JI1049 (96%) at 164 h

and on JI1050 (93%) at 240 h (Table 17) (Fig. 10). Significance tests showed that 22 and 44 h there was no difference between frequencies of mature appressoria on cultivars BS10, JI1047, JI1048 and JI1049, only at 22 h on BS10 and JI1050 and only at 44 h on BS10 and BS12 (Table 20). The proportions of conidia producing mature appressoria were significantly higher on cultivar BS10 than on the resistant cultivars at 68 h, higher than on BS12, JI1048 and JI1050 at 92 h and at 164 h higher only than on JI1048 and JI1050.

4 Investigation of the structure of epicuticular waxes on the adaxial epidermes of susceptible and resistant cultivars by scanning electron microscopy.

4.1 Introduction

The detailed investigation of the effect of cultivar on germination of conidia and formation of appressoria showed effect both in the early (0-8 h) (CIIa 2.3) and in the later stages (22-240 h) (CIIa 3.3) of the primary infection process. The frequency of germination was higher on BS10 than on all the resistant cultivars except JI1049 in the first 8 h of the infection period and higher than on all the resistant cultivars in the later stages. Appressorium formation was more frequent on BS10 than on the resistant cultivars until 4 h. Greater proportions of conidia remained at the appressorial-initial stage on the resistant cultivars in the later stages resulting in a delay in reaching maximum frequencies of appressorium

Table II Germination of conidia and formation of initial and mature appressoria on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Samples were taken 1, 2, 3, 4, 6 and 8 h after inoculation. The results are expressed as a % of total conidia.

| CULTIVAR | Sampling time (h) | GERMINATION (total) | "SHORT" GERM TUBE ONLY | "NORMAL" GERM TUBE ONLY | APPRESSORIAL INITIAL | MATURE APPRESSORIUM |
|---|---|---|---|---|---|---|
| BS10 | 1 | 0.2 | 0.2 | 0 | 0 | 0 |
| | 2 | 11.4 | 7.9 | 3.5 | 0 | 0 |
| | 3 | 39.2 | 15.5 | 20.6 | 3.2 | 0 |
| | 4 | 59.9 | 0 | 11.8 | 9.9 | 38.2 |
| | 6 | 68.3 | 0 | 4.7 | 6.6 | 57.0 |
| | 8 | 78.9 | 0 | 12.7 | 14.5 | 51.7 |
| BS12 | 1 | 0.3 | 0.3 | 0 | 0 | 0 |
| | 2 | 8.9 | 5.9 | 3.0 | 0 | 0 |
| | 3 | 22.1 | 9.1 | 13.0 | 0 | 0 |
| | 4 | 21.9 | 7.0 | 13.4 | 1.5 | 0 |
| | 6 | 30.8 | 1.2 | 12.0 | 5.2 | 12.4 |
| | 8 | 42.6 | 8.1 | 11.7 | 10.3 | 12.5 |
| JI1047 | 1 | 0.05 | 0 | 0 | 0 | 0 |
| | 2 | 3.0 | 0 | 0 | 0 | 0 |
| | 3 | 41.6 | 4.0 | 36.5 | 1.1 | 0 |
| | 4 | 43.5 | 0 | 8.3 | 11.6 | 23.6 |
| | 6 | 48.9 | 0 | 7.1 | 5.6 | 36.0 |
| | 8 | 53.2 | 0 | 1.2 | 5.3 | 40.7 |
| JI1048 | 1 | 5.9 | 3.4 | 2.5 | 0 | 0 |
| | 2 | 5.1 | 1.9 | 3.2 | 0 | 0 |
| | 3 | 14.1 | 7.9 | 6.2 | 0 | 0 |
| | 4 | 24.3 | 3.3 | 19.0 | 1.9 | 0.1 |
| | 6 | 38.0 | 1.1 | 20.3 | 5.3 | 9.9 |
| | 8 | 46.5 | 2.5 | 13.6 | 8.9 | 21.5 |
| JI1049 | 1 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 50.1 | 45.5 | 4.6 | 0 | 0 |
| | 3 | 72.2 | 0 | 18.6 | 27.7 | 24.9 |
| | 4 | 68.2 | 0 | 14.5 | 17.6 | 36.1 |
| | 6 | 81.0 | 0 | 10.3 | 15.6 | 55.1 |
| | 8 | 70.0 | 0 | 14.2 | 26.0 | 29.8 |
| JI1050 | 1 | 3.6 | 1.5 | 2.4 | 0 | 0 |
| | 2 | 4.6 | 1.7 | 2.9 | 0 | 0 |
| | 3 | 11.1 | 5.2 | 5.7 | 0.2 | 0 |
| | 4 | 27.1 | 3.1 | 17.4 | 2.9 | 3.7 |
| | 6 | 29.9 | 0.6 | 16.0 | 6.2 | 7.1 |
| | 8 | 38.5 | 3.4 | 13.3 | 6.7 | 15.1 |

Table 12 Germination of conidia and formation of initial and mature appressoria on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Samples were takne 1, 2, 3, 4, 6 and 8 h after inoculation. The results are expressed as a % of germinated conidia.

| CULTIVAR | Sampling time (h) | "SHORT" GERM TUBE ONLY | "NORMAL" GERM TUBE ONLY | APPRESSORIAL INITIAL | MATURE APPRESSORIUM |
|---|---|---|---|---|---|
| BS10 | 1 | 100 | 0 | 0 | 0 |
| | 2 | 67.8 | 32.2 | 0 | 0 |
| | 3 | 37.8 | 54.3 | 7.9 | 0 |
| | 4 | 0 | 17.5 | 13.9 | 68.6 |
| | 6 | 0 | 6.5 | 9.7 | 83.8 |
| | 8 | 0 | 16.0 | 17.6 | 65.9 |
| BS12 | 1 | 100 | 0 | 0 | 0 |
| | 2 | 64.1 | 35.9 | 0 | 0 |
| | 3 | 41.1 | 58.9 | 0 | 0 |
| | 4 | 29.8 | 62.5 | 7.7 | 0 |
| | 6 | 4.2 | 41.8 | 15.4 | 38.6 |
| | 8 | 19.6 | 27.5 | 23.7 | 29.2 |
| JI1047 | 1 | 100 | 0 | 0 | 0 |
| | 2 | 100 | 0 | 0 | 0 |
| | 3 | 9.5 | 87.9 | 7.6 | 0 |
| | 4 | 0 | 18.7 | 26.7 | 54.6 |
| | 6 | 0 | 15.2 | 11.3 | 73.5 |
| | 8 | 0 | 13.2 | 9.9 | 76.9 |
| JI1048 | 1 | 54.7 | 45.3 | 0 | 0 |
| | 2 | 35.9 | 64.1 | 0 | 0 |
| | 3 | 57.2 | 42.8 | 0 | 0 |
| | 4 | 15.8 | 76.2 | 7.4 | 0.6 |
| | 6 | 3.0 | 55.9 | 13.9 | 26.8 |
| | 8 | 5.3 | 28.9 | 19.3 | 46.5 |
| JI1049 | 1 | 0 | 0 | 0 | 0 |
| | 2 | 90.5 | 9.5 | 0 | 0 |
| | 3 | 0 | 26.6 | 38.4 | 35.0 |
| | 4 | 0 | 21.1 | 25.9 | 53.0 |
| | 6 | 0 | 12.5 | 19.2 | 68.3 |
| | 8 | 0 | 20.2 | 36.9 | 42.9 |
| JI1050 | 1 | 43.3 | 56.7 | 0 | 0 |
| | 2 | 37.8 | 62.2 | 0 | 0 |
| | 3 | 50.8 | 47.4 | 1.8 | 0 |
| | 4 | 9.7 | 68.5 | 10.9 | 10.9 |
| | 6 | 1.9 | 54.2 | 20.6 | 23.3 |
| | 8 | 9.1 | 34.8 | 17.6 | 38.5 |

Table 13 Statistical analysis of the data presented in Table 11 to compare the effect of cultivar on germination of conidia on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Data were compared 2, 3, 4, 6 and 8 h after inoculation. The results are expressed as a % of total conidia and probability values (p) obtained from Student's t test.

| Sampling time (h) | GERMINATION (total) | | |
|---|---|---|---|
| | BS10 | BS12 | P |
| 2 | 11.4 | 8.9 | 0.2 |
| 3 | 39.2 | 22.1 | 0.01 |
| 4 | 59.9 | 21.9 | 0.001 |
| 6 | 68.3 | 30.8 | 0.001 |
| 8 | 78.9 | 42.6 | 0.001 |
| | BS10 | JI1047 | P |
| 2 | 11.4 | 3.0 | 0.001 |
| 3 | 39.2 | 41.6 | 0.4 |
| 4 | 59.9 | 43.5 | 0.001 |
| 6 | 68.3 | 48.9 | 0.01 |
| 8 | 78.9 | 53.2 | 0.001 |
| | BS10 | JI1048 | P |
| 2 | 11.4 | 5.1 | 0.001 |
| 3 | 39.2 | 14.1 | 0.001 |
| 4 | 59.9 | 24.3 | 0.001 |
| 6 | 68.3 | 38.0 | 0.01 |
| 8 | 78.9 | 46.5 | 0.001 |
| | BS10 | JI1049 | P |
| 2 | 11.4 | 50.1 | 0.001 |
| 3 | 39.2 | 72.2 | 0.001 |
| 4 | 59.9 | 68.2 | 0.02 |
| 6 | 68.3 | 81.0 | 0.05 |
| 8 | 78.9 | 70.0 | 0.1 |
| | BS10 | JI1050 | P |
| 2 | 11.4 | 4.6 | 0.001 |
| 3 | 39.2 | 11.1 | 0.001 |
| 4 | 59.9 | 27.1 | 0.001 |
| 6 | 68.3 | 29.9 | 0.001 |
| 8 | 78.9 | 38.5 | 0.001 |

Fig. 7 Comparisons of germination frequencies on susceptible and resistant cultivars of pea in the early stages of the infection process (0-8 h) following "evening"- inoculation.

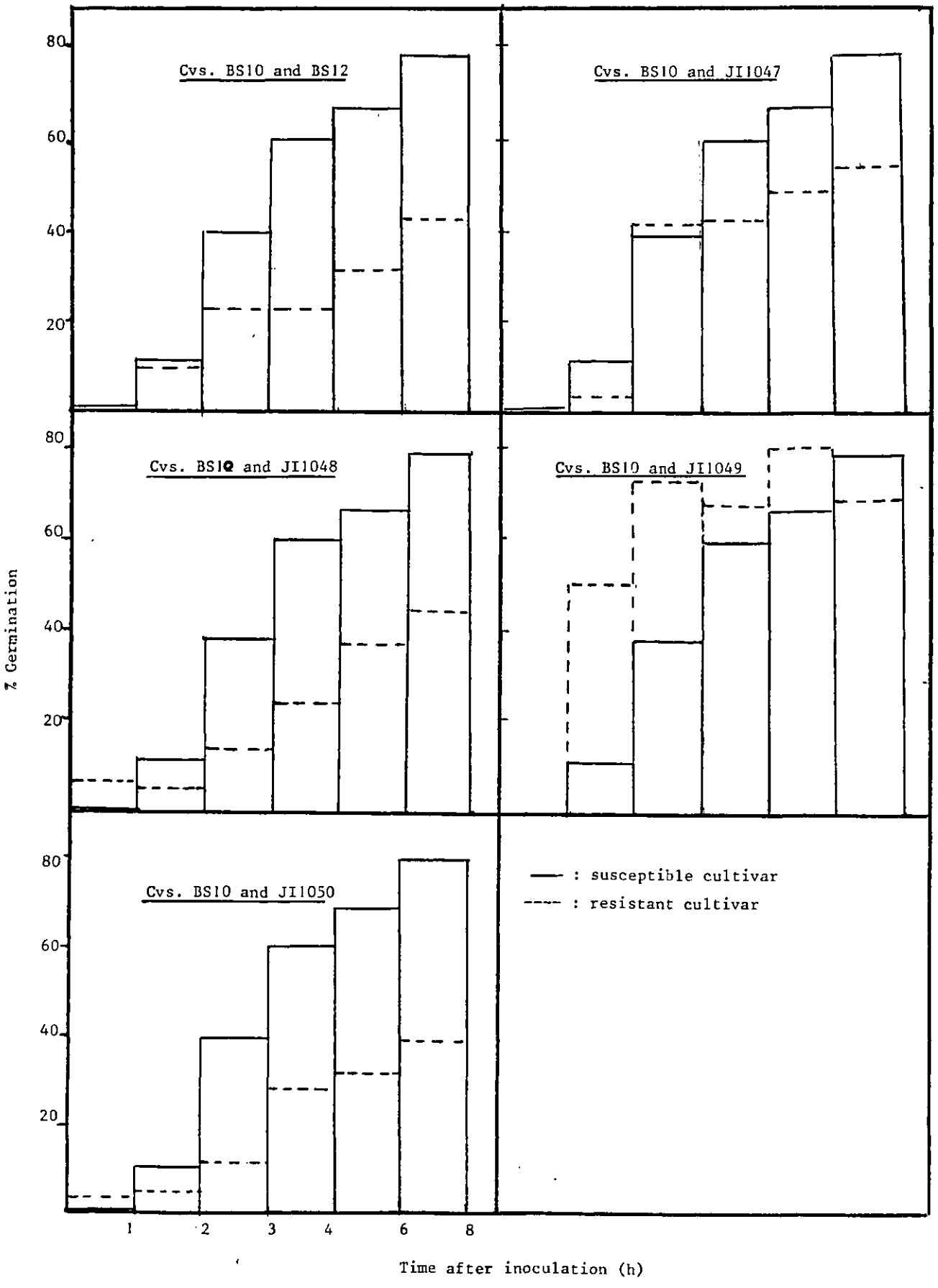


Table 14 Statistical analysis of the data presented in Table 12 to compare the effect of cultivar on the formation of appressorial initials on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Data were compared 3, 4, 6 and 8 h after inoculation. The results are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| Sampling time (h) | APPRESSORIAL INITIAL | | |
|---|---|---|---|
| | BS10 | BS12 | p |
| 3 | 7.9 | 0 | 0.01 |
| 4 | 13.9 | 7.7 | 0.2 |
| 6 | 9.7 | 15.4 | 0.7 |
| 8 | 17.6 | 23.7 | 0.2 |
| | BS10 | JI1047 | p |
| 3 | 7.9 | 7.6 | > 0.9 |
| 4 | 13.9 | 26.7 | 0.01 |
| 6 | 9.7 | 11.3 | 0.6 |
| 8 | 17.6 | 9.9 | 0.1 |
| | BS10 | JI1048 | p |
| 3 | 7.9 | 0 | 0.01 |
| 4 | 13.9 | 7.4 | > 0.9 |
| 6 | 9.7 | 13.9 | 0.3 |
| 8 | 17.6 | 19.3 | 0.7 |
| | BS10 | JI1049 | p |
| 3 | 7.9 | 38.4 | 0.001 |
| 4 | 13.9 | 25.9 | 0.01 |
| 6 | 9.7 | 19.2 | 0.01 |
| 8 | 17.6 | 36.9 | 0.01 |
| | BS10 | JI1050 | p |
| 3 | 7.9 | 1.8 | 0.05 |
| 4 | 13.9 | 10.9 | 0.6 |
| 6 | 9.7 | 20.6 | 0.01 |
| 8 | 17.6 | 17.6 | > 0.9 |

Table 15 Statistical analysis of the data presented in Table 12 to compare the effect of cultivar on formation of mature appressoria on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Data were compared 3, 4, 6 and 8 h after inoculation. The results are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| Sampling time (h) | MATURE APPRESSORIUM | | |
|---|---|---|---|
| | BS10 | BS12 | p |
| 3 | 0 | 0 | |
| 4 | 68.6 | 0 | 0.001 |
| 6 | 83.8 | 38.6 | 0.001 |
| 8 | 65.9 | 29.2 | 0.001 |
| | BS10 | JI1047 | p |
| 3 | 0 | 0 | |
| 4 | 68.6 | 54.6 | 0.01 |
| 6 | 83.8 | 73.5 | 0.01 |
| 8 | 65.9 | 76.9 | 0.1 |
| | BS10 | JI1048 | p |
| 3 | 0 | 0 | |
| 4 | 68.6 | 0.6 | 0.001 |
| 6 | 83.8 | 26.8 | 0.001 |
| 8 | 65.9 | 46.5 | 0.2 |
| | BS10 | JI1049 | p |
| 3 | 0 | 35.0 | 0.001 |
| 4 | 68.6 | 53.0 | 0.01 |
| 6 | 83.8 | 68.3 | 0.01 |
| 8 | 65.9 | 42.9 | 0.02 |
| | BS10 | JI1050 | p |
| 3 | 0 | 0 | |
| 4 | 68.6 | 10.9 | 0.001 |
| 6 | 83.8 | 23.3 | 0.001 |
| 8 | 65.9 | 38.5 | 0.01 |

Fig. 8 Comparisons of frequencies of mature appressorium formation (expressed as a% of germinated conidia) on susceptible and resistant cultivars of pea in the early stages of the infection process (0-8 h) following "evening"- inoculation.

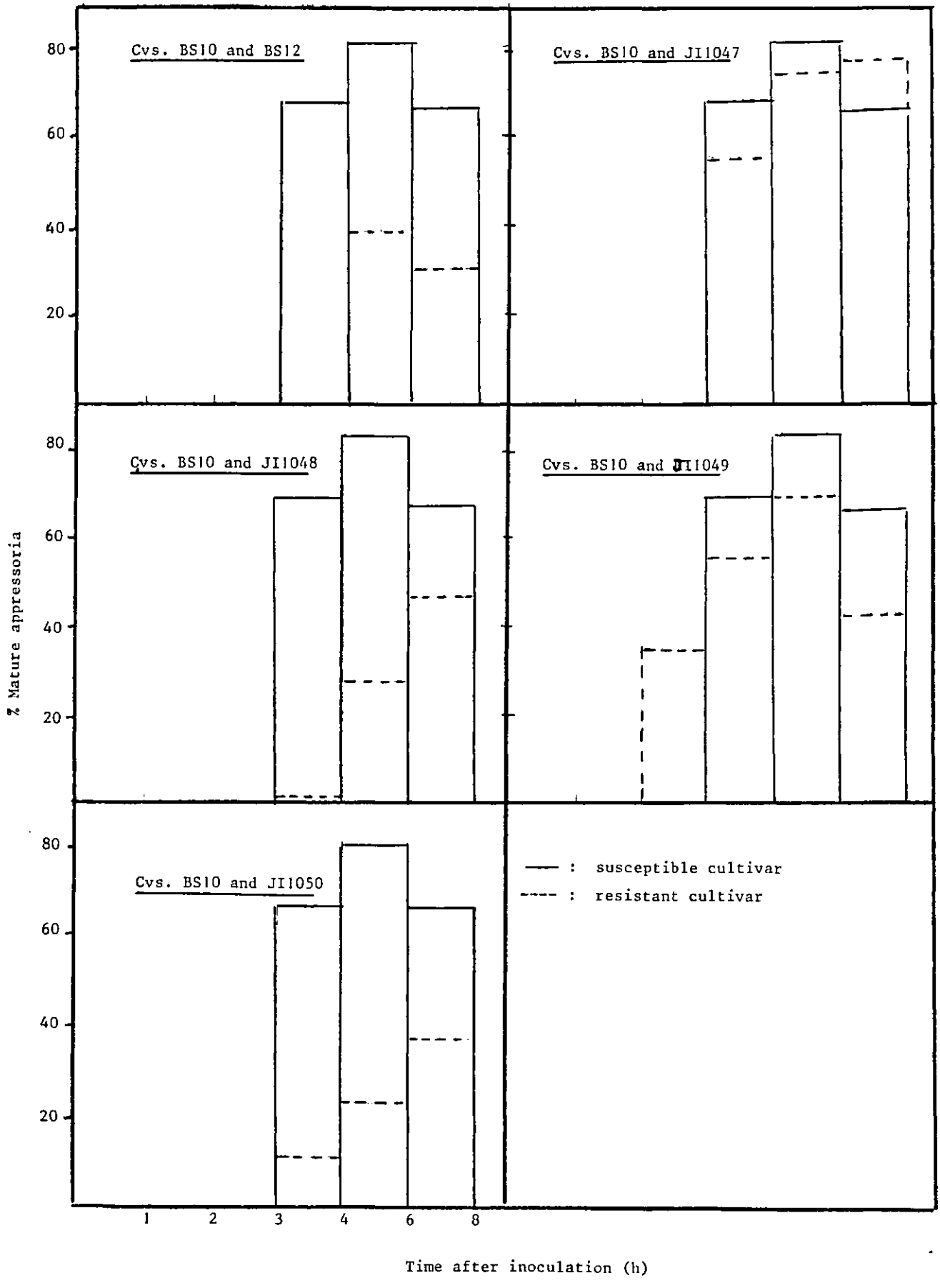


Table 16 Germination of conidia and formation of initial and mature appressoria on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Samples were taken 22, 44, 68, 92, 164 and 240 h after inoculation. The results are expressed as a % of total conidia.

| CULTIVAR | Sampling time (h) | GERMINATION (total) | GERM TUBE ONLY | APPRESSORIAL INITIAL | MATURE APPRESSORIUM |
|---|---|---|---|---|---|
| BS10 | 22 | 38.7 | 8.1 | 1.5 | 29.1 |
| | 44 | 66.1 | 4.2 | 1.8 | 60.1 |
| | 68 | 60.7 | 0.6 | 0.4 | 59.7 |
| | 92 | 91.9 | 2.1 | 3.8 | 86.0 |
| | 164 | 87.2 | 0.9 | 1.2 | 85.1 |
| | 240 | 91.1 | 0.9 | 2.4 | 87.8 |
| BS12 | 22 | 62.8 | 2.5 | 5.6 | 84.7 |
| | 44 | 47.6 | 2.5 | 3.5 | 42.8 |
| | 68 | 38.0 | 1.4 | 3.3 | 33.4 |
| | 92 | 47.2 | 7.7 | 6.9 | 31.5 |
| | 164 | 38.7 | 1.1 | 1.1 | 36.5 |
| | 240 | 41.0 | 2.2 | 3.4 | 36.4 |
| JI1047 | 22 | 59.3 | 2.3 | 8.6 | 48.4 |
| | 44 | 51.7 | 2.2 | 5.5 | 44.0 |
| | 68 | 54.4 | 3.1 | 5.6 | 45.7 |
| | 92 | 57.7 | 1.0 | 2.9 | 53.8 |
| | 164 | 57.6 | 1.9 | 4.3 | 51.4 |
| | 240 | 54.7 | 7.6 | 10.8 | 34.5 |
| JI1048 | 22 | 65.2 | 1.1 | 9.6 | 54.5 |
| | 44 | 60.5 | 2.1 | 6.6 | 51.8 |
| | 68 | 44.9 | 3.5 | 5.4 | 36.0 |
| | 92 | 35.9 | 0 | 7.6 | 28.3 |
| | 164 | 40 2 | 0.4 | 2.4 | 37.4 |
| | 240 | 67.7 | 5.4 | 0 | 62.3 |
| JI1049 | 22 | 54.6 | 1.5 | 7.6 | 45.5 |
| | 44 | 51.6 | 3.1 | 13.6 | 41.9 |
| | 68 | 53.8 | 2.3 | 7.4 | 44.1 |
| | 92 | 56.8 | 1.0 | 3.1 | 52.7 |
| | 164 | 53.1 | 0.6 | 1.2 | 51.3 |
| | 240 | 58.4 | 1.3 | 3.1 | 54.0 |
| JI1050 | 22 | 48.8 | 1.6 | 8.8 | 35.4 |
| | 44 | 39.0 | 0.1 | 9.3 | 28.6 |
| | 68 | 34.0 | 1.5 | 11.9 | 20.6 |
| | 92 | 58.8 | 9.5 | 15.3 | 34.0 |
| | 164 | 45.5 | 4.0 | 6.3 | 35.2 |
| | 240 | 50.1 | 0.6 | 3.0 | 46.5 |

Table 17 Germination of conidia and formation of initial and mature appressoria on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Samples were taken 22, 44, 68, 92, 164 and 240 h after inoculation. The results are expressed as a % of germinated conidia.

| CULTIVAR | Sampling time (h) | GERM TUBE ONLY | APPRESSORIAL INITIAL | MATURE APPRESSORIUM |
|---|---|---|---|---|
| BS10 | 22 | 20.3 | 4.0 | 75.7 |
| | 44 | 6.1 | 2.4 | 91.5 |
| | 68 | 2.0 | 0.6 | 98.4 |
| | 92 | 2.3 | 4.2 | 93.5 |
| | 164 | 1.0 | 2.1 | 97.7 |
| | 240 | 1.1 | 2.5 | 96.4 |
| BS12 | 22 | 3.8 | 9.0 | 87.2 |
| | 44 | 3.2 | 9.2 | 78.6 |
| | 68 | 3.9 | 9.7 | 86.4 |
| | 92 | 16.0 | 17.1 | 66.9 |
| | 164 | 2.0 | 3.0 | 95.0 |
| | 240 | 5.4 | 5.8 | 88.8 |
| JI1047 | 22 | 4.0 | 14.7 | 81.3 |
| | 44 | 4.5 | 11.6 | 83.9 |
| | 68 | 3.6 | 10.7 | 74.8 |
| | 92 | 1.7 | 5.0 | 93.3 |
| | 164 | 3.4 | 7.8 | 88.8 |
| | 240 | 13.8 | 19.7 | 66.5 |
| JI1048 | 22 | 1.6 | 15.0 | 83.4 |
| | 44 | 3.3 | 7.0 | 85.7 |
| | 68 | 5.1 | 11.0 | 83.9 |
| | 92 | 2.4 | 18.8 | 78.8 |
| | 164 | 1.1 | 6.0 | 92.9 |
| | 240 | 3.3 | 4.7 | 92.0 |
| JI1049 | 22 | 3.9 | 13.9 | 83.2 |
| | 44 | 6.7 | 12.5 | 80.8 |
| | 68 | 4.4 | 13.7 | 81.9 |
| | 92 | 1.7 | 5.6 | 92.7 |
| | 164 | 1.0 | 2.6 | 96.4 |
| | 240 | 2.2 | 5.4 | 92.4 |
| JI1050 | 22 | 3.4 | 17.8 | 78.8 |
| | 44 | 2.3 | 31.4 | 59.3 |
| | 68 | 4.3 | 14.8 | 80.9 |
| | 92 | 16.7 | 26.0 | 57.3 |
| | 164 | 8.8 | 13.7 | 77.5 |
| | 240 | 1.2 | 6.1 | 92.7 |

Table 18 Statistical analysis of the data presented in Table 16 to compare the effect of cultivar on germination of conidia on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Data were compared 22, 44, 68, 92, 240 h after inoculation. The results are expressed as a % of total conidia and probability values (p) obtained from Student's t test.

| Sampling time (h) | GERMINATION (total) | | |
|---|---|---|---|
| | BS10 | BS12 | p |
| 22 | 38.7 | 62.8 | 0.001 |
| 44 | 66.1 | 47.6 | 0.02 |
| 68 | 60.7 | 38.0 | 0.01 |
| 92 | 91.9 | 47.2 | 0.001 |
| 164 | 87.2 | 38.7 | 0.001 |
| 240 | 91.1 | 41.0 | 0.001 |
| | BS10 | JI1047 | p |
| 22 | 38.7 | 59.3 | 0.01 |
| 44 | 66.1 | 51.7 | 0.01 |
| 68 | 60.7 | 54.4 | 0.01 |
| 92 | 91.9 | 57.7 | 0.001 |
| 164 | 87.2 | 57.6 | 0.001 |
| 240 | 91.1 | 54.7 | 0.001 |
| | BS10 | JI1048 | p |
| 22 | 38.7 | 65.2 | 0.001 |
| 44 | 66.1 | 60.5 | 0.02 |
| 68 | 60.7 | 44.9 | 0.001 |
| 92 | 91.9 | 35.9 | 0.001 |
| 164 | 87.2 | 40.2 | 0.001 |
| 240 | 91.1 | 67.7 | 0.001 |
| | BS10 | JI1049 | p |
| 22 | 38.7 | 54.6 | 0.05 |
| 44 | 66.1 | 51.6 | 0.01 |
| 68 | 60.7 | 53.8 | 0.001 |
| 92 | 91.9 | 56.8 | 0.001 |
| 164 | 87.2 | 53.1 | 0.001 |
| 240 | 91.1 | 58.4 | 0.001 |
| | BS10 | JI1050 | p |
| 22 | 38.7 | 48.8 | 0.01 |
| 44 | 66.1 | 39.0 | 0.01 |
| 68 | 60.7 | 34.0 | 0.02 |
| 92 | 91.9 | 58.8 | 0.001 |
| 164 | 87.2 | 45.5 | 0.001 |
| 240 | 91.1 | 50.1 | 0.001 |

Table 19 Statistical analysis of the data presented in Table 17 to compare the effect of cultivar on the formation of appressorial initials on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Data were compared 22, 44, 68, 92, 164 and 240 h after inoculation. The results are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| Sampling time (h) | GERMINATION (total) | | |
|---|---|---|---|
| | BS10 | BS12 | p |
| 22 | 4.0 | 9.0 | 0.01 |
| 44 | 2.4 | 9.2 | 0.01 |
| 68 | 0.6 | 9.7 | 0.001 |
| 92 | 4.2 | 17.1 | 0.001 |
| 164 | 2.1 | 3.0 | 0.5 |
| 240 | 2.5 | 5.8 | 0.05 |
| | BS10 | JI1047 | p |
| 22 | 4.0 | 14.7 | 0.01 |
| 44 | 2.4 | 11.6 | 0.01 |
| 68 | 0.6 | 10.7 | 0.01 |
| 92 | 4.2 | 5.0 | 0.6 |
| 164 | 2.1 | 7.8 | 0.05 |
| 240 | 2.5 | 19.7 | 0.001 |
| | BS10 | JI1048 | p |
| 22 | 4.0 | 15.0 | 0.01 |
| 44 | 2.4 | 7.0 | 0.01 |
| 68 | 0.6 | 11.0 | 0.001 |
| 92 | 4.2 | 18.8 | 0.01 |
| 164 | 2.1 | 6.0 | 0.3 |
| 240 | 2.5 | 4.7 | 0.2 |
| | BS10 | JI1049 | p |
| 22 | 4.0 | 13.9 | 0.02 |
| 44 | 2.4 | 12.5 | 0.02 |
| 68 | 0.6 | 13.7 | 0.001 |
| 92 | 4.2 | 5.6 | 0.5 |
| 164 | 2.1 | 2.6 | 0.9 |
| 240 | 2.5 | 5.4 | 0.01 |
| | BS10 | JI1050 | p |
| 22 | 4.0 | 17.8 | 0.001 |
| 44 | 2.4 | 31.4 | 0.01 |
| 68 | 0.6 | 14.8 | 0.01 |
| 92 | 4.2 | 26.0 | 0.001 |
| 164 | 2.1 | 13.7 | 0.001 |
| 240 | 2.5 | 6.1 | 0.2 |

Fig. 9 Comparisons of germination frequencies on susceptible and resistant cultivars of pea in the total infection period (22-240 h) following "evening" inoculation.

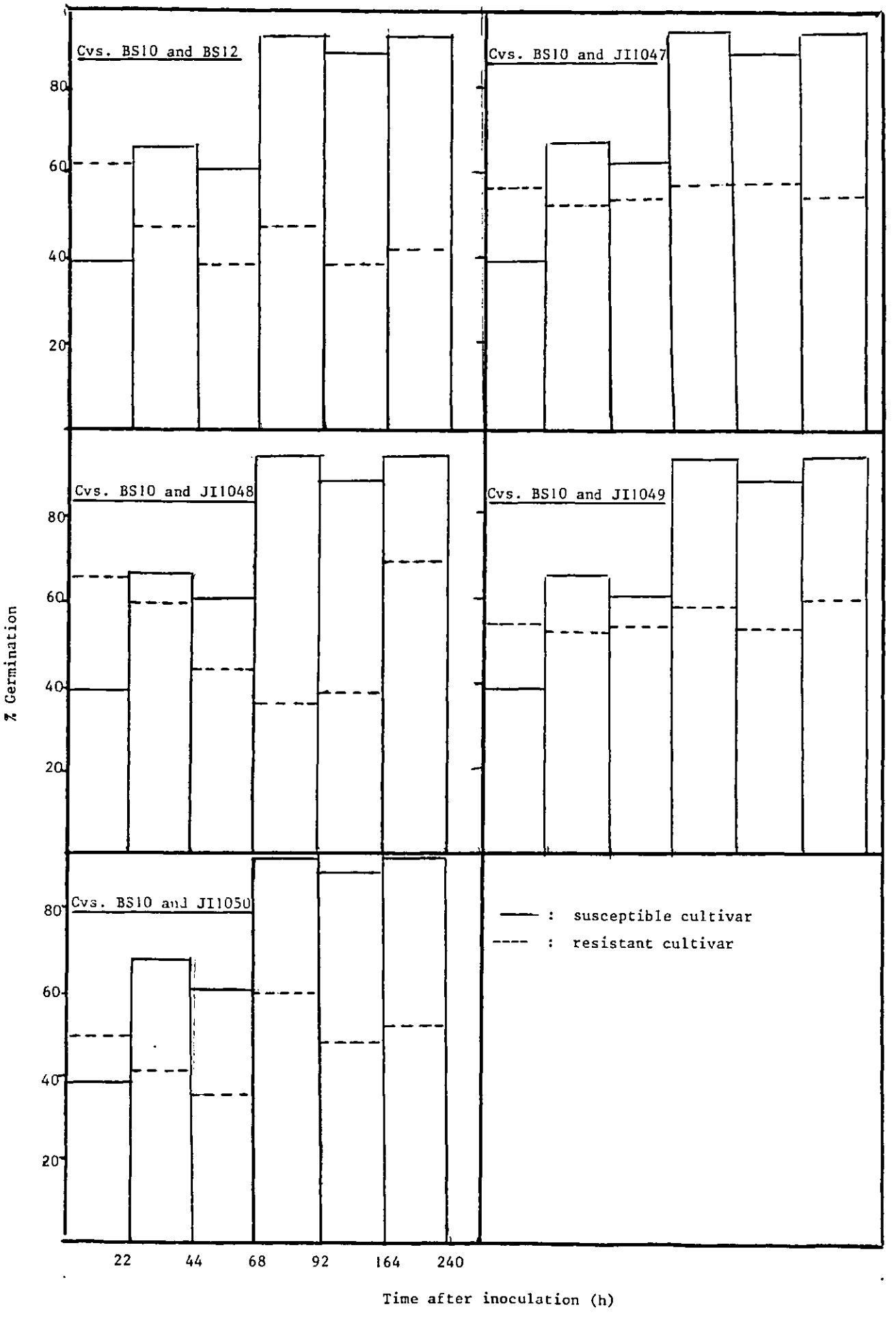


Table 20 Statistical analysis of the data presented in Table 17 to compare the effect of cultivar on the formation of mature appressoria on the oldest leaves of plants 22 days after sowing following "evening"-inoculation. Data were compared 22, 44, 68, 92, 164 and 240 h after inoculation. The results are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| Sampling time (h) | MATURE APPRESSORIUM | | |
|---|---|---|---|
| | BS10 | BS12 | p |
| 22 | 75.7 | 87.2 | 0.01 |
| 44 | 91.5 | 78.6 | 0.1 |
| 68 | 98.4 | 86.4 | 0.001 |
| 92 | 93.5 | 66.9 | 0.001 |
| 164 | 97.7 | 95.0 | 0.1 |
| 240 | 96.4 | 88.0 | 0.01 |
| | BS10 | JI1047 | p |
| 22 | 75.7 | 81.3 | 0.2 |
| 44 | 91.5 | 83.9 | 0.1 |
| 68 | 98.4 | 74.8 | 0.01 |
| 92 | 93.5 | 93.3 | 0.9 |
| 164 | 97.7 | 88.8 | 0.05 |
| 240 | 96.4 | 66.5 | 0.001 |
| | BS10 | JI1048 | p |
| 22 | 75.7 | 83.4 | 0.1 |
| 44 | 91.5 | 85.7 | 0.2 |
| 68 | 98.4 | 83.9 | 0.01 |
| 92 | 93.5 | 78.8 | 0.01 |
| 164 | 97.7 | 92.9 | 0.02 |
| 240 | 96.4 | 92.0 | 0.001 |
| | BS10 | JI1049 | p |
| 22 | 75.7 | 83.2 | 0.1 |
| 44 | 91.5 | 80.8 | 0.05 |
| 68 | 98.4 | 81.9 | 0.001 |
| 92 | 93.5 | 92.7 | 0.9 |
| 164 | 97.7 | 96.4 | 0.3 |
| 240 | 96.4 | 92.4 | 0.3 |
| | BS10 | JI1050 | p |
| 22 | 75.7 | 78.8 | 0.4 |
| 44 | 91.5 | 59.3 | 0.01 |
| 68 | 98.4 | 80.9 | 0.001 |
| 92 | 93.5 | 57.3 | 0.001 |
| 164 | 97.7 | 77.5 | 0.001 |
| 240 | 96.4 | 92.7 | 0.01 |

Fig. 10 Comparisons of frequencies of mature appressorium formation (expressed as a % of germinated conidia) on susceptible and resistant cultivars of pea in the total infection period (22-240 h) following "evening"- inoculation.

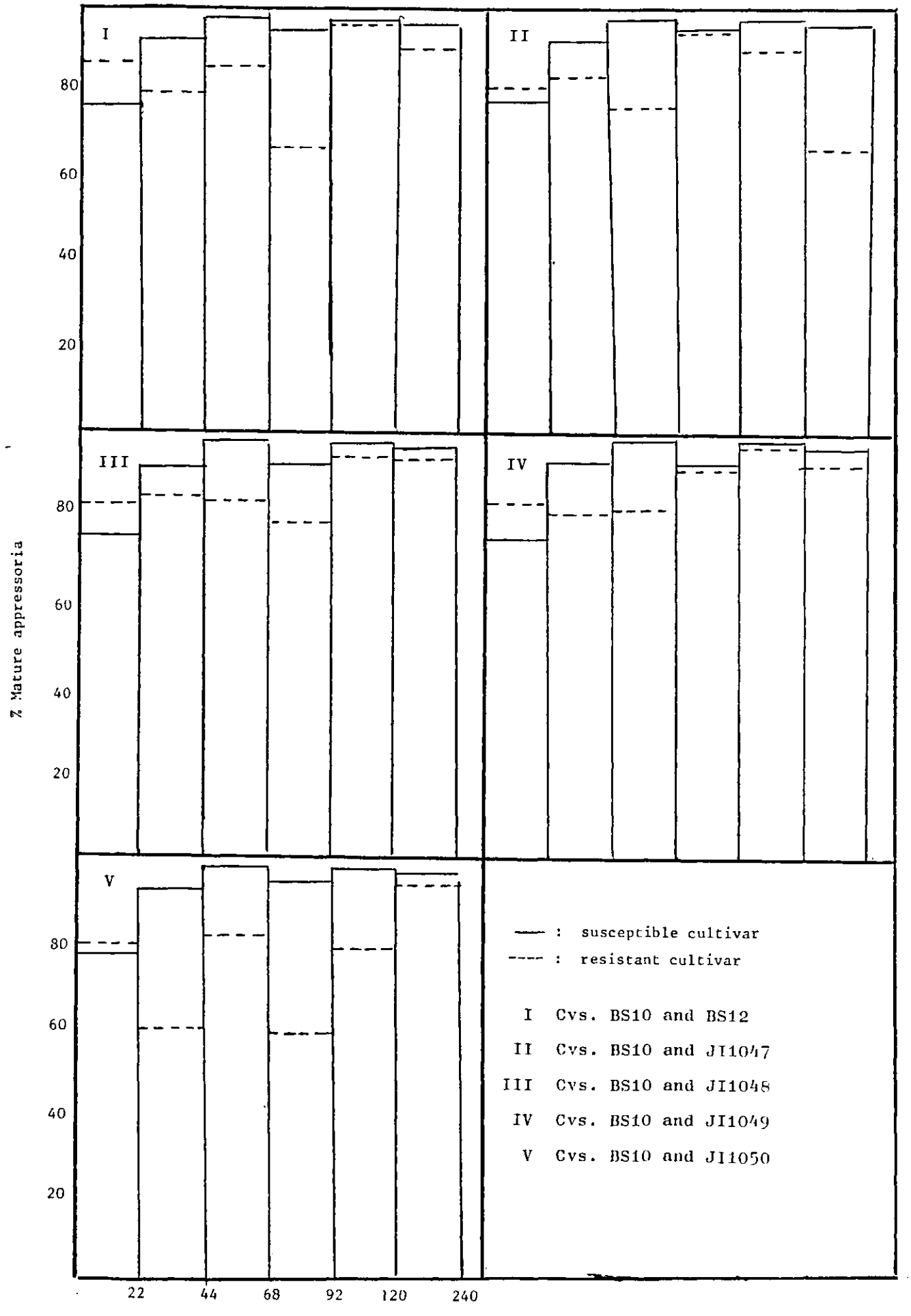
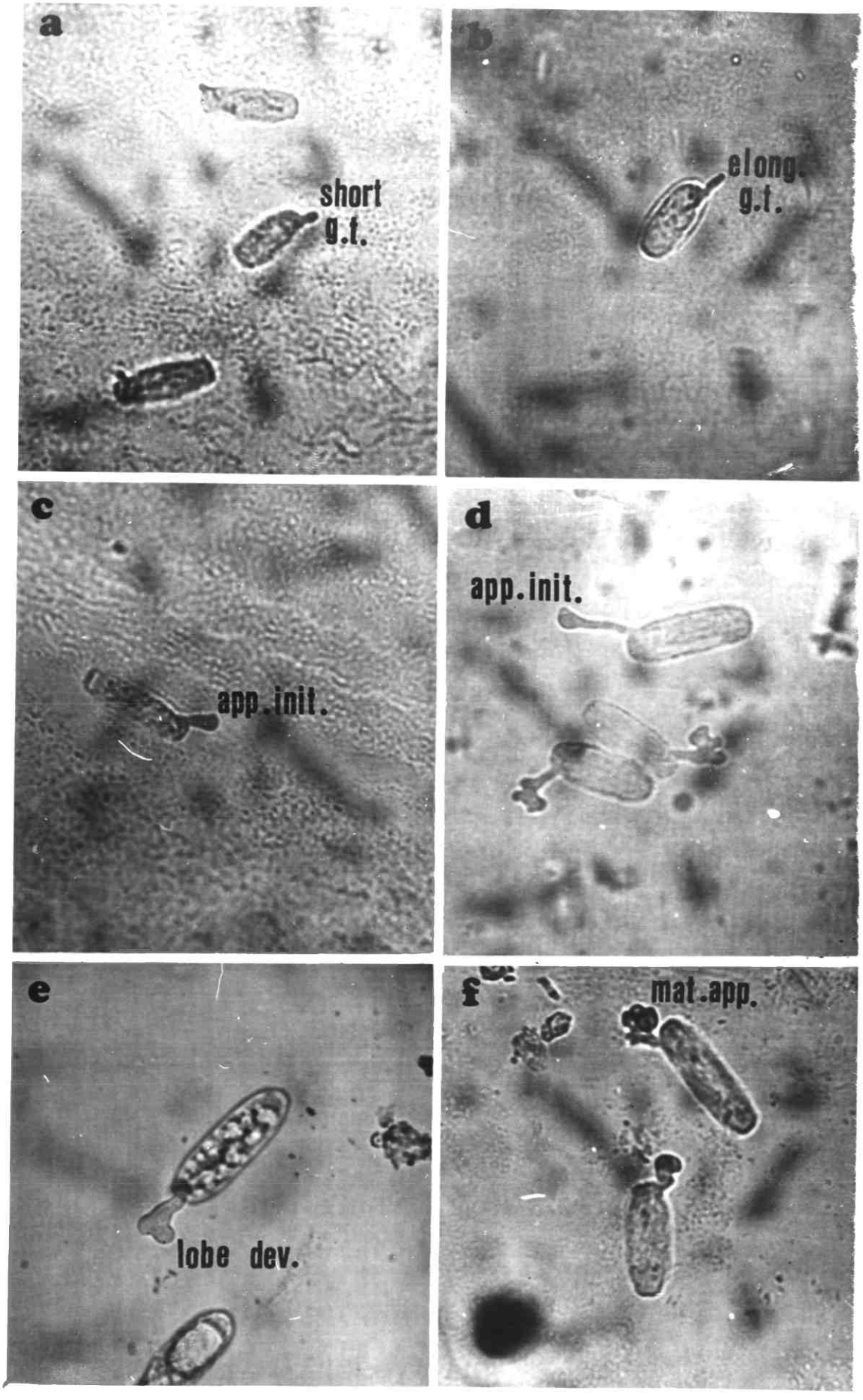


Plate 1. Light micrographs of conidium germination and appressorium formation by *E. pisi* on *P. sativum*. x 340

a. Conidia with short primary germ tubes.
b. Conidium with an elongating primary germ tube.
c. Conidium with an appressorial initial.
d. Conidia with appressoria at the initial and lobed stages.
e. Conidium with a lobed appressorium.
f. Conidium with a mature appressorium.


a
short
g.t.
b
elong.
g.t.
c
app. init.
d
app. init.
e
lobe dev.
f
mat. app.

maturation. It has been suggested that germination of conidia and formation of appressoria may be affected by the chemical constituents of epicuticular waxes either directly or by their influence on the passage of stimulatory or inhibitory chemicals from within the leaf (Martin, 1964, Martin and Juniper, 1970). Other workers have postulated that the size and spatial distribution of the wax subunits may influence appressodium formation (Dickinson, 1970, Emmett and Parbery 1975). Thus, the morphology of the epicuticular waxes on the adaxial epidermis of leaves of the susceptible and resistant cultivars was studied to discover if there were any differences which could be correlated with the frequencies of germination and appressorium formation on them.

4.2 Experimental

The oldest leaves from uninfected 15- and 22- day plants of the six test cultivars were prepared for scanning electron microscopy by air-drying as described above (IV I).

4.3 Results

The physical structure of the epicuticular waxes on the adaxial leaf surfaces of the six test cultivars was of three types. Waxes on cultivars BS10 and BS12 (Plate 2 ) consisted of densely packed, thin, pointed, randomly orientated rods projecting from the cuticular surface. The number and size of the epicuticular projections were markedly smaller over the subsidiary and guard cells. Cultivars JI1047, JI1048 and JI1050 (Plate 3 ) exhibited similar wax structures which were in the form of densely packed jagged platelets projecting from the cuticle surface

but shorter than the projections seen on BS10 and BS12. As for the latter cultivars, the density was lower around the stomata. The waxes on cultivar JI1049 (Plate 4) consisted of a sculptured reticulum with small projections which assumed the form of flakes from which projected very short rods.

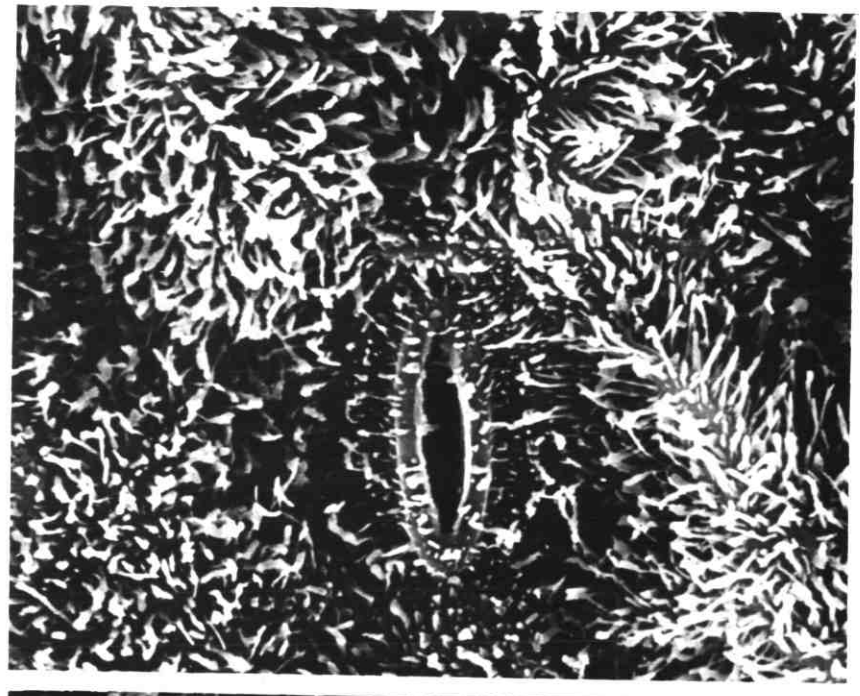
There was no difference in the morphology or density of the waxes on 15- and 22- day plants.

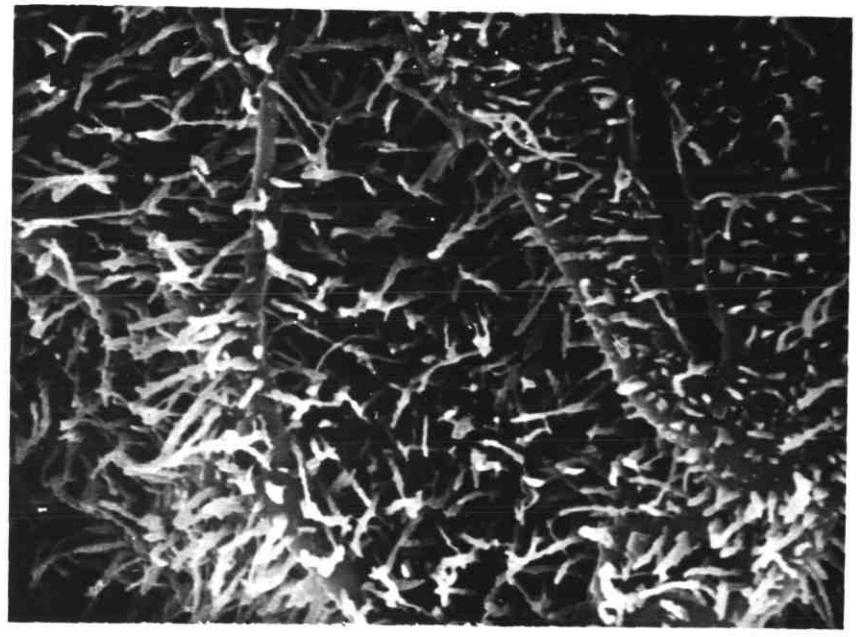
Plate 2. Scanning electron micrographs of epicuticular waxes on the adaxial leaf epidermis of cultivars BS10 and BS12 of Pisum sativum.

a. x 2,500.

b. x 5,000.

c. x 10,000.





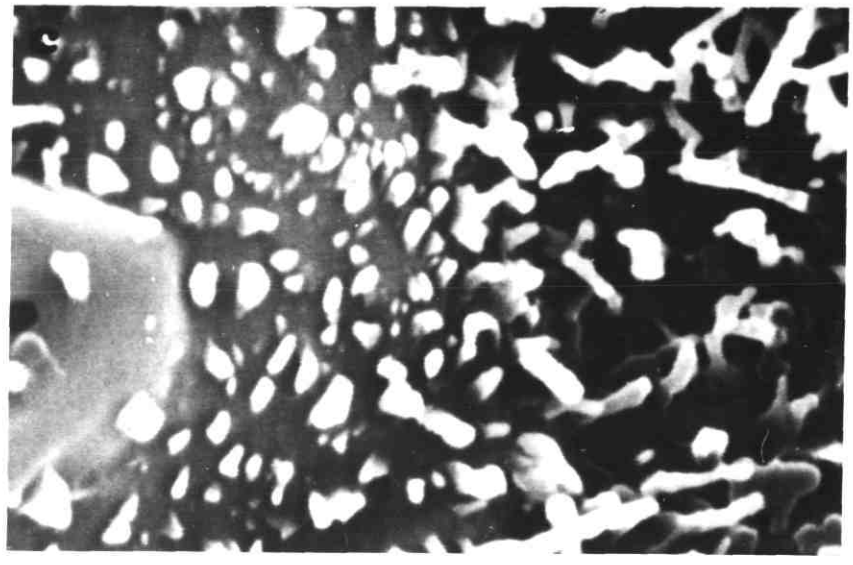


Plate 3. Scanning electron micrographs of epicuticular waxes on the adaxial leaf epidermis of cultivars JI1047, JI1048 and JI1050 of Pisum sativum.

a. x 2,500.

b. x 5,000.

c. x 10,000.

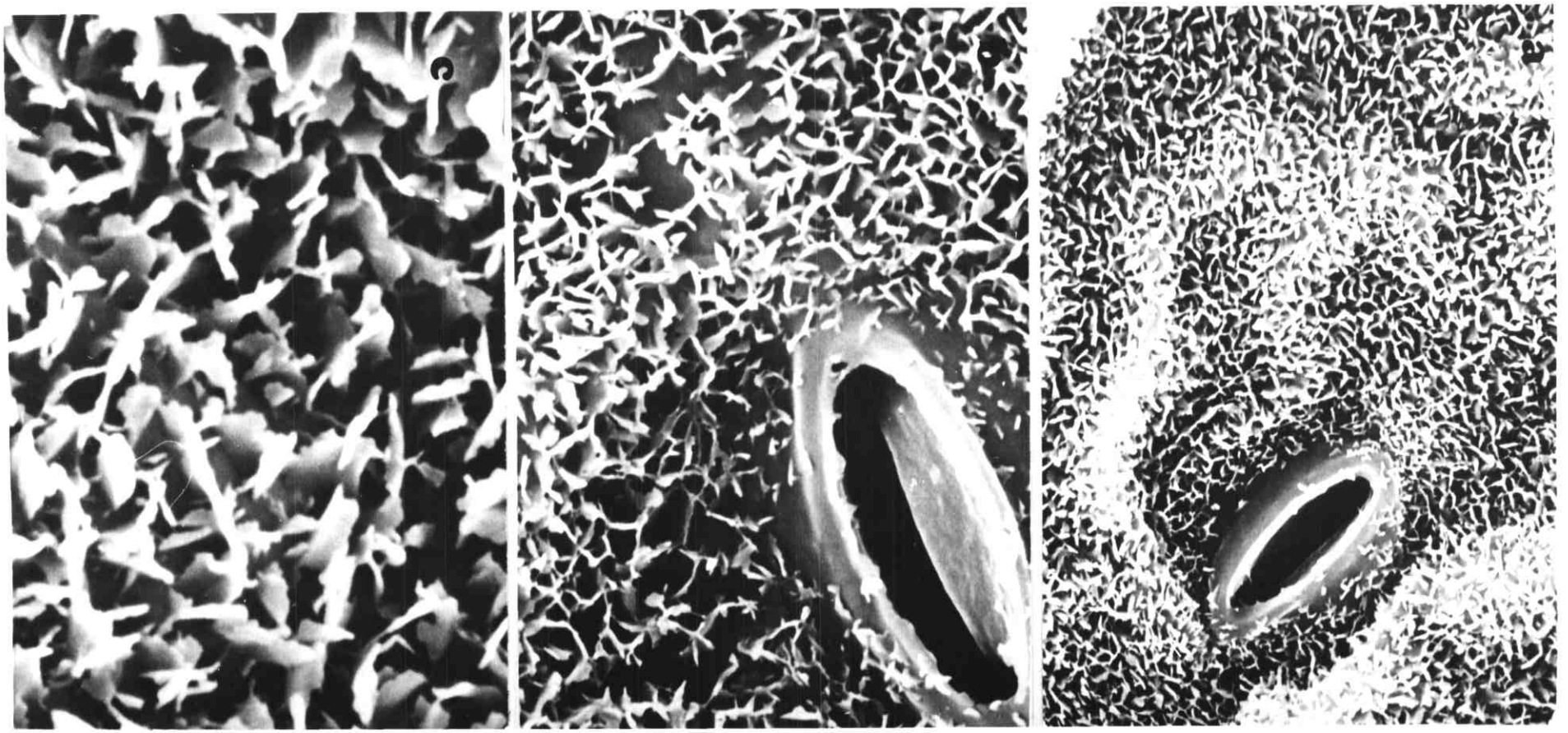
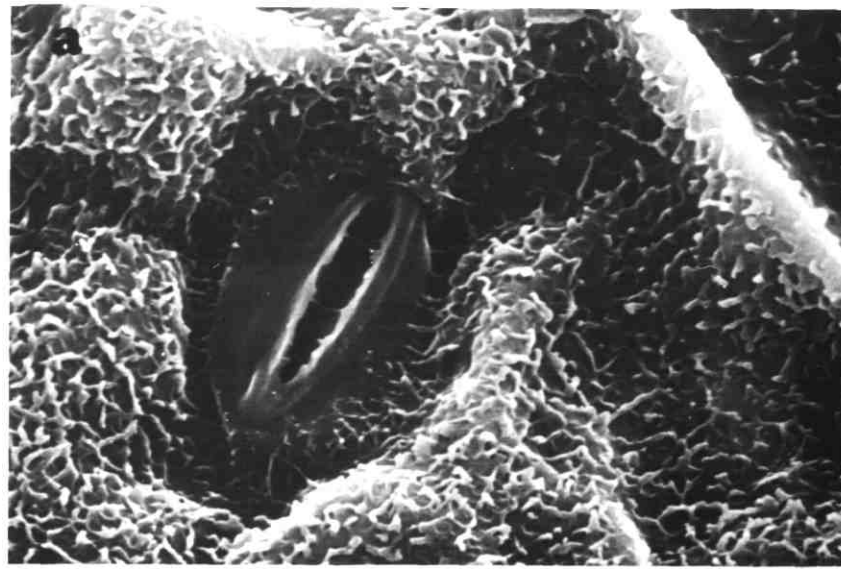

C
P
B

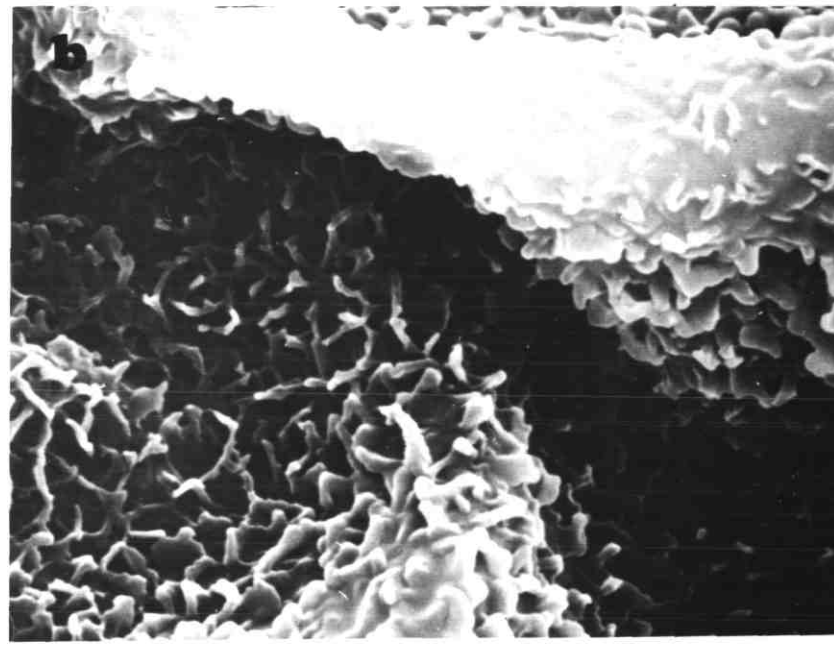
Plate 4. Scanning electron micrographs of epicuticular waxes on the adaxial leaf epidermis of cultivar JI1049 of *Pisum sativum*.

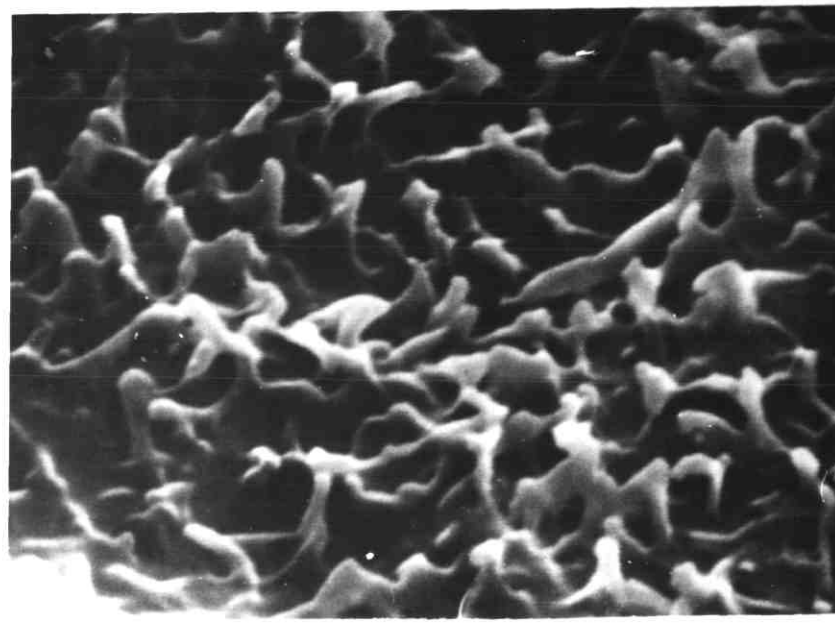
a. x 2,500.

b. x 5,000.

c. x 10,000.


a


b



b Production of haustoria

1 Investigation of the effect of cultivar on the development of haustoria using bright field light microscopy

1.1 Introduction

The purpose of these investigations was to discover the effect of cultivar on the initiation and development of haustoria. The lengths and widths of haustoria, the surface area of the extra haustorial membranes and the enclosed volumes were assessed.

1.2 Experimental

After "evening"- inoculation (BI 5.2) the six 22-day test cultivars were incubated in a greenhouse with 16 h light and 8 h dark periods. Samples were taken 12, 14, 17, 18, 19 and 20 h after inoculation by removing epidermal strips (BII 4.1) which were than stained with acid fuchsin in lactophenol (BII 1.4). The presence or absence of haustoria in each cultivar were recorded. Measurements were made, using a micrometer eyepiece, of the lengths and widths of at least twenty haustoria in each time sample and mean values are recorded in Table 21. In the 12 and 14 h samples the haustoria were very small and they were not measured. The mean surface area of the extra haustorial membrane and enclosed volume in each cultivar were calculated from the data, using the following formulae (Manners, 1979):

$$\text{Surface area: } = 4\pi\ (w/2)^2 + 2\pi\ (w/2)\ (l-w)$$

$$\text{Volume : } = 4/3\pi\ (w/2)^3 + \pi\ (w/2)^2\ (l-w)$$

where l = length and w = width.

The calculated results are recorded in Tables 22a and 22b, respectively. The experiment was repeated taking samples after 24 h and the data obtained are entered into Table 21.

1.3 Results

Haustoria were first observed between 12 and 14 h on all the cultivars and it was clear that they were more frequent on BS10. Quantitative data on this point are given in section 2. Lobe development was noted by 16 h on cultivars BS10, JI1047 and JI1049 and by 18 h on the other cultivars. There was no difference in the degree of lobe development on haustoria in cultivars BS10, JI1047 and JI1049, nor between those in the other three cultivars. At 20 h the lobes in the first group were more extensive than in the other cultivars and this is correlated with their earlier initiation. At 17 h the longest haustoria occurred in cultivars BS10 and JI1047 (Table 21). The mean lengths of haustoria in all cultivars increased gradually throughout the experimental period the greatest rates of increase occurring between 20 and 24 h in cultivars BS10 and BS12 and between 19 and 20 h in JI1047. There was no rapid increase in length in the other cultivars.

By also taking account of the widths of haustoria the mean volumes calculated showed increases on all the cultivars throughout the experimental period (Table 22a). However, the growth rates varied and at 17 h the volumes of haustoria were greatest on cultivars BS10 and JI1047 (68.4 and 60.1 $\mu m^3$, respecitvely). By 24 h the highest value was recorded in cultivars BS10 (897.9 $\mu m^3$) and the lowest values in JI1048, JI1049 and JI1050 (38.1, 103.1 and 67.3 $\mu m^3$, respec-

Table 21 Mean lengths and widths of haustorial complexes produced in the oldest leaves of 22-day plants following "evening"-inoculation. Samples were taken 17, 18 19, 20 and 24 h after inoculation. The 24 h sample was from a separate experiment.

| | CULTIVAR | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BS10 | | BS12 | | JI1047 | | JI1048 | | JI1049 | | JI1050 | |
| Sampling time (h) | Length (µm) | Width (µm) | Length (µm) | Width (µm) | Length (µm) | Width (µm) | Length (µm) | Width (µm) | Length (µm) | Width (µm) | Length (µm) | Width (µm) |
| 17 | 7.3 | 3.8 | 5.2 | 3.1 | 7.1 | 3.6 | 5.6 | 3.7 | 6.2 | 3.4 | 5.2 | 3.0 |
| 18 | 7.1 | 3.5 | 6.8 | 3.0 | 7.5 | 4.0 | 6.5 | 4.7 | 6.8 | 3.4 | 5.1 | 3.2 |
| 19 | 8.4 | 4.1 | 7.4 | 4.0 | 8.5 | 3.8 | 6.0 | 4.2 | 6.9 | 3.8 | 6.1 | 3.2 |
| 20 | 7.9 | 4.0 | 8.0 | 4.2 | 15.5 | 3.8 | 6.8 | 4.8 | 7.2 | 3.7 | 6.7 | 3.6 |
| 24 | 12.8 | 10.6 | 12.1 | 8.2 | 13.1 | 9.2 | 5.8 | 3.2 | 7.1 | 4.9 | 7.2 | 3.8 |

Tables 22a and b Mean surface areas ($\mu m^2$) if the extra-haustorial membrane around individual haustoria (Table 22a) and mean volumes ($\mu m^3$) enclosed by it (Table 22b) in the oldest leaves of 22-day plants following "evening"-inoculation. The values are calculated from data in Table 21 using the formulae given in b1.2.

Table 22a

| Sampling time (h) | CULTIVAR | | | | | |
|---|---|---|---|---|---|---|
| | BS10 | BS12 | JI1047 | JI1048 | JI1049 | JI1050 |
| 17 | 87.1 | 50.6 | 80.3 | 65.1 | 66.2 | 49.0 |
| 18 | 78.0 | 64.1 | 94.2 | 101.6 | 72.6 | 51.3 |
| 19 | 108.1 | 92.9 | 100.2 | 79.1 | 82.3 | 61.3 |
| 20 | 99.2 | 96.7 | 184.9 | 102.5 | 83.7 | 75.7 |
| 24 | 426.0 | 311.7 | 378.5 | 58.3 | 109.3 | 85.9 |

Table 22b

| Sampling time (h) | CULTIVAR | | | | | |
|---|---|---|---|---|---|---|
| | BS10 | BS12 | JI1047 | JI1048 | JI1049 | JI1050 |
| 17 | 68.4 | 31.4 | 60.1 | 47.0 | 46.0 | 29.7 |
| 18 | 57.1 | 40.9 | 77.5 | 85.6 | 51.4 | 32.5 |
| 19 | 92.8 | 76.2 | 80.9 | 63.7 | 63.9 | 40.5 |
| 20 | 82.5 | 78.4 | 161.4 | 94.1 | 63.0 | 56.1 |
| 24 | 898.0 | 494.6 | 667.0 | 38.1 | 103.1 | 67.3 |

tively). Similarly, the mean surface area of the extra haustorial membranes of haustoria increased throughout the experimental period (Table 22b) and by 24 h was highest in cultivar BS10 (426 $\mu m^2$). Lower but similar areas were found with cultivars BS12 and JI1047 (311.7 and 378 $\mu m^2$, respectively). The smallest areas were in cultivars JI1048, JI1049 and JI1050 where they were approximately only 1/8 to 1/4 of those around individual haustoria on BS10.

2 Numbers of haustoria in susceptible and resistant cultivars

2.1 Introduction

Results from the previous experiment (CIIIb 1.3) indicated that haustoria were less frequent in the resistant cultivars at the early stages of haustorium formation. It was necessary to find out if this was due to a delay in the formation of haustoria or due to failure by some conidia to produce haustoria in these cultivars. In addition, in Erysiphe graminis the effect of the time of inoculation with respect to the photocycle has been shown to influence germination (Nair and Ellingboe, 1962, 1965), formation of appressoria (Masri and Ellingboe, 1963, 1966) and haustorium development (Hirata, 1967) and, therefore, experiments were carried out to quantify the production of haustoria in susceptible and resistant cultivars throughout the infection period following "morning"- and "evening"- inoculation.

2.2 Experimental

After "morning"- (BI 5.1) and "evening"- (BI 5.2) inoculation 22-day old plants were incubated in a Fison's

Fitotron, 600G3/THTL with 16 h light and 8 h dark periods. The oldest leaves of the plants were sampled after 24, 48, 72 and 96 h in the "morning"- batch and after 16, 20 and 41 h in the "evening"- batch, with give replicates in each case. The surface fungal structures were prepared for observation on Necoloidine films (BII 3.2) and stained in lactophenol cotton blue (BII 1.2). The outline of each leaf was drawn onto a transparent plastic film. The leaf diagrams were then overlayed with graph paper on an illuminated viewing screen and the leaf outlines traced. Most of the epidermis was stripped from each leaf, stained in acid fuchsin (BII 1.4) and stored in lactophenol (BII 1.1). The stripped areas were recorded in the leaf outlines already prepared and also on the Necoloidine films so that counts of conidia could be made within the areas corresponding to stripped epidermis. The total leaf area and that of the epidermal strips was calculated from the diagrams on the graph paper. Counts were made of the haustoria in the epidermal strips, of the germinated conidia and of conidia with mature appressoria on the whole Necoloidine films and on the film areas corresponding to the stripped epidermis. The numbers of conidia producing appressoria (expressed as a percentage of germinated conidia) and conidia producing haustoria (expressed as a percentage of the mature appressoria) are recorded in Tables 23 and 24 for "morning"- and "evening"- inoculated plants, respectively. The data for each sample is based upon 60-150 $mm^2$ of leaf.

## 2.3 Results

### 2.3.1 Effect of cultivar on the appressorium-haustorium ratio following "evening"-inoculation

Appressoria were formed more frequently on cultivar BS10 throughout the experimental period than on all the resistant cultivars except JI1048 at 96 h (Table 23). Maximum frequencies were reached at 48 h on cultivars BS10 (94.3%) and JI1050 (80.0%), at 72 h on BS12 (82.5%) and at 96 h JI1047 (79.1%), JI1048 (97.0%) and JI1049 (52.8%). The proportions of conidia producing appressoria on the resistant cultivars were 10-15% lower than on BS10 at 24 h. At 48 h they were approximately 50% the BS10 value on all the resistant cultivars except JI1050 where they were only approximately 15% fewer. By 72 h the frequency of appressorium formation on cultivar JI1049 remained at about 50% of the BS10 value but intermediate values were obtained with the other cultivars. By 96 h there was no difference in the frequency of appressorium formation on cultivars BS10 and JI1048 but 42% lower values occurred with JI1049 and the other cultivars gave intermediate results.

Throughout the experimental period appressoria formed haustoria in the highest proportions in cultivar BS10. Haustoria had already formed from 50% of the appressoria at the first sampling time (24 h) and by 72 h all appressoria had haustoria. In the resistant cultivars haustoria were not recorded until 48 h when similar frequencies occurred in cultivars BS12 (5.3%), JI1047 (8.3%) and JI1050 (3.3%) and higher values in JI1048 (20.2%) and JI1049 (16.7%).

During the next 24 h the greatest increase in haustoria formation occurred in cultivar JIJ047 and the least in JI1048, so that 72 h similar frequencies were recorded in cultivars BS12 (28.8%), JI1048 (26.3%) and JI1050 (28.5%), those in JI1047 (48.0%) and JI1049 (45.3%) being greater. At 96 h the highest frequency of haustorium formation occurred in cultivar JI1047 (62.0%) and the lowest in JI1048 (34.2%) and JI1050 (32.7%).

2.3.2 Effect of cultivar on the appressorium-haustorium ration following "morning"-inoculation

The susceptible and two resistant cultivars, JI1047 and JI1049, were tested. Appressoria were formed more frequently on BS10 than on the two resistant cultivars throughout the experimental period (Table 24). Maximum frequencies were reached at 41 h on cultivars BS10 (87.4%) and JI1047 (51.2%) but no increase occurred on JI1049 after 20 h when 47.6% of germinating conidia had formed appressoria. In all time samples following "morning"-inoculation, also, the frequencies of haustorium formation were greater in BS10 than on the two resistant cultivars tested and by 20 and 41 h they were approximately 20% and 30% higher than for JI1049 and JI1047, respectively.

Thus, the results indicate that the likelihood of appressorium formation varied with the different cultivars and that high proportions of appressoria on the resistant cultivars are in some way prevented from producing haustoria after both "morning"- and "evening"- inoculation.

2.3.3 Effect of time of inoculation with respect to the photocycle on the appressorium-haustoria ratio

Although the times of sampling were not identical, the results from the three cultivars used (BS10, JI1047 and JI1049) do not suggest differences in appressorium numbers arising from time of inoculation. Haustoria had formed by 16 h in all cultivars of the "morning"-inoculated batch but their formation was delayed by "evening"-inoculation. The delay was especially marked in the resistant cultivars where the first production of haustoria occurred between 24 and 48 h after inoculation. Furthermore, the frequencies of haustorium formation were greater at 41 h on cultivars JI1047 (38.8%) and JI1049 (42.7%) following "morning"- inoculation than at 48 h in the "evening"- batch (JI1047 - 8.3%, JI1049 - 16.7%).

The results indicate that the formation of haustoria is affected by photocycle. This is clearly shown in the resistant cultivars. Haustoria were produced earlier in the "morning"- inoculated plants and within the period during which comparisons can be made (up to 41 h) their numbers were greater than after "evening"- inoculation.

Table 23 Proportions of conidia producing mature appressoria (expressed as a % of germinated conidia) and of appressoria giving rise to haustoria (expressed as a % of total appressoria) on the oldest leaves of 22-day plants following "evening"- inoculation each result is based upon 60-150mm$^2$ of leaf.

| | CULTIVAR | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BS10 | | BS12 | | JI1047 | | JI1048 | | JI1049 | | JI1050 | |
| Sampling time (h) | Mature appressoria | Haustoria | Mature appressoria | Haustoria | Mature appressoria | Haustoria | Mature appressoria | Haustoria | Mature appressoria | Haustoria | Mature appressoria | Haustoria |
| 24 | 51.4 | 50.0 | 38.9 | 0 | 35.6 | 0 | 41.6 | 0 | 43.4 | 0 | 40.9 | 0 |
| 48 | 94.3 | 68.4 | 44.7 | 5.3 | 41.9 | 8.3 | 41.9 | 20.2 | 44.3 | 16.7 | 80.0 | 3.3 |
| 72 | 95.7 | 100 | 82.5 | 28.8 | 72.8 | 48.0 | 82.6 | 26.3 | 46.3 | 45.3 | 74.1 | 28.5 |
| 96 | 94.1 | 100 | 78.9 | 41.3 | 79.1 | 62.0 | 97.0 | 34.2 | 52.8 | 47.5 | 75.0 | 32.7 |

Table 24 Proportions of conidia producing mature appressoria (expressed as a % of germinated conidia) and of appressoria giving rise to haustoria (expressed as a % of total appressoria) on the oldest leaves of 22-day plants following "morning"-inoculation. Each result is 60-150 $mm^2$ of leaf.

| Sampling time (h) | BS10 | | JI1047 | | JI1049 | |
|---|---|---|---|---|---|---|
| | Mature appressoria | Haustoria | Mature appressoria | Haustoria | Mature appressoria | Haustoria |
| 16 | 48.2 | 57.0 | 28.3 | 27.0 | 30.3 | 28.3 |
| 20 | 63.1 | 62.4 | 39.8 | 29.1 | 47.6 | 43.4 |
| 41 | 87.4 | 65.0 | 51.2 | 38.8 | 46.3 | 42.7 |

## c Production of secondary hyphae

### 1 Introduction

The purpose of these investigations was to discover the effect of cultivar, host plant age and time of inoculation with respect to the photocycle on the production of secondary hyphae. A secondary hypha can be defined as a germ-tube, other than that produced during germination (primary germ tube). It arises from a mature appressorium or directly from a conidium. However, for ease of reference in the tables the secondary hyphae are assigned to the conidia.

### 2 Investigation of the effect of cultivar, age of host plants and time of inoculation with respect to photocycle on the production of secondary hyphae

#### 2.1 Experimental

After "morning"- inoculation (BI 5.1) 15- and 22- day old plants of the six test cultivars were incubated in a greenhouse with 16 h light and 8 h dark periods. The oldest leaves were sampled after 24, 30 and 48 h, with five replicates at each time. The experiment was repeated but with "evening"- (BI 5.2) inoculation of 15- and 22- day old plants and sampling after 17, 19, 24 and 48 h. The fungal structures were prepared for observation on sellotape strips (BII 3.1) and stained in lactophenol cotton blue (BII 1.2). Counts were made of total conidia, those producing mature appressoria and those producing secondary hyphae. These data, expressed as a percentage of total and germinated conidia, are recorded in Tables 25 and 26,

respectively, for the "morning"- inoculated batch and in Tables 28 and 29, respectively, for the "evening"- inoculated batch. Counts were also made of the number of secondary hyphae produced by each conidium and these are expressed as a percentage of germinated conidia. These results are recorded for the "morning"- and "evening"- inoculated batches in Tables 27 and 30, respectively. Statistical analysis of the frequency of germinated conidia producing secondary hyphae on 15- and 22-day old plants was carried out for "morning"- and "evening"- inoculation using Student's t test (BII[1]) and the results are recorded in Tables 31 and 32, respectively. Statistical analysis was also made of the frequency of germinated conidia producing secondary hyphae after "morning"- and "evening"- inoculation for the 15- and 22- day old plants and the results are recorded in Tables 33 and 34 respectively.

2.2 Results

2.2.1 Effect of cultivar on the production of secondary hyphae on 15-day old plants following "morning"- inoculation

Proportions of germinated conidia (Table 25) and of those producing appressoria (Table 26) remained higher on cultivar BS10 than on the resistant cultivars throughout the experimental period. Secondary hyphae were first seen at 24 h on cultivars BS10 and JI1050, at 30 h on BS12 and JI1049 and at 48 h on JI1047 and JI1048 (Table 25). The proportions of conidia with secondary hyphae on cultivar BS10 was consistently higher than on the resistant cultivars and almost all germinating conidia on BS10 had secondary

hyphae throughout the experimental period (Table 26). Of the resistant cultivars, the highest numbers of conidia producing secondary hyphae at 48 h occurred on BS12 (20.0%); lower values were recorded on JI1047, JI1049 and JI1050 (10.0%, 10.7% and 14.5% respectively) and even fewer occurred on JI1048 (0.8%).

By 24 h most conidia on cultivar BS10 had produced three secondary hyphae but a few conidia on JI1050 produced only one (Table 27). A few conidia produced four secondary hyphae by 30 h on cultivar BS10 but by 48 h the majority of germinating conidia had produced four. Secondary hyphae were first seen on cultivars BS12 and JI1049 by 30 h. On BS12 they numbered from one to four per conidium, all at low frequencies; on the other hand, on JI1049 almost equal numbers of conidia produced one or two secondary hyphae. By 48 h, when secondary hyphae had been produced on all the resistant cultivars, only one was seen on a very few conidia on cultivar JI1048, but up to two were produced by larger numbers of conidia on the other cultivars.

2.2.2. Effect of cultivar on the production of secondary hyphae on 22-day old plants following "morning"- inoculation.

The proportions of germinated conidia (Table 25) and of those producing appressoria (Table 26) remained higher on cultivar BS10 than on the resistant cultivars throughout the experimental period. Secondary hyphae were seen on all the cultivars by 24 h (Table 25) and the numbers of conidia with secondary hyphae were consistently higher on

BS10 than on the resistant cultivars, almost all the germinated conidia on BS10 producing secondary hyphae by 24 h (Table 26). The proportions of conidia with secondary hyphae were very low after 24 h on cultivars BS12, JI1047 and JI1048 (1.4%, 3.2% and 1.7%, respectively) but were higher on JI1049 and JI1050 (17.6% and 18.8%, respectively). The values increased throughout the experimental period on all resistant cultivars except JI1050 where there was little change after 24 h. The highest values recorded on the resistant cultivars occurred on BS12 and JI1049 (33.2% and 61.5%, respectively).

Most conidia on cultivar BS10 had produced four secondary hyphae by the beginning of the experimental period (24 h) (Table 27). At this time conidia on cultivar JI1047 produced only one secondary hypha whereas on BS12 and JI1048 they all formed three. Up to three secondary hyphae per conidium were seen on cultivar JI1049 at this stage, while conidia on JI1050 produced from two to four secondary hyphae. By 48 h low frequencies of conidia on all the resistant cultivars except BS12 and JI1050 had produced up to four secondary hyphae.

2.2.3. Effect of cultivar on the production of secondary hyphae on 15-day old plants following "evening"- inoculation

The proportion of germinated conidia was higher on cultivar BS10 than on BS12, JI1048 and JI1049 at 17 h but was higher than on all the cultivars throughout the rest of the experimental period (Table 28). Proportions of conidia producing appressoria were approximately equal on

all the cultivars throughout the experimental period (Table 29). Secondary hyphae were seen by 17 h on cultivar BS10 but not until 48 h on all resistant cultivars except JI1050 where none was produced (Table 28). The numbers of conidia producing secondary hyphae on cultivar BS10 were consistently higher than on the resistant cultivars and by 48 h almost all conidia on BS10 had produced them (Table 29). The maximum proportion of germinated conidia producing secondary hyphae on resistant cultivars was less than 6% (JI1047).

On cultivar BS10 only one secondary hypha per conidium was recorded at 17 h, up to two were seen by 19 h and by 48 h the majority of germinated conidia had produced four secondary hyphae (Table 30). The number of secondary hyphae per conidium was restricted to one on the resistant cultivars.

2.2.4. Effect of cultivar on the production of secondary hyphae on 22-day old plants following "evening"- inoculation

The frequency of germination was higher on cultivar BS10 than on JI1047, JI1048, JI1049 and JI1050 at 17 h but was higher than on all the cultivars throughout the rest of the experimental period (Table 28). The proportions of conidia producing appressoria were approximately equal on all the cultivars throughout the experimental period (Table 29). Secondary hyphae were seen on cultivar BS10 and fewer occurred on JI1049 at 17 h; on JI1047 they first occurred at 19 h and on BS12 and JI1050 at 24 h; none was produced on JI1048 (Table 28). On BS10 the numbers of germinated conidia with secondary hyphae were consistently

higher than on the resistant cultivars, almost all the conidia on BS10 producing them by 24 h (Table 29). Except for JI1047 there was no increase in the number of conidia with secondary hyphae on any resistant cultivar after they were first seen. The highest values on resistant cultivars were reached by 48 h on JI1047 (22.9%) and after 19 h on JI1049 (25.0%).

Conidia on cultivar BS10 produced only one secondary hypha at 17 h, up to three per conidium were seen by 24 h and at 48 h the majority of germinated conidia had produced four (Table 30). The number of secondary hyphae per conidium on the resistant cultivars was restricted to one.

2.2.5. Effect of host plant age on the production of secondary hyphae following "morning"-inoculation

Similar frequencies of appressorium formation were produced in both age groups on cutlivars BS10 and BS12 but on the other cultivars the frequencies were greater on 22-day plants (Table 26). Secondary hyphae were recorded on cultivar BS10 at 24 h on 15- and 22-day plants (Table 25) and statistical analysis showed no significant difference in the proportions of conidia producing them (Table 31) but by 30 h their number was significantly hgiher on 22-day old plants. Secondary hyphae were produced on all 22-day old resistant cultivars by 24 h but only on JI1050 in the 15-day group. They were recorded in the 15-day group at 30 h on cultivars BS12 and JI1049 but only at 48 h on JI1047 and JI1048. The proportions of conidia producing secondary hyphae were significantly higher in the 22-day

group throughout the experimental period on cultivars JI1048, JI1049 and JI1050, but only at 24 and 30 h on JI1047. Age of host plant did not affect the production of secondary hyphae on cultivar BS12.

The average number of secondary hyphae produced by each conidium was affected by host plant age on all the cultivars (Table 27). An average of up to four secondary hyphae per conidium was produced on 22-day plants of cultivar BS10 throughout the experimental period whereas in the 15-day group only three per conidium were produced until 48 h after which most conidia had produced four. The conidia on all 15-day old resistant cultivars except BS12 produced no more than two secondary hyphae throughout the experimental period, up to four being seen on BS12. The conidia in the 22-day group, however, had produced up to three secondary hyphae on cultivars BS12 and JI1050 by 48 h and up to four on the other resistant cultivars.

2.2.6. Effect of host plant age on the production of secondary hyphae following "evening"-inoculation

By 24 h the frequency of appressorium formation was approximately similar in both age groups. Secondary hyphae were seen 17 h after inoculation on cultivar BS10 in both age groups (Table 28). Statistical analysis showed that the proportion of conidia producing secondary hyphae was higher on 22-day old plants of BS10 at 17, 19 and 24 h but was higher on the 15-day plants at 48 h (Table 32). Secondary hyphae were not recorded until 48 h after inoculation on all the resistant cultivars except JI1050 in the

15-day group. On the 22-day plants secondary hyphae were seen at 17 h on cultivar JI1049, at 19 h on JI1047 and at 24 h on BS12 and JI1050, none being produced by 48 h on JI1048. Statistical analysis showed that the proportion of conidia producing secondary hyphae was higher in the 22-day group on all resistant cultivars except JI1048. The average number of secondary hyphae produced by each conidium was also affected by host plant age on cultivar BS10. Conidia on 22-day plants of BS10 had produced up to three secondary hyphae by 19 h and a few gave rise to four by 24 h (Table 30). The number of secondary hyphae per conidium on 15-day plants of BS10 was restricted to two until 48 h when most conidia produced four. Only one secondary hypha per conidium was produced in both age groups on all the resistant cultivars except JI1048, where equal numbers of conidia on 15-day old plants produced one and two secondary hyphae.

2.2.7. Effect of time of inoculation with respect to photocycle on the production of secondary hyphae on 15-day old plants

Significantly higher proportions of conidia produced secondary hyphae in the "morning"- inoculated cultivars of BS10 and JI1050 at 24 h and on all the cultivars at 48 h (Table 33). Up to three secondary hyphae per conidium were seen on cultivar BS10 at 24 h after both "morning"- and "evening"- inoculation and by 48 h most conidia in btoh batches had produced four secondary hyphae (Tables 27 and 30). "Morning"- inoculation resulted in the production of two secondary hyphae per conidium by 48 h

on all the resistant cultivars. One secondary hypha per conidium was the maximum produced after "evening"- inoculation on all cultivars except JI1050 where none was seen.

2.2.8. Effect of time of inoculation with respect to photocyle on production of secondary hyphae on 22-day old plants

Significantly higher proportions of conidia produced secondary hyphae on cultivar BS10 at 24 h after "morning"- inoculation (Table 34). At 48 h, there was also a significant difference between the results on the "morning"- and "evening"- inoculated groups. Significantly higher proportions of conidia produced secondary hyphae after "morning"- inoculation on cultivars JI1048, and JI1050 at 24 and 48 h but only at 24 h on BS12. "Evening"- inoculation, however, resulted in significantly higher frequencies of secondary hyphae formation on cultivar JI1047 at 24 and 48 h and at 24 h on JI1049.

Most conidia had produced four secondary hyphae by 24 h on cultivar BS10 after "morning"- inoculation when only a low proportion did so after "evening"- inoculation and it was only at 48 h that most of these conidia produced four secondary hyphae (Tables 27 and 30). Conidia on cultivars BS12 and JI1050 had produced up to three secondary hyphae by 48 h after "morning"- inoculation and on cultivars JI1047, JI1048 and JI1049 they produced up to four. After "evening"- inoculation, however, conidia on all the resistant cultivars except JI1048, where no secondary hyphae were seen, had produced only one secondary

Table 25 Germination of conidia, formation of appressoria and of secondary hyphae on the oldest leaves of plants 15- and 22- day plants following "morning"- inoculation. Samples were taken 24, 30 and 48 h after inoculation. The results are expressed as a % total conidia.

| CULTIVAR | Sampling time (h) | GERMINATION Age of Plants (days) 15 | 22 | CONIDIA WITH MATURE APPRESSORIA Age of Plants (days) 15 | 22 | CONIDIA WITH SECONDARY HYPHAE Age of Plants (days) 15 | 22 |
|---|---|---|---|---|---|---|---|
| BS10 | 24 | 81.3 | 72.1 | 77.0 | 68.0 | 66.0 | 66.4 |
| | 30 | 72.4 | 80.4 | 67.6 | 80.4 | 65.7 | 78.3 |
| | 48 | 73.2 | 94.2 | 72.4 | 90.4 | 70.7 | 78.8 |
| BS12 | 24 | 31.7 | 39.7 | 22.4 | 29.8 | 0 | 0.5 |
| | 30 | 54.1 | 25.9 | 52.1 | 18.5 | 4.8 | 1.4 |
| | 48 | 48.5 | 82.6 | 36.2 | 30.0 | 33.1 | 11.2 |
| JI1047 | 24 | 50.8 | 46.3 | 35.0 | 38.1 | 0 | 1.5 |
| | 30 | 54.9 | 39.9 | 49.7 | 28.7 | 0 | 3.2 |
| | 48 | 53.0 | 55.5 | 45.1 | 44.9 | 5.4 | 4.8 |
| JI1048 | 24 | 55.2 | 37.0 | 44.5 | 23.2 | 0 | 0.6 |
| | 30 | 57.0 | 33.5 | 45.1 | 11.2 | 0 | 5.6 |
| | 48 | 60.7 | 40.4 | 57.7 | 28.1 | 0.5 | 9.0 |
| JI1049 | 24 | 47.3 | 40.9 | 35.3 | 37.9 | 0 | 7.2 |
| | 30 | 46.2 | 40.0 | 37.6 | 30.4 | 4.3 | 14.3 |
| | 48 | 58.6 | 47.1 | 50.9 | 41.1 | 6.3 | 29.0 |
| JI1050 | 24 | 39.3 | 61.5 | 33.8 | 36.5 | 3.0 | 11.5 |
| | 30 | 59.2 | 46.0 | 52.4 | 38.5 | 0 | 8.9 |
| | 48 | 52.0 | 61.8 | 48.9 | 40.2 | 2.3 | 11.2 |

Table 26 Formation of appressoria and of secondary hyphae on the oldest leaves of 15- and 22- day plants following "morning"-inoculation. Samples were taken 24, 30 and 48 h after inoculation. The results are expressed as a % of germinated conidia.

| CULTIVAR | Sampling time (h) | CONIDIA WITH MATURE APPRESSORIA | | CONIDIA WITH SECONDARY HYPHAE | |
|---|---|---|---|---|---|
| | | Age of Plants (days) | | Age of Plants (days) | |
| | | 15 | 22 | 15 | 22 |
| BS10 | 24 | 94.8 | 94.3 | 81.2 | 92.0 |
| | 30 | 93.5 | 100 | 90.9 | 97.4 |
| | 48 | 98.9 | 91.9 | 95.6 | 97.6 |
| BS12 | 24 | 70.6 | 75.4 | 0 | 1.4 |
| | 30 | 96.9 | 71.4 | 8.8 | 7.1 |
| | 48 | 68.0 | 48.7 | 20.0 | 33.2 |
| JI1047 | 24 | 68.8 | 82.2 | 0 | 3.2 |
| | 30 | 90.5 | 71.9 | 0 | 7.9 |
| | 48 | 85.0 | 80.9 | 10.0 | 8.5 |
| JI1048 | 24 | 80.6 | 62.7 | 0 | 1.7 |
| | 30 | 79.0 | 33.4 | 0 | 16.7 |
| | 48 | 95.0 | 69.4 | 0.8 | 22.2 |
| JI1049 | 24 | 74.6 | 92.6 | 0 | 17.6 |
| | 30 | 81.4 | 76.0 | 9.3 | 36.0 |
| | 48 | 86.7 | 77.1 | 10.7 | 61.5 |
| JI1050 | 24 | 86.0 | 59.4 | 8.0 | 18.8 |
| | 30 | 88.6 | 83.7 | 10.0 | 14.1 |
| | 48 | 94.1 | 66.6 | 14.5 | 18.3 |

Table 27 Percentage of germinated conidia producing various numbers of secondary hyphae on the oldest leaves of 15- and 22- day plants following "morning"- inoculation. Samples were taken 24, 30 and 48 h after inoculation.

| CULTIVAR | Sampling time (h) | NUMBER OF SECONDARY HYPHAE: ONE — Age of Plants (days) 15 | ONE — 22 | TWO — Age of Plants (days) 15 | TWO — 22 | THREE — Age of Plants (days) 15 | THREE — 22 | FOUR — Age of Plants (days) 15 | FOUR — 22 |
|---|---|---|---|---|---|---|---|---|---|
| BS10 | 24 | 10.6 | 8.0 | 14.2 | 0 | 56.4 | 0 | 0 | 84.0 |
| | 30 | 1.3 | 0 | 0 | 0 | 73.7 | 1.3 | 15.9 | 92.3 |
| | 48 | 2.8 | 0 | 0.7 | 2.0 | 4.5 | 6.1 | 87.6 | 71.5 |
| BS12 | 24 | 0 | 0 | 0 | 0 | 0 | 1.4 | 0 | 0 |
| | 30 | 2.5 | 0 | 1.3 | 0 | 3.7 | 7.1 | 1.3 | 0 |
| | 48 | 2.0 | 4.0 | 18.0 | 2.6 | 0 | 26.6 | 0 | 0 |
| JI1047 | 24 | 0 | 3.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 30 | 0 | 5.8 | 0 | 2.1 | 0 | 0 | 0 | 0 |
| | 48 | 3.8 | 0 | 4.0 | 0 | 0 | 4.3 | 0 | 4.2 |
| JI1048 | 24 | 0 | 0 | 0 | 0 | 0 | 1.7 | 0 | 0 |
| | 30 | 0 | 0 | 0 | 0 | 0 | 11.1 | 0 | 5.6 |
| | 48 | 0.8 | 0 | 0 | 0 | 0 | 11.1 | 0 | 11.1 |
| JI1049 | 24 | 0 | 4.4 | 0 | 11.8 | 0 | 1.4 | 0 | 0 |
| | 30 | 4.7 | 0 | 4.6 | 0 | 0 | 36.0 | 0 | 0 |
| | 48 | 4.1 | 0 | 6.6 | 10.3 | 0 | 34.6 | 0 | 16.6 |
| JI1050 | 24 | 8.0 | 0 | 0 | 12.5 | 0 | 3.15 | 0 | 3.15 |
| | 30 | 10.0 | 0 | 0 | 4.1 | 0 | 0 | 0 | 0 |
| | 48 | 12.5 | 5.0 | 2.5 | 6.7 | 0 | 6.6 | 0 | 0 |

Table 28 Germination of conidia, formation of appressoria and of secondary hyphae on the oldest leaves of 15- and 22- day plants following "evening"-inoculation. Samples were taken 17, 19, 24 and 48 h after inoculation. The results are expressed as a % of total conidia.

| CULTIVAR | Sampling time (h) | GERMINATION Age of Plants (days) 15 | GERMINATION Age of Plants (days) 22 | CONIDIA WITH MATURE APPRESSORIA Age of Plants (days) 15 | CONIDIA WITH MATURE APPRESSORIA Age of Plants (days) 22 | CONIDIA WITH SECONDARY HYPHAE Age of Plants (days) 15 | CONIDIA WITH SECONDARY HYPHAE Age of Plants (days) 22 |
|---|---|---|---|---|---|---|---|
| BS10 | 17 | 47.3 | 66.5 | 35.3 | 49.0 | 17.3 | 43.0 |
| | 19 | 65.8 | 80.6 | 47.4 | 77.4 | 25.0 | 48.4 |
| | 24 | 68.8 | 77.5 | 53.3 | 67.2 | 40.0 | 58.6 |
| | 48 | 93.3 | 83.3 | 89.9 | 70.8 | 84.9 | 61.4 |
| BS12 | 17 | 41.7 | 68.8 | 23.4 | 60.5 | 0 | 0 |
| | 19 | 52.8 | 58.5 | 41.6 | 43.4 | 0 | 0 |
| | 24 | 45.8 | 58.9 | 32.2 | 44.7 | 0 | 3.6 |
| | 48 | 53.3 | 53.2 | 43.4 | 48.9 | 0.8 | 3.9 |
| JI1047 | 17 | 55.4 | 27.7 | 41.6 | 23.1 | 0 | 0 |
| | 19 | 40.9 | 44.1 | 27.3 | 27.7 | 0 | 2.9 |
| | 24 | 46.7 | 44.6 | 21.9 | 38.6 | 0 | 6.3 |
| | 48 | 59.6 | 48.0 | 50.8 | 69.2 | 3.5 | 11.0 |
| JI1048 | 17 | 27.7 | 59.3 | 25.0 | 46.5 | 0 | 0 |
| | 19 | 44.6 | 45.7 | 25.0 | 34.6 | 0 | 0 |
| | 24 | 51.7 | 51.1 | 27.6 | 24.4 | 0 | 0 |
| | 48 | 49.5 | 52.1 | 41.7 | 59.2 | 1.7 | 0 |
| JI1049 | 17 | 28.2 | 31.1 | 18.9 | 24.1 | 0 | 6.9 |
| | 19 | 35.9 | 34.5 | 22.1 | 22.4 | 0 | 8.6 |
| | 24 | 36.9 | 39.7 | 26.1 | 22.0 | 0 | 14.4 |
| | 48 | 44.0 | 43.7 | 27.0 | 53.8 | 1.3 | 15.1 |
| JI1050 | 17 | 58.1 | 29.4 | 46.6 | 29.4 | 0 | 0 |
| | 19 | 62.1 | 37.2 | 36.2 | 18.6 | 0 | 0 |
| | 24 | 57.5 | 48.8 | 41.5 | 27.0 | 0 | 5.7 |
| | 48 | 51.6 | 52.8 | 38.5 | 47.4 | 0 | 4.3 |

Table 29 Formation of appressoria and of secondary hyphae on the oldest leaves of 15- and 22- day plants following "evening" inoculation. Samples were taken 17, 19, 24 and 48 h after inoculation. The results are expressed as a % of germinated conidia.

| CULTIVAR | Sampling time (h) | CONIDIA WITH MATURE APPRESSORIA Age of Plants (days) 15 | 22 | CONIDIA WITH SECONDARY HYPHAE Age of Plants (days) 15 | 22 |
|---|---|---|---|---|---|
| BS10 | 17 | 74.6 | 73.7 | 36.5 | 64.7 |
| | 19 | 72.0 | 85.7 | 38.0 | 63.6 |
| | 24 | 77.4 | 86.7 | 58.0 | 68.2 |
| | 48 | 96.4 | 86.0 | 91.0 | 75.1 |
| BS12 | 17 | 88.0 | 87.9 | 0 | 0 |
| | 19 | 78.3 | 74.2 | 0 | 0 |
| | 24 | 70.3 | 75.8 | 0 | 6.1 |
| | 48 | 81.6 | 78.2 | 1.6 | 6.3 |
| JI1047 | 17 | 75.0 | 83.3 | 0 | 0 |
| | 19 | 66.6 | 40.0 | 0 | 6.7 |
| | 24 | 46.9 | 69.5 | 0 | 11.1 |
| | 48 | 85.3 | 80.0 | 5.9 | 22.9 |
| JI1048 | 17 | 90.0 | 78.4 | 0 | 0 |
| | 19 | 86.1 | 75.7 | 0 | 0 |
| | 24 | 53.4 | 77.8 | 0 | 0 |
| | 48 | 84.2 | 96.4 | 3.5 | 0 |
| JI1049 | 17 | 67.2 | 77.8 | 0 | 22.2 |
| | 19 | 61.6 | 65.0 | 0 | 25.0 |
| | 24 | 70.7 | 55.5 | 0 | 20.1 |
| | 48 | 63.7 | 86.4 | 3.0 | 23.6 |
| JI1050 | 17 | 80.0 | 100 | 0 | 0 |
| | 19 | 58.4 | 50.0 | 0 | 0 |
| | 24 | 72.1 | 55.4 | 0 | 11.7 |
| | 48 | 74.5 | 79.0 | 0 | 10.5 |

Table 30 Percentages of germinated conidia producing various numbers of secondary hyphae on the oldest leaves of 15- and 22- day plants following "evening"- inoculation. Samples were taken 17, 19, 24 and 48 h after inoculation.

| CULTIVAR | Sampling time (h) | NUMBER OF SECONDARY HYPHAE: ONE — Age of Plants (days) 15 | ONE — 22 | TWO — 15 | TWO — 22 | THREE — 15 | THREE — 22 | FOUR — 15 | FOUR — 22 |
|---|---|---|---|---|---|---|---|---|---|
| BS10 | 17 | 36.5 | 64.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 19 | 20.0 | 10.7 | 18.0 | 28.6 | 0 | 24.3 | 0 | 0 |
| | 24 | 25.8 | 11.1 | 32.2 | 14.8 | 0 | 40.0 | 0 | 2.3 |
| | 48 | 3.5 | 0.4 | 9.0 | 3.5 | 8.2 | 3.8 | 70.3 | 67.4 |
| BS12 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 0 | 6.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 1.6 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| JI1047 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 19 | 0 | 6.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 0 | 11.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 5.9 | 22.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| JI1048 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 1.8 | 0 | 1.7 | 0 | 0 | 0 | 0 | 0 |
| JI1049 | 17 | 0 | 22.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 19 | 0 | 25.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 0 | 20.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 3.0 | 23.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| JI1050 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 0 | 11.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 0 | 10.5 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 31 Statistical analysis of data presented in Table 26 comparing the effect of host plant age on the formation of secondary hyphae following "morning"- inoculation. Samples were taken 24, 30 and 48 h after inoculation. The results are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| CULTIVAR | Sampling time (h) | CONIDIA WITH SECONDARY HYPHAE 15-day plants | 22-day plants | P |
|---|---|---|---|---|
| BS10 | 24 | 81.2 | 92.0 | 0.1 |
| | 30 | 90.9 | 97.4 | 0.001 |
| | 48 | 95.6 | 97.6 | 0.1 |
| BS12 | 24 | 0 | 1.4 | 0.001 |
| | 30 | 8.8 | 7.1 | 0.6 |
| | 48 | 20.0 | 33.2 | 0.001 |
| JI1047 | 24 | 0 | 3.2 | 0.001 |
| | 30 | 0 | 7.9 | 0.001 |
| | 48 | 10.0 | 8.5 | 0.001 |
| JI1048 | 24 | 0 | 1.7 | 0.001 |
| | 30 | 0 | 16.7 | 0.001 |
| | 48 | 0.8 | 22.2 | 0.001 |
| JI1049 | 24 | 0 | 17.6 | 0.001 |
| | 30 | 9.3 | 36.0 | 0.001 |
| | 48 | 10.7 | 61.5 | 0.001 |
| JI1050 | 24 | 8.0 | 18.8 | 0.001 |
| | 30 | 10.0 | 14.1 | 0.001 |
| | 48 | 14.5 | 18.3 | 0.001 |

Table 32 Statistical analysis of data presented in Table 29 comparing the effect of host plant age on the formation of secondary hyphae following "evening"-inoculation. Samples were taken 17, 19, 24 and 48 h after inoculation. The results are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| CULTIVAR | Sampling time (h) | CONIDIA WITH SECONDARY HYPHAE 15-day plants | 22-day plants | P |
|---|---|---|---|---|
| BS10 | 17 | 36.5 | 64.7 | 0.001 |
| | 19 | 38.0 | 63.6 | 0.001 |
| | 24 | 58.0 | 68.2 | 0.001 |
| | 48 | 91.0 | 75.1 | 0.01 |
| BS12 | 17 | 0 | 0 | |
| | 19 | 0 | 0 | |
| | 24 | 0 | 6.1 | 0.001 |
| | 48 | 1.6 | 6.3 | 0.001 |
| JI1047 | 17 | 0 | 0 | |
| | 19 | 0 | 6.7 | 0.001 |
| | 24 | 0 | 11.1 | 0.001 |
| | 48 | 5.9 | 22.9 | 0.001 |
| JI1048 | 17 | 0 | 0 | |
| | 19 | 0 | 0 | |
| | 24 | 0 | 0 | |
| | 48 | 3.5 | 0 | 0.001 |
| JI1049 | 17 | 0 | 22.2 | 0.001 |
| | 19 | 0 | 25.0 | 0.001 |
| | 24 | 0 | 20.1 | 0.001 |
| | 48 | 3.0 | 23.6 | 0.001 |
| JI1050 | 17 | 0 | 0 | |
| | 19 | 0 | 0 | |
| | 24 | 0 | 11.7 | 0.001 |
| | 48 | 0 | 10.5 | 0.001 |

Table 33 Statistical analysis of data presented in Table 26 and 29 comparing the effect of time inoculation on the formation of secondary hyphae on 15- day plants. Data compared at 24 and 48 h, are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| CULTIVAR | Sampling time (h) | CONIDIA WITH SECONDARY HYPHAE "Morning"- inoculation | "Evening"- inoculation | P |
|---|---|---|---|---|
| BS10 | 24 | 81.2 | 58.0 | 0.001 |
| | 48 | 95.6 | 91.0 | 0.001 |
| BS12 | 24 | 0 | 0 | |
| | 48 | 20.0 | 1.6 | 0.001 |
| JI1047 | 24 | 0 | 0 | |
| | 48 | 10.0 | 5.9 | 0.001 |
| JI1048 | 24 | 0 | 0 | |
| | 48 | 0.8 | 3.5 | 0.001 |
| JI1049 | 24 | 0 | 0 | |
| | 48 | 10.7 | 3.0 | 0.001 |
| JI1050 | 24 | 8.0 | 0 | 0.001 |
| | 48 | 14.5 | 0 | 0.001 |

Table 34 Statistical analysis of data presented in Tables 26 and 29 comparing effects of time of inoculation on the formation of secondary hyphae on 22- day plants. Data, compared at 24 and 48 h, are expressed as a % of germinated conidia and probability values (p) obtained from Student's t test.

| CUTLIVAR | Sampling time (h) | CONIDIA WITH SECONDARY HYPHAE "Morning"-inoculation | "Evening" inoculation | P |
|---|---|---|---|---|
| BS10 | 24 | 92.0 | 75.6 | 0.001 |
| | 48 | 97.6 | 78.9 | 0.001 |
| BS12 | 24 | 1.4 | 6.1 | 0.001 |
| | 48 | 33.2 | 6.3 | 0.001 |
| JI1047 | 24 | 3.2 | 11.1 | 0.001 |
| | 48 | 8.5 | 22.9 | 0.001 |
| JI1048 | 24 | 1.7 | 0 | 0.001 |
| | 48 | 22.2 | 0 | 0.001 |
| JI1049 | 24 | 17.6 | 11.1 | 0.001 |
| | 48 | 61.5 | 13.6 | 0.001 |
| JI1050 | 24 | 18.8 | 11.7 | 0.001 |
| | 48 | 18.3 | 10.5 | 0.001 |

hypha by 48 h.

2.2.9 Summary

The production of secondary hyphae was delayed on the resistant cultivars except on 22-day plants following "morning"- inoculation. Higher proportions of conidia produced secondary hyphae on BS10 than on the resistant cultivars. The number of secondary hyphae produced per conidium was restricted on the resistant cultivars.

The production of secondary hyphae was delayed on 15-day old plants. In general higher proportions of conidia produced secondary hyphae on 22-day old plants. Greater numbers of secondary hyphae were produced per conidium on 22-day plants of the resistant cultivars.

Higher proportions of conidia produced secondary hyphae on all "morning"- inoculated cultivars in the 15-day group.

## 3 Investigation of the effect of cultivar on the production of haustoria and secondary hyphae

### 3.1 Introduction

The purpose of this experiment was to discover the proportions of haustoria in susceptible and resistant cultivars giving rise (indirectly) to secondary hyphae.

### 3.2 Experimental

The Necoloidine films prepared in the investigations of the effect of cultivar on the frequency of haustorium formation after "morning"- (BI 5.1) and "evening"- (BI 5.2) inoculation (Cb 2.2) were examined and counts made of conidia producing secondary hyphae. The number of haustoria

calculated in the above experiment(s) and of conidia producing secondary hyphae were expressed as a percentage of mature appressoria, the data for each sample being based on 60 to 150 $mm^2$ of leaf. The results obtained after "morning"- and "evening"- inoculation are recorded in Tables 35 and 36, respectively.

3.3. Results

3.3.1 Correlation of the proportions of conidia producing haustoria and secondary hyphae on susceptible and resistant cultivars following "evening"- inoculation.

More than half of the conidia with haustoria in cultivar BS10 had produced secondary hyphae by 24 h and by 48 h almost all of them had done so (Table 35). Haustoria were first recorded at 48 h in all the resistant cultivars. Secondary hyphae were not produced on cultivars JI1048, JI1049 and JI1050 and only at 96 h on BS12 and JI1047. Almost all of the haustoria in cultivar BS12 gave rise (indirectly) to secondary hyphae but only about one fifth of haustoria did so in JI1047.

3.3.2. Correlation of the proportions of conidia producing haustoria and secondary hyphae on susceptible and resistant cultivars following "morning"- inoculation

Haustoria were produced in all three cultivars tested by 16 h but secondary hyphae were seen only on cultivar BS10 at this time (Table 36). Less than a quarter of the conidia on BS10 producing haustoria had given rise to secondary hyphae at this stage. By 20 h approximately

half of the conidia producing haustoria in cultivar BS10 had produced secondary hyphae and at 41 h almost all such conidia had done so. Very low proportions of conidia producing haustoria gave rise to secondary hyphae on cultivar JI1047 at 20 and 41 h and none was recorded on JI1049.

Table 35 Formation of haustoria and secondary hyphae on the oldest leaves of 22- day plants following "evening"-inoculation. Samples were taken 24, 48, 72 and 96 h after inoculation. The results are expressed as a % of the total appressoria and each result is based upon 60-150 $mm^2$ of leaf.

| | CULTIVAR | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BS10 | | BS12 | | JI1047 | | JI1048 | | JI1049 | | JI1050 | |
| Sampling time (h) | Haust. | $2^o$ hyphae | Haust. | $2^o$ hyphae | Haust. | $2^o$ hyphae | Haust. | $2^o$ hyphae | Haust. | $2^o$ hyphae | Haust. | $2^o$ hyphae |
| 24 | 50.0 | 34.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 68.4 | 66.5 | 5.3 | 0 | 8.3 | 0 | 20.2 | 0 | 16.7 | 0 | 3.3 | 0 |
| 72 | 100 | 88.0 | 28.8 | 0 | 48.0 | 0 | 26.3 | 0 | 45.3 | 0 | 28.5 | 0 |
| 96 | 100 | 97.3 | 41.3 | 38.4 | 62.0 | 14.3 | 34.2 | 0 | 47.5 | 0 | 32.7 | 0 |

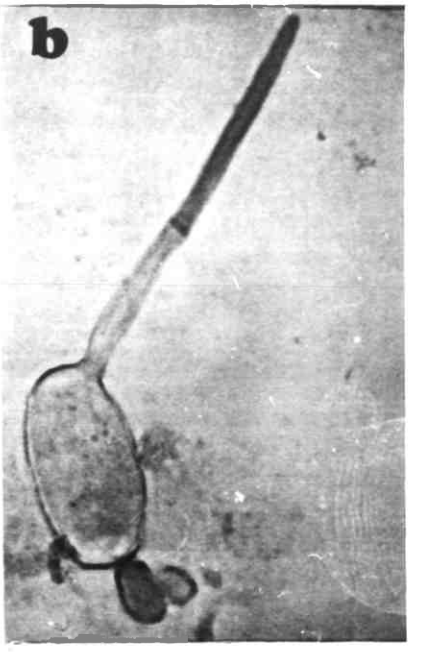
Table 36 Formation of haustoria and secondary hyphae on the oldest leaves of 22- day plants following "morning"-inoculation. Samples were taken 16, 20 and 41 h after inoculation. The results are expressed as a % of total appressoria and each result in based upon 60-150 $mm^2$ of leaf.

| Sampling time (h) | BS10 | | JI1047 | | JI1049 | |
|---|---|---|---|---|---|---|
| | Haust. | 2° Hyphae | Haust. | 2° Hyphae | Haust. | 2° Hyphae |
| 16 | 57.0 | 17.6 | 27.0 | 0 | 28.3 | 0 |
| 20 | 62.4 | 33.3 | 29.1 | 0.3 | 43.4 | 0 |
| 41 | 65.0 | 60.1 | 38.8 | 1.3 | 42.7 | 0 |

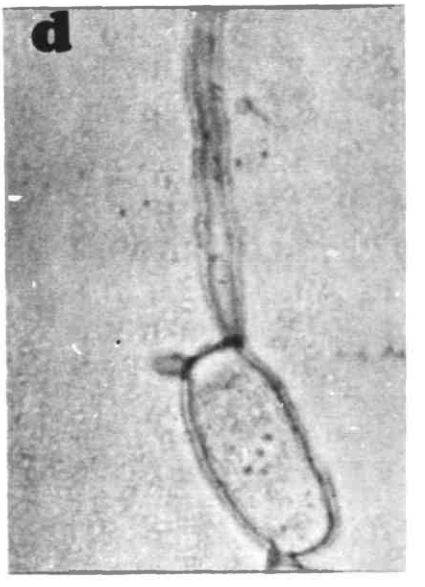
Plate 5. Light micrographs of developing elongating secondary hyphae of *E. pisi* on *P. sativum*. x 385.

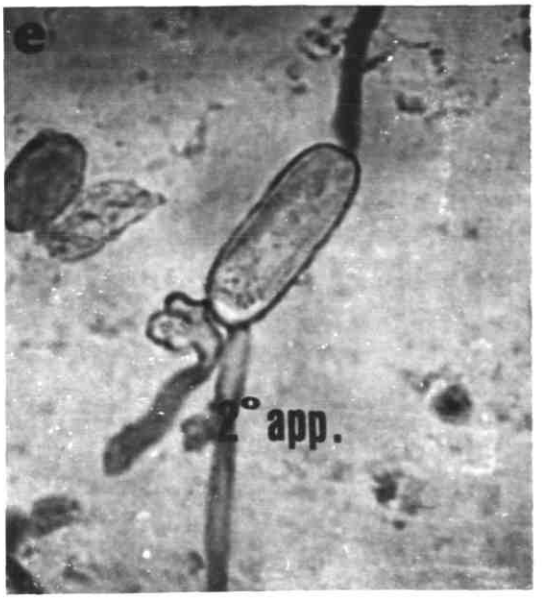
a. Elongating secondary hypha produced from a mature appressorium.
b. Elongating secondary hypha produced directly from the conidium and opposite to the appressorium.
c. Elongating secondary hypha produced directly from the conidium adjacent to the appressorium.
d. Elongating secondary hypha and a secondary hyphal initial produced directly from the conidium.
e. Elongating secondary hyphae produced from each end of a conidium. One hypha has formed a secondary appressorium.
f. Conidium with four elongating secondary hyphae.


a
mat.
app.
2°
hypha


b


c


d


e
2° app.


f

d Development of colonies

1 Introduction

Results of the investigation of the effect of cultivar on the production of secondary hyphae (CIIc 2.2.1.4) showed that on resistant cultivars germinated conidia produced no or fewer secondary hyphae than on the susceptible cultivar (BS10) and the appearance was delayed. Further investigation necessitated a quantitative assessment of colony development to discover the effect of cultivar on hyphal extension rates. As the production of secondary hyphae was affected by host plant age (CIII c 2.2.5.6) and by the time of inoculation with respect to the photocycle (CIIIc 2.2.7.8.) the average lengths of hyphae and their rates of extension were calculated using 15- and 22-day old plants following "morning"- and "evening"-inoculations.

2 Experimental

Following "morning"- (BI 5.1) and "evening"- (BI 5.2) inoculations of the six test cultivars, 15- and 22- day old plants were incubated in growth rooms with 16 h light and 8 h dark periods. Samples were taken from the "morning"-inoculated batch after 24, 30, 48, 72 and 96 h and from the "evening"- inoculated batch after 17, 19, 24, 48, 72 and 96 h, with four replicates in each case. The surface fungal structures were prepared for observation on sellotape strips (BII 3.1) and stained in lactophenol cotton blue (BII 1.2). Counts were made of the total germinated conidia, of conidia producing micro-colonies and producing colonies. Micro-colonies are defined as comprising up to

four unbranched secondary hyphae and colonies comprise more than four secondary hyphae. The total length of hyphae produced per germinated conidium were measured from micrographs using a matrix digitizor linked to a Hewlett Packard calculator programmed to record measured lengths (BII 5.1). The proportions of conidia producing a micro-colony or a colony expressed as a percentage of germinated conidia, and the average length of hyphae in each colony at each sampling time are recorded in Tables 37-40. The calculated number of epidermal cells crossed by hyphae, (minimal and maximal), calculated from the mean lengths and widths of epidermal cells and the average hyphal lengths produced by each germinated conidium on each cultivar are also recorded in these tables. Statistical analysis of the effect of cultivar in each batch on the average hyphal lengths was carried out using Student's t test (BII 1 ) and the results are recorded in Tables 41-44. The results of statistical analysis of the effect of host plant age and of the time of inoculation on the average hyphal lengths produced by each germinated conidium are recorded in Tables 45-48.

## 3 Results

### 3.1 Effect of cultivar on the development of colonies on 15-day old plants following "morning"-inoculation

Micro-colonies had developed on cultivar BS10 and JI1050 by 24 h, on BS12 and JI1049 by 30 h and on JI1047 and JI1048 by 48 h (Table 37). The proportion of germinated conidia which had formed micro-colonies was

greater at 24 h on BS10 (81.2%) than on the resistant cultivars at any time during the experimental period (96 h). By 30 h 90.9% of germinated conidia had formed micro-colonies on cultivar BS10 but only 10-20% on BS12, JI1049 and JI1050. The status of colonies was reached by 48 h on cultivar BS10 by all the germinated conidia but not until 72 h on BSi2 and JI1048 and 96 h on JI1047 and JI1050. The growths did not progress beyond micro-colonies on cultivar JI1049. The proportions of germinated conidia producing colonies on cultivar BS10 did not increase after 48 h (95.6%). No increase in the frequency of colony formation occurred after 72 h on cultivar BS12 (62.0%) and only a small increase on JI1048 (6.2% to 8.5%).

The average hyphal length produced by each germinated conidium on BS10 was significantly higher than on any resistant cultivars throughout the experimental period (Table 41). The values recorded on cultivar BS10 increased and almost doubled in 24 h period between 30 and 48 h and then trebled between 72 and 96 h. A nineteen-fold increase in the average hyphal length occurred on cultivar BS12 between 30 and 72 h. On the other cultivars the average hyphal length increased until 96 h. The maximum value recorded on cultivar BS10 (1·25 mm) was nearly three times greater than on JI1047, at least four times greater than on BS12, JI1048 and JI1050 and twelve times greater than on JI049.

The rate of hyphal extension was higher on BS10 than on the resistant cultivars throughout the experimental period (Fig. 11). The rate increased on cultivar BS10

until 48 h and again after 72 h, reaching a value of 0·07 mm $h^{-1}$ but on BS12 the rate reached an 0·008 mm $h^{-1}$ and growth ceased at 72 h. On cultivars JI1047 and JI1048 the rates were still lower and declined further after 48 h. JI1047 showed a slight increase after 72 h but the rate remained constant on JI1048. The rates on cultivars JI1049 and JI1050 declined after 30 h but increased again after 48 h, remaining constant on JI1049 thereafter. By 96 h the rate recorded on BS10 was seven times higher than on JI1047, almost twelve times higher than on JI1050 and thirty five times higher than on JI1048 and JI1049.

### 3.2 Effect of cultivar on the development of colonies on 22-day old plants following "morning"-inoculation

Micro-colonies had developed on all the cultivars by 24 h but the proportion of germinated conidia producing them was greater on BS10 (92.0%) than on the resistant cultivars where the values ranged from 1.4% - 3.2% on BS12, JI1047 and JI1048 and were approximately 18.0% on JI1049 and JI1050 (Table 38). By 30 h 97.4% of germinated conidia had formed micro-colonies on BS10 but only 4.1%-16.7% on the resistant cultivars. None of the growths reached the status of colonies until 48 h when the value was 97.6% on BS10, 33.2% on BS12 and 1.5% on JI1047. No colonies were recorded on JI1048 and JI1050 until 72 h and not until 96 h on JI1049. No increase in the frequency of colony formation occurred after 48 h on cultivar BS10 (97.6%) after 72 h on BS12 (65.0%) and by 96 h the values were 10.4% on JI1047, 9.2% on JI1048,

67.8% on JI1049 and 20.4% on JI1050.

The average hyphal lengths produced by germinated conidia on cultivars BS10, BS12 and JI1047 were similar until 48 h. They were significantly higher than on the other cultivars during this period (Table 42); thereafter, the values recorded on BS10 were significantly higher than on all the resistant cultivars. The maximum value reached at 96 h on cultivar BS10 (0.71 mm) was almost double the values recorded on BS12, JI1047 and JI1048 and approximately three time greater than on JI1049 and the rates of hyphal extension remained constant until 48 h on cultivars BS10 (0.003 mm $h^{-1}$), BS12 (0.002 mm $h^{-1}$) and JI1050 (0.005 mm $h^{-1}$) after which they increased on BS10 and declined on all the other cultivars (Fig. 12 ). On JI1050 the decline continued until 96 h but on the other cultivars some fluctuations in the rate occurred during the latter part of the experimental period. By 96 h the rate of hyphal extension on cultivar BS10 was more than three times higher than on BS12, JI1047 and JI1048 and six times higher than on JI1049 and JI1050.

3.3 Effect of cultivar on the development of colonies on 15-day old plants following "evening"-inoculation

Micro-colonies had developed by 17 h on cultivar BS10 but not until 48 h on BS12, JI1047, JI1048 and JI1049 and not until 72 h on JI1050 (Table 39). By 48 h 91.0% of germinated conidia on cultivar BS10 had formed micro-colonies but only 1.6%-5.9% on BS12, JI1047, JI1048 and JI1049. The status of colonies was reached at 72 h

by all germinated conidia on cultivars BS10 and BS12, but not until 96 h on JI1047, JI1048 and JI1050; the growths did not progress beyond micro-colonies on JI1049. The frequency of colony formation on cultivar BS10 at 96 h (97.2%) was approximately four times greater than on JI1048 and almost ten times greater than on BS12, JI1047 and JI1050.

The average hyphal lengths produced by germinated conidia on BS10 was significantly higher than on the resistant cultivars throughout the experimental period (Table 43). The values increased until 96 h on all the cultivars. They approximately doubled between every sampling time on cultivar BS10 except between 48 and 72 h when they increased four-fold. The increments in average hyphal length on the resistant cultivars were much smaller. The average hyphal length reached on cultivar BS10 by 96 h (0.82 mm) was approximately three times higher than on JI1047, JI1048 and JI1050 and approximately four times higher than on BS12 and JI1049.

The rates of hyphal extension also were higher on cultivar BS10 than on the resistant cultivars throughout the experimental period except between 24 and 48 h when the rates on BS10 and JI1048 were the same (0.001 mm $h^{-1}$) less than on JI1049 (0.002 mm $h^{-1}$) (Fig. 13 ). The rate increased until 24 h on BS10 (0.007 mm $h^{-1}$) but declined to 0.001 mm $h^{-1}$ between 24 and 48 h and increased thereafter. A gradual decline in the rate of hyphal extension occurred after 48 h on cultivars BS12 and JI1050. On cultivars JI1047 and JI1048 the rate increased until 72 h and then

declined again but on JI1049 it rose until 48 h and remained constant. By 96 h the rate recorded on cultivar BS10 was almost seven times greater than on BS12, JI1047, JI1048 and JI1050 and ten times greater than on JI1048.

3.4 Effect of cultivar on the development of colonies on 22-day old plants following "evening"-inoculation

Micro-colonies had developed by 17 h on cultivars BS10 and JI1049, by 19 h on JI1047 by 24 h on BS12 and JI1050 and by 72 h on JI1048 (Table 40). By 19 h approximately 64% of germinated conidia had produced micro-colonies on cultivar BS10 but only 7% and 25% had formed them on JI1047 and JI1049, respectively. The status of colony was reached at 24 h by all germinated conidia on cultivar BS10 but not until 48 h on BS12 and at 72 h on JI1047, JI1049 and JI1050; the growths did not progress beyond micro-colonies on JI1048. The maximum frequency of colony formation on cultivar BS10 (94.1%) was almost three times greater than on JI1047, almost five times greater than on JI1049 and JI1050 and almost six times greater than on BS12.

The average hyphal lengths produced per germinated conidium were significantly hgiher on BS10 than on the resistant cultivars throughout the experimental period except at 19 and 24 h on BS12 when the values were similar (Table 44). The values increased until 96 h on all the cultivars. They doubled between 17 and 19 h and again between 19 and 24 h on cultivar BS10 but increased in smaller increments thereafter. The increases were smaller on all the resistant cultivars except JI1048 where the

average hyphal length doubled between 72 and 96 h. The length reached by 96 h on cultivar BS10 (1·07 mm) was almost double that on JI1047, more than three times higher than on BS12, JI1049 and JI1050 and almost five times greater than on JI1048.

The rates of hyphal extension were higher on cultivar BS10 than on the resistant cultivars throughout the experimental period (Fig. 14 ). The rate increased until 24 h on cultivar BS10, declined between 24 and 48 h, increased between 48 and 72 h and then declined again. A decline occurred between 17 and 24 h on cultivars BS12, JI1047 and JI1049 but increased after this on BS12 and JI1049, declining again between 72 and 96 h on BS12. The rate of hyphal extension remained constant on cultivar JI1048. By 90 h the rate calculated for cultivar BS10 (0.006 mm $h^{-1}$) was 1½ times greater than on JI1048 (0.004 mm $h^{-1}$), twice the rate on JI1047 (0.003 mm $h^{-1}$), three times greater than on JI1049 (0.002 mm $h^{-1}$) and six times greater than on BS12 and JI1050 (0.001 mm $h^{-1}$).

### 3.5 Effect of host plant age on the development of colonies following "morning"- inoculation

Micro-colonies had developed by 24 h on all the 22-day cultivars but only on BS10 and JI1050 on 15-day plants at this time (Table 45). Micro-colonies were present by 30 h on 15- day plants of cultivars BS12 and JI1049 and by 48 h on JI1047 and JI1048. The frequencies of micro-colony formation were greater on all the 22-day old cultivars at 24 h and on all except BS12 at 30 h. The status of colony was reached at 48 h on 15-day plants of cultivar

BS10, at 72 h on BS12 and JI1048 and at 96 h on JI1047 and JI1050; the growths did not progress to colonies on JI1049. In the 22-day group colonies had developed by 48 h on cultivars BS10, BS12 and JI1047, by 72 h on JI1048 and by 96 h on JI1049. The maximum frequencies of colony formation on 15- and 22-day plants were similar on cultivars BS10 (96.5% and 97.2%, respectively), BS12 (62.0% and 65.0%, respectively) and JI1048 (8.5% and 9.2%, respectively) at 96 h. However, higher values were recorded on 15-day plants of JI1047 (28.2%) and JI1050 (14.5%) at this time.

The average hyphal lengths produced per germinated conidium were similar in both age groups on cultivar BS10 at 24 and 30 h but by 96 h significantly higher values were recorded on 22-day plants. The values recorded on the other cultivars were significantly higher on 22-day plants until 72 h the effect of host plant age becoming insignificant thereafter.

The rates of hyphal extension were often higher on 15-day cultivars of BS10, BS12, JI1047 and JI1048 throughout the experimental period and by 96 h on all 15-day cultivars (Fig. 15).

However, since there were periods inthe growth of some of the cultivars when no extension occurred, the high rates do not necessarily co-incide with the longest hyphal systems.

### 3.6 Effect of host plant age on the development of colonies following "evening"- inoculation

Micro-colonies were recorded at 17 h on cultivar BS10 in both age groups (Table 46). On 22-day plants they were produced earlier on JI1049 (at 17 h), JI1047 (at 19 h) and BS12 and JI1050 (at 24 h), but with 15-day plants they were produced first on JI1048 (at 48 h). On 15-day plants all germinated conidia had reached the status of colonies by 72 h on cultivars BS10 and BS12 and by 96 h on JI1047, JI1048 and JI1050 but the growths did not progress beyond micro-colonies on JI1049. On 22-day plants colonies had developed from all germinated conidia by 24 h on cultivar BS10 and by 72 h on all the other cultivars except JI1048 where micro-colonies were present to the end of the experimental period. By 96 h similar frequencies of colony formation were recorded on cultivar BS10 in both age groups but the values were higher on 22-day plants of BS12 (15.8%), JI1047 (34.7%) and JI1050 (20.8%).

The average hyphal lengths produced per germinated conidium were significantly higher on 22-day plants of all cultivars except JI1048 throughout the experimental period (Table 46). By 96 h the values recorded on 22-day plants of cultivars JI1047 (0.59 mm) and JI1049 (0.39 mm) were approximately double those of 15-day plants. On cultivar JI1048, however, the average hyphal length on 15-day plants (0.26 mm) was higher than on the older plants.

The rates of hyphal extension were higher on 22-day cultivars of BS10, JI1047 and JI1049 between 17 and 19 h.

Between 19 and 24 h higher rates were calculated for 22-day cultivars of BS12, JI1047, JI1049 and JI1050 (Fig. 16). By 48 h, however, the rates were higher on 15-day plants of all cultivars except BS10. The results indicated that the rates of hyphal extension could not be conclusively attributed to the effect of host plant age.

3.7 Effect of time of inoculation on development of colonies on 15-day old plants

Micro-colonies had developed by 24 h on "morning"- and "evening"- inoculated plants of cultivar BS10 and by 48 h on BS12, JI1047, JI1048 and JI1049 in both batches; micro-colonies were recorded at 24 h on "morning"- inoculated plants of JI1050 but not until 72 h after "evening"- inoculation (Table 47). On "morning"- inoculated plants the status of colonies was reached by 48 h on cultivar BS10, by 72 h on BS12 and JI1048 and by 96 h on JI1047 and JI1050. Colony development was delayed on BS10 and JI1048 but unaffected on the other cultivars by "evening"- inoculation. The growths on cultivar JI1049 did not progress beyond micro-colonies in either group. By 96 h the frequencies of colony formation were similar after both inoculation times on BS10 (97.0%); the values on the "morning"- inoculated plants were higher on cultivars BS12, JI1047 and JI1050 but higher on JI1048 after "evening"- inoculation.

The average hyphal lengths produced per germinated conidium were significantly higher on the "morning"- inoculated plants of cultivar BS10 at 24, 48 and 96 h (Table 47). The values recorded on cultivars BS12 and

JI1047 were significantly higher after "morning"- inoculation throughout the experimental period and by 96 h on JI1048. The average hyphal lengths were significantly higher after "evening"- inoculation on cultivar JI1049 throughout the experimental period but on JI1050 the effect of photoperiod had become insignificant by 96 h.

The rates of hyphal extension were higher on cultivars BS10, BS12, JI1047 and JI1048 after "morning"- inoculation between 24 and 48 h (Fig. 17). By 72 h the rates were higher on the "morning"- inoculated plants of all the cultivars except JI1048 where the same rate occurred after both inoculation times; by 96 h the higher rates occurred on all "morning"- inoculated cultivars except JI1049.

3.8 Effect of time of inoculation on the development of colonies on 22-day old plants

Micro-colonies had developed by 24 h on "morning" and "evening"- inoculated cultivars of BS12, JI1047, JI1049 and JI1050 (Table 48). Germinated conidia had produced micro-colonies by 24 h on cultivar BS10 after "morning"- inoculation when on "evening"- inoculated plants the growths had already progressed to become colonies. After "morning"- inoculation of cultivar JI1048 micro-colonies were recorded at 24 h but not until 72 h after "evening"- inoculation. At 24 h the frequencies of micro-colony formation were greater on the "evening"- inoculated cultivars of BS12, JI1047 and JI1049 but higher values were recorded on "morning"- inoculated JI1050.

As stated above, "evening"- inoculation of BS10

had already resulted in colonies by 24 h. "Morning"-inoculation of BS10, BS12 and JI1047 resulted in colonies by 48 h and in all other cases colonies were not produced until at least 72 h. Almost consistently throughout the experimental period the proportions of germinated conidia producing colonies were similar on cultivars BS10 and JI1050 in both batches, higher after "morning"-inoculation on BS12 and JI1049 but higher after "evening"-inoculation on JI1047.

The average hyphal lengths produced by each germinated conidium were significantly higher after "morning"-inoculation on cultivars BS10, JI1047, JI1049 and JI1050 throughout the experimental period; however, they were higher after "evening"- inoculation on JI1048 and the effect of photoperiod was insignificant on BS12. The lengths increased until 96 h on all the cultivars after both inoculation times.

The rates of hyphal extension were higher on all "morning"- inoculated cultivars except JI1049 between 72 and 96 h (Fig. 18 ).

## 4 Conclusions

The frequencies of micro-colony and colony formation were higher on BS10 than on the resistant cultivars irrespective of host plant age and inoculation time. The average length of hypha was significantly higher on BS10 than on the resistant cultivars and reflected the lower rate of hyphal extensionand restricted numbers of secondary hyphae produced by each germinated conidium, resulting in a delay on the resistant cultivars in the transition from

the status of micro-colony to colony.

Table 37 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia), the average hyphal length produced per germinated conidium and the calculated number of epidermal cells (minimal and maximal) crossed by hyphae on the oldest leaves of 15- day plants following "morning"- inoculation. Samples were taken 24, 30, 48, 72 and 96 h after inoculation.

| CULTIVAR | Sampling time (h) | Micro-colonies (%) | Colonies (%) | Average hyphal length ($mm \times 10^{-1}$) | Calculated number of epidermal cells crossed by hyphae | |
|---|---|---|---|---|---|---|
| | | | | | Minimum | Maximum |
| BS10 | 24 | 81.2 | - | 1.8 | 30 | 45 |
| | 30 | 90.9 | - | 2.1 | 35 | 53 |
| | 48 | - | 95.6 | 4.2 | 70 | 105 |
| | 72 | - | 95.5 | 4.1 | 68 | 103 |
| | 96 | - | 96.5 | 12.5 | 208 | 313 |
| BS12 | 24 | 0 | - | 0 | 0 | 0 |
| | 30 | 8.8 | - | 0.2 | 3 | 6 |
| | 48 | 20.0 | - | 1.7 | 25 | 47 |
| | 72 | - | 62.0 | 3.7 | 54 | 103 |
| | 96 | - | 60.0 | 3.1 | 46 | 86 |
| JI1047 | 24 | 0 | - | 0 | 0 | 0 |
| | 30 | 0 | - | 0 | 0 | 0 |
| | 48 | 10.0 | - | 1.9 | 38 | 63 |
| | 72 | 24.6 | - | 2.3 | 46 | 77 |
| | 96 | - | 28.2 | 4.9 | 98 | 163 |
| JI1048 | 24 | 0 | - | 0 | 0 | 0 |
| | 30 | 0 | - | 0 | 0 | 0 |
| | 48 | 0.8 | - | 1.0 | 15 | 23 |
| | 72 | - | 6.2 | 1.5 | 22 | 35 |
| | 96 | - | 8.5 | 3.5 | 52 | 81 |
| JI1049 | 24 | 0 | - | 0 | 0 | 0 |
| | 30 | 9.3 | - | 0.1 | 2 | 2 |
| | 48 | 109 | - | 0.2 | 3 | 4 |
| | 72 | 10.6 | - | 0.6 | 10 | 12 |
| | 96 | 40.0 | - | 1.1 | 18 | 22 |
| JI1050 | 24 | 8.0 | - | 0.5 | 8 | 13 |
| | 30 | 10.0 | - | 0.6 | 0 | 0 |
| | 48 | 14.5 | - | 0.7 | 12 | 18 |
| | 72 | 15.2 | - | 1.3 | 22 | 33 |
| | 96 | - | 14.5 | 2.3 | 45 | 68 |

Fig. 11

Comparisons of average hyphal lengths in colonies of E. pisi on six 15-day cultivars of pea following "morning"-inoculation.

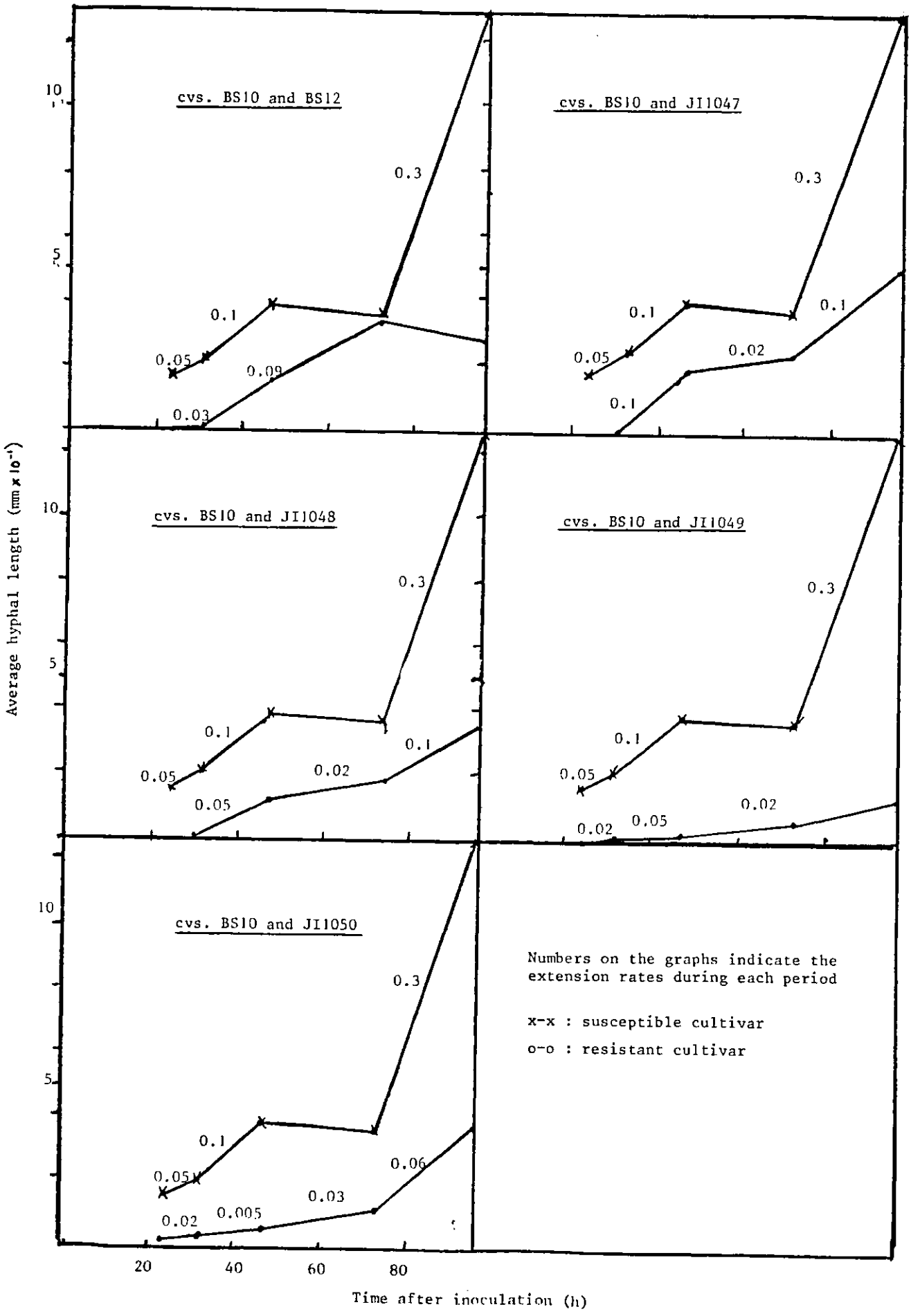


Table 38 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia), the average hyphal length produced per germinated conidium and the calculated number of epidermal cells (minimal and maximal) crossed by hyphae on the oldest leaves of 22- day plants following "morning"- inoculation. Samples were taken 24, 30, 48, 72 and 96 h after inoculation.

| CULTIVAR | Sampling time (h) | Micro-colonies (%) | Colonies (%) | Average hyphal length ($mm \times 10^{-1}$) | Calculated number of epidermal cells crossed by hyphae | |
|---|---|---|---|---|---|---|
| | | | | | minimum | maximum |
| BS10 | 24 | 92.0 | - | 1.9 | 32 | 48 |
| | 30 | 97.4 | - | 2.1 | 35 | 53 |
| | 48 | - | 97.6 | 2.7 | 45 | 68 |
| | 72 | - | 97.8 | 5.9 | 98 | 148 |
| | 96 | - | 97.2 | 7.1 | 118 | 178 |
| BS12 | 24 | 1.4 | - | 1.9 | 28 | 53 |
| | 30 | 7.1 | - | 2.0 | 29 | 56 |
| | 48 | - | 33.2 | 2.6 | 38 | 72 |
| | 72 | - | 65.0 | 2.9 | 43 | 81 |
| | 96 | - | 64.7 | 3.8 | 56 | 106 |
| JI1047 | 24 | 3.2 | - | 1.6 | 32 | 53 |
| | 30 | 7.9 | - | 2.6 | 52 | 87 |
| | 48 | - | 8.5 | 2.3 | 46 | 77 |
| | 72 | - | 8.8 | 2.8 | 56 | 93 |
| | 96 | - | 10.4 | 3.8 | 76 | 127 |
| JI1048 | 24 | 1.7 | - | 0.7 | 10 | 16 |
| | 30 | 16.7 | - | 1.6 | 24 | 37 |
| | 48 | 22.2 | - | 2.3 | 34 | 54 |
| | 72 | - | 27.3 | 2.8 | 42 | 65 |
| | 96 | - | 29.3 | 3.8 | 57 | 88 |
| JI1049 | 24 | 17.6 | - | 0.8 | 13 | 16 |
| | 30 | 36.0 | - | 1.2 | 20 | 24 |
| | 48 | 61.5 | - | 1.6 | 27 | 32 |
| | 72 | 60.8 | - | 2.1 | 35 | 42 |
| | 96 | - | 67.8 | 2.8 | 47 | 56 |
| JI1050 | 24 | 18.8 | - | 0.5 | 8 | 13 |
| | 30 | 14.1 | - | 0.8 | 13 | 20 |
| | 48 | 18.3 | - | 1.7 | 28 | 43 |
| | 72 | - | 15.3 | 2.4 | 40 | 60 |
| | 96 | - | 20.4 | 2.8 | 47 | 70 |

Fig. 12

Comparisons of average hyphal lengths in colonies of E. pisi on six 22-day cultivars of pea following "morning"-inoculation.

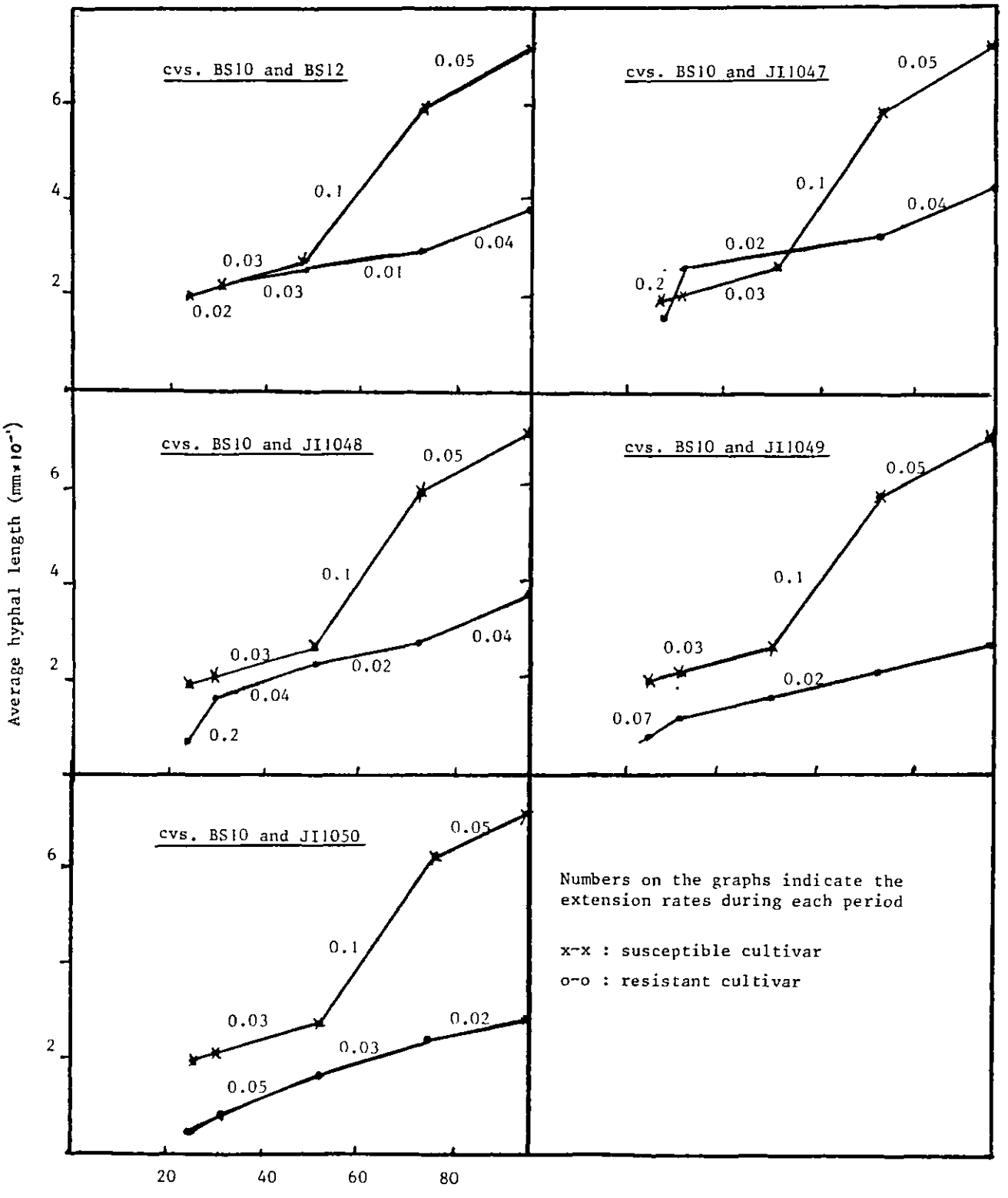


Table 39 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia), the average hyphal length produced per germinated conidium and the calculated number of epidermal cells (minimal and maximal) crossed by hyphae on the oldest leaves of 15- day plants following "evening"- inoculation. Samples were taken 17, 19, 24, 48, 72 and 96 h after inoculation.

| CULTIVAR | Sampling time (h) | Micro-colonies (%) | Colonies (%) | Average hyphal length (mm $\times 10^{-1}$) | Calculated number of epidermal cells crossed by hyphae | |
|---|---|---|---|---|---|---|
| | | | | | minimum | maximum |
| BS10 | 17 | 36.5 | - | 0.2 | 3 | 5 |
| | 19 | 38.0 | - | 0.26 | 4 | 7 |
| | 24 | 58.0 | - | 0.59 | 10 | 15 |
| | 48 | 91.0 | - | 0.9 | 15 | 23 |
| | 72 | - | 96.3 | 4.1 | 68 | 103 |
| | 96 | - | 97.2 | 8.2 | 137 | 205 |
| BS12 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 0 | - | 0 | 0 | 0 |
| | 48 | 1.6 | - | 0.2 | 3 | 6 |
| | 72 | - | 6.8 | 1.4 | 21 | 39 |
| | 96 | - | 9.2 | 2.1 | 31 | 58 |
| JI1047 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 0 | - | 0 | 0 | 0 |
| | 48 | 5.9 | - | 0.2 | 4 | 7 |
| | 72 | 8.6 | - | 2.0 | 40 | 67 |
| | 96 | - | 8.8 | 2.7 | 54 | 90 |
| JI1048 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 0 | - | 0 | 0 | 0 |
| | 48 | 3.5 | - | 0.3 | 5 | 7 |
| | 72 | 15.8 | - | 2.1 | 31 | 49 |
| | 96 | - | 25.1 | 2.6 | 39 | 60 |
| JI1049 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 0 | - | 0 | 0 | 0 |
| | 48 | 3.0 | - | 0.5 | 8 | 10 |
| | 72 | 20.2 | - | 1.3 | 22 | 26 |
| | 96 | 32.8 | - | 1.9 | 32 | 38 |
| JI1050 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 0 | - | 0 | 0 | 0 |
| | 48 | 0 | - | 0 | 0 | 0 |
| | 72 | 4.1 | - | 2.2 | 37 | 55 |
| | 96 | - | 9.6 | 2.8 | 47 | 70 |

Fig. 13

Comparisons of average hyphal lengths in colonies of E. pisi on six 15-day cultivars of pea following "evening"-inoculation.

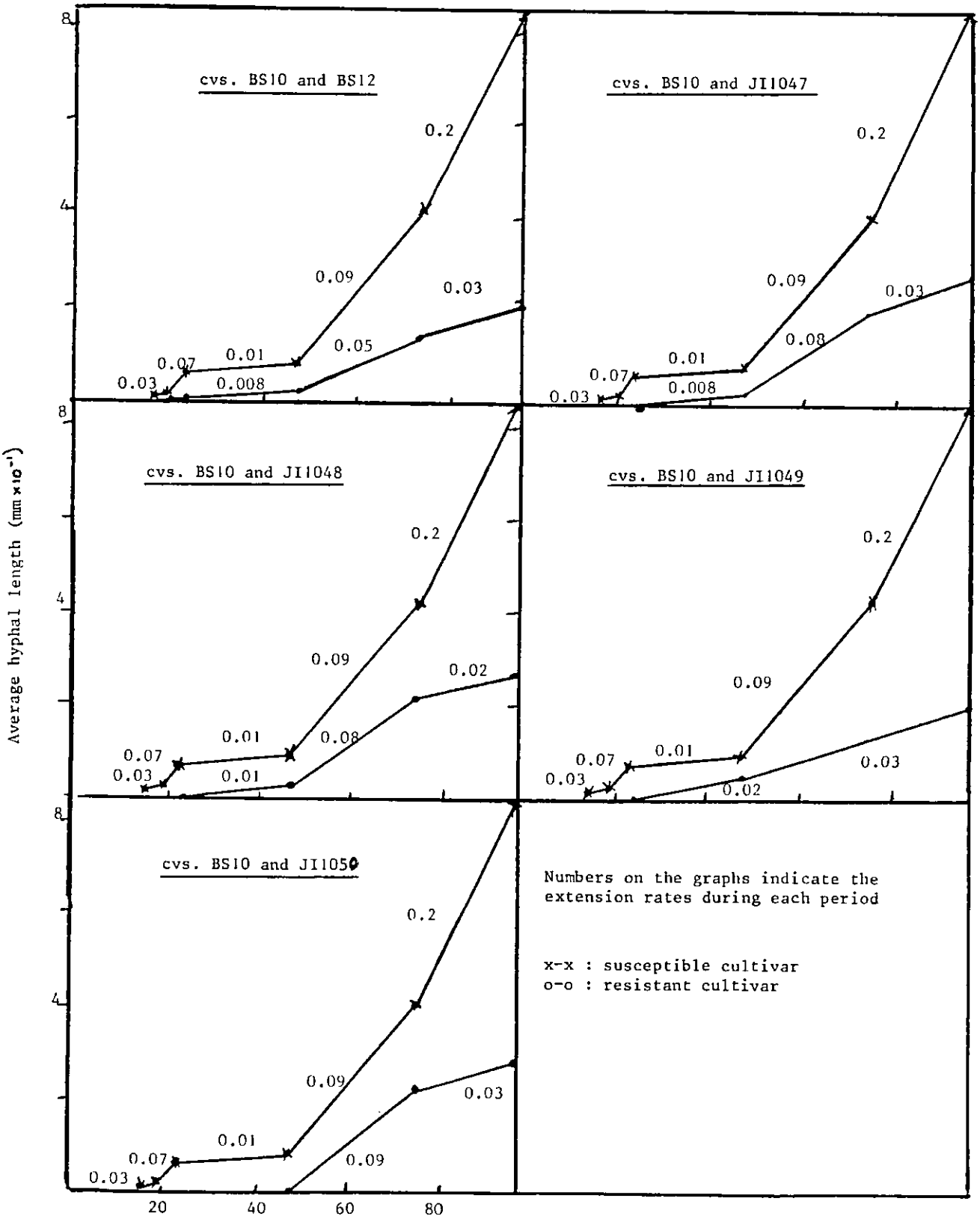


Table 40 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia), the average hyphal length produced per germinated conidium and the calculated number of epidermal cells (minimal and maximal) crossed by the hyphae on the oldest leaves of 22- day plants following "evening"- inoculation. Samples were taken 17, 19, 24, 48, 72 and 96 h after inoculation.

| CULTIVAR | Sampling time (h) | Micro-colonies (%) | Colonies (%) | Average hyphal length ($mm \times 10^{-1}$) | Calculated number of epidermal cells crossed by hyphae | |
|---|---|---|---|---|---|---|
| | | | | | minimum | maximal |
| BS10 | 17 | 64.7 | - | 1.1 | 18 | 28 |
| | 19 | 63.6 | - | 2.4 | 40 | 60 |
| | 24 | - | 68.2 | 5.1 | 85 | 128 |
| | 48 | - | 75.1 | 6.8 | 113 | 170 |
| | 72 | - | 90.1 | 9.3 | 155 | 232 |
| | 96 | - | 94.1 | 10.7 | 178 | 268 |
| BS12 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 6.1 | - | 1.8 | 26 | 50 |
| | 48 | 6.3 | - | 2.1 | 31 | 58 |
| | 72 | - | 10.4 | 2.8 | 37 | 78 |
| | 96 | - | 15.8 | 3.1 | 46 | 86 |
| JI1047 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 6.7 | - | 2.0 | 40 | 67 |
| | 24 | 11.1 | - | 4.3 | 86 | 143 |
| | 48 | 22.9 | - | 4.8 | 96 | 160 |
| | 72 | - | 28.9 | 5.2 | 104 | 173 |
| | 96 | - | 34.7 | 5.9 | 118 | 197 |
| JI1048 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 0 | - | 0 | 0 | 0 |
| | 48 | 0 | - | 0 | 0 | 0 |
| | 72 | 5.9 | - | 0.9 | 13 | 21 |
| | 96 | 6.7 | - | 1.8 | 27 | 42 |
| JI1049 | 17 | 22.2 | - | 0.3 | 5 | 6 |
| | 19 | 25.0 | - | 0.9 | 15 | 18 |
| | 24 | 20.1 | - | 2.7 | 45 | 54 |
| | 48 | 23.6 | - | 2.9 | 48 | 58 |
| | 72 | - | 20.7 | 3.4 | 57 | 68 |
| | 96 | - | 20.9 | 3.9 | 60 | 78 |
| JI1050 | 17 | 0 | - | 0 | 0 | 0 |
| | 19 | 0 | - | 0 | 0 | 0 |
| | 24 | 11.7 | - | 1.8 | 20 | 45 |
| | 48 | 10.5 | - | 2.2 | 37 | 55 |
| | 72 | - | 15.7 | 2.9 | 48 | 73 |
| | 96 | - | 20.8 | 3.2 | 53 | 80 |

Fig. 14

Comparisons of average hyphal lengths in colonies of E. pisi on six 22-day cultivars on pea following "evening" inoculation.

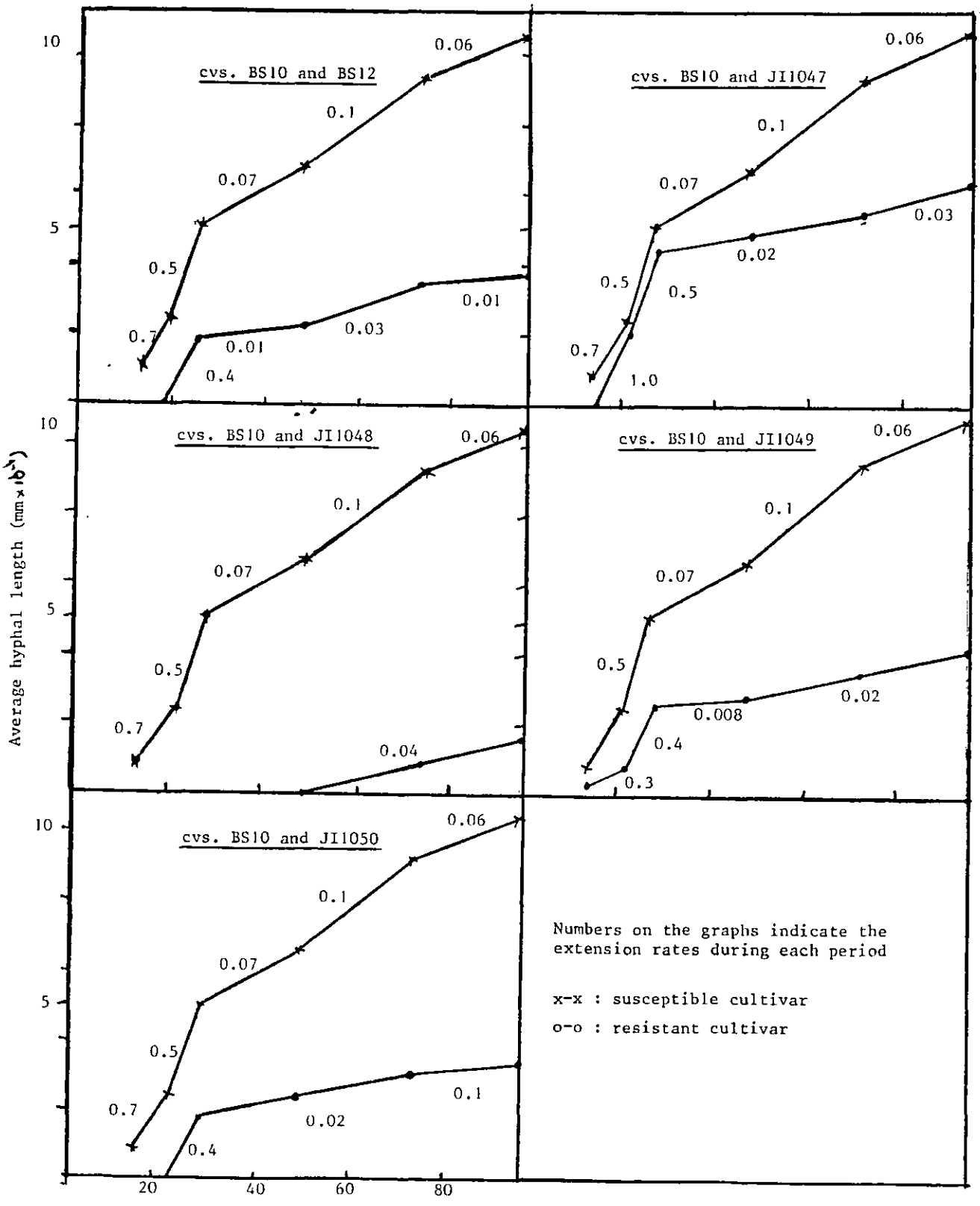


Table 41 Statistical analysis of data presented in Table 37 comparing the effect of cultivar on the average hyphal length produced per germinated conidium on the oldest leaves of 15- day plants following "morning"-inoculation. Samples were taken 24, 30, 48, 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| | AVERAGE HYPHAL LENGTH ($mm \times 10^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Cultivar | | | | | |
| Sampling time (h) | BS10 | BS12 | JI1047 | JI1048 | JI1049 | JI1050 |
| 24 | 1.8 | 0 *(0.001) | 0 (0.001) | 0 (0.001) | 0 (0.001) | 0.5 (0.001) |
| 30 | 2.1 | 0.2 (0.001) | 0 (0.001) | 0 (0.001) | 0.1 (0.001) | 0.6 (0.001) |
| 48 | 4.2 | 1.7 (0.001) | 1.9 (0.001) | 1.0 (0.001) | 0.2 (0.001) | 0.7 (0.001) |
| 72 | 4.1 | 3.7 (0.01) | 2.3 (0.001) | 1.5 (0.001) | 0.6 (0.001) | 1.3 (0.001) |
| 96 | 12.5 | 3.1 (0.001) | 4.9 (0.001) | 3.5 (0.001) | 1.1 (0.001) | 2.7 (0.001) |

* p : Significance of differences in lengths of hyphae on BS10 and resistant cultivars

Table 42 Statistical analysis of data presented in Table 38 comparing the effect of cultivars on the average hyphal length produced per germinated conidium on the oldest leaves of 22- day plants following "morning"-inoculation. Samples were taken 24, 30, 48 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| | AVERAGE HYPHAL LENGTH ($mm \times 10^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Cultivar | | | | | |
| Sampling time (h) | BS10 | BS12 | JI1047 | JI1048 | JI1049 | JI1050 |
| 24 | 1.9 | 1.9 | 1.6 (0.01) | 0.7 (0.001) | 0.8 (0.001) | 0.5 (0.001) |
| 30 | 2.1 | 2.0 *(0.1) | 2.6 (0.01) | 1.6 (0.001) | 1.2 (0.001) | 0.8 (0.001) |
| 48 | 2.7 | 2.6 (0.1) | 2.9 (0.1) | 2.3 (0.01) | 1.6 (0.001) | 1.7 (0.001) |
| 72 | 5.9 | 2.9 (0.01) | 3.3 (0.01) | 2.8 (0.01) | 2.1 (0.001) | 2.4 (0.001) |
| 96 | 7.1 | 3.8 (0.001) | 4.2 (0.001) | 3.8 (0.001) | 2.8 (0.001) | 2.8 (0.001) |

* p : Significance of differences in lengths of hyphae on BS10 and resistant cultivars

Table 43 Statistical analysis of data presented in Table 39 comparing the effect of cultivar on the average hyphal length produced per germinated conidium on the oldest leaves of 15- day plants following "evening"- inoculation. Samples were taken 17, 19, 24, 48, 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| | AVERAGE HYPHAL LENGTH ($mm \times 10^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Cultivar | | | | | |
| Sampling time (h) | BS10 | BS12 | JI1047 | JI1048 | JI1049 | JI1050 |
| 17 | 0.2 | 0 * (0.001) | 0 (0.001) | 0 (0.001) | 0 (0.001) | 0 (0.001) |
| 19 | 0.26 | 0 (0.001) | 0 (0.001) | 0 (0.001) | 0 (0.001) | 0 (0.001) |
| 24 | 0.59 | 0 (0.001) | 0 (0.001) | 0 (0.001) | 0 (0.001) | 0 (0.001) |
| 48 | 0.9 | 0.2 (0.001) | 0.2 (0.001) | 0.3 (0.01) | 0.5 (0.01) | 0 (0.001) |
| 72 | 4.1 | 1.4 (0.001) | 2.0 (0.001) | 2.1 (0.001) | 1.3 (0.001) | 2.2 (0.001) |
| 96 | 8.2 | 2.1 (0.001) | 2.7 (0.001) | 2.6 (0.001) | 1.9 (0.001) | 2.8 (0.001) |

* p : Significance of differences in lengths of hyphae on BS10 and resistant cultivars

Table 44 Statistical analysis of data presented in Table 40 comparing the effect of cultivar on the average hyphal length produced per germinated conidium on the oldest leaves of 22- day plants following "evening"- inoculation. Samples were taken 17, 19, 24, 48, 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| | AVERAGE HYPHAL LENGTH ($mm \times 10^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Cultivar | | | | | |
| Sampling time (h) | BS10 | BS12 | JI1047 | JI1048 | JI1059 | JI1050 |
| 17 | 1.1 | 0 (0.001) | 0 (0.001) | 0 (0.001) | 0.3 (0.001) | 0 (0.001) |
| 19 | 2.4 | 0 (0.001) | 2.0 (0.5) | 0 (0.001) | 0.9 (0.001) | 0 (0.001) |
| 24 | 5.1 | 1.8 (0.001) | 4.3 (0.5) | 0 (0.001) | 2.7 (0.001) | 1.8 (0.001 |
| 48 | 6.8 | 2.1 (0.001) | 4.8 (0.001) | 0 (0.001 | 2.9 (0.001) | 2.2 (0.001) |
| 72 | 9.3 | 2.8 (0.001) | 5.2 (0.001) | 0.9 (0.001) | 3.4 (0.001) | 2.9 (0.001) |
| 96 | 10.7 | 3.1 (0.001) | 5.9 (0.001) | 1.8 (0.001) | 3.9 (0.001) | 3.2 (0.001) |

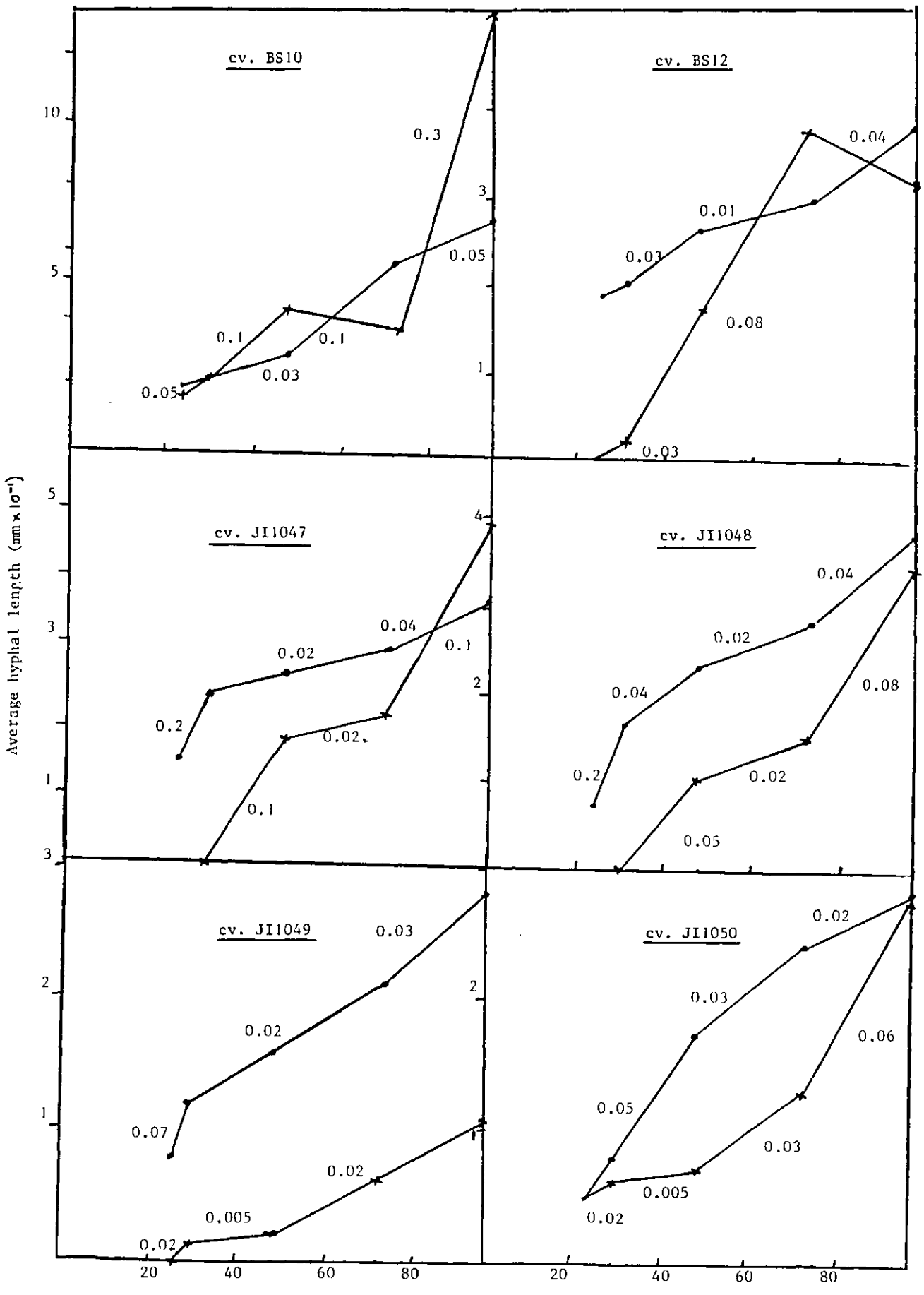
* p : Significance of differences in lengths of hyphae on BS10 and resistant cultivars

Table 45 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia) and statistical analysis comparing the effect of host plant age on the average hyphal length produced per germinated conidium on the oldest leaves of plants following "morning"-inoculation. Samples were taken 24, 30, 48, 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| | | 15- DAY PLANTS | | | 22- DAY PLANTS | | | |
|---|---|---|---|---|---|---|---|---|
| CULTIVAR | Sampling time (h) | Micro-colonies (%) | Colonies (%) | Average hyphal length (mm×10⁻²) | Micro-colonies (%) | Colonies (%) | Average hyphal length (mm×10⁻²) | Significance of differences in length of hyphae P |
| BS10 | 24 | 81.2 | - | 1.8 | 92.0 | - | 1.9 | 0.1 |
| | 30 | 90.9 | - | 2.1 | 97.4 | - | 2.1 | 0.1 |
| | 48 | - | 95.6 | 4.2 | - | 97.6 | 2.7 | 0.001 |
| | 72 | - | 95.5 | 4.1 | - | 97.8 | 3.9 | 0.5 |
| | 96 | - | 96.5 | 12.5 | - | 97.2 | 7.1 | 0.01 |
| BS12 | 24 | 0 | - | 0 | 1.4 | - | 1.9 | 0.001 |
| | 30 | 8.8 | - | 0.2 | 7.1 | - | 2.0 | 0.001 |
| | 48 | 20.0 | - | 1.7 | - | 33.2 | 2.6 | 0.001 |
| | 72 | - | 62.0 | 3.7 | - | 65.0 | 2.9 | 0.001 |
| | 96 | - | 60.0 | 3.1 | - | 64.7 | 3.8 | 0.05 |
| JI1047 | 24 | 0 | - | 0 | 3.2 | - | 1.6 | 0.001 |
| | 30 | 0 | - | 0 | 7.9 | - | 2.6 | 0.001 |
| | 48 | 10.0 | - | 1.9 | - | 8.5 | 2.9 | 0.001 |
| | 72 | 24.6 | - | 2.3 | - | 8.8 | 3.3 | 0.001 |
| | 96 | - | 28.2 | 4.9 | - | 10.4 | 4.2 | 0.5 |
| JI1048 | 24 | 0 | - | 0 | 1.7 | - | 0.7 | 0.001 |
| | 30 | 0 | - | 0 | 16.7 | - | 1.6 | 0.001 |
| | 48 | 0.8 | - | 1.0 | 22.2 | - | 2.3 | 0.001 |
| | 72 | - | 6.2 | 1.5 | - | 27.3 | 2.8 | 0.001 |
| | 96 | - | 8.5 | 3.5 | - | 29.3 | 3.8 | 0.5 |
| JI1049 | 24 | 0 | - | 0 | 17.6 | - | 0.8 | 0.001 |
| | 30 | 9.3 | - | 0.1 | 36.0 | - | 1.2 | 0.001 |
| | 48 | 10.7 | - | 0.2 | 61.5 | - | 1.6 | 0.001 |
| | 72 | 10.6 | - | 0.6 | 60.8 | - | 2.1 | 0.001 |
| | 96 | 40.0 | - | 1.1 | - | 67.8 | 2.8 | 0.001 |
| JI1050 | 24 | 8.0 | - | 0.5 | 18.8 | - | 0.5 | - |
| | 30 | 10.0 | - | 0.6 | 14.1 | - | 0.8 | 0.01 |
| | 48 | 14.5 | - | 0.7 | 18.3 | - | 1.7 | 0.001 |
| | 72 | 15.2 | - | 1.3 | - | 15.3 | 2.4 | 0.001 |
| | 96 | - | 14.5 | 2.7 | - | 20.4 | 2.8 | 0.1 |

Fig. 15

Comparisons of average hyphal lengths in colonies of E. pisi on six 15- and 22- day cultivars of pea following "morning"- inoculation.




Numbers on the graphs indicate the extension rates during each period

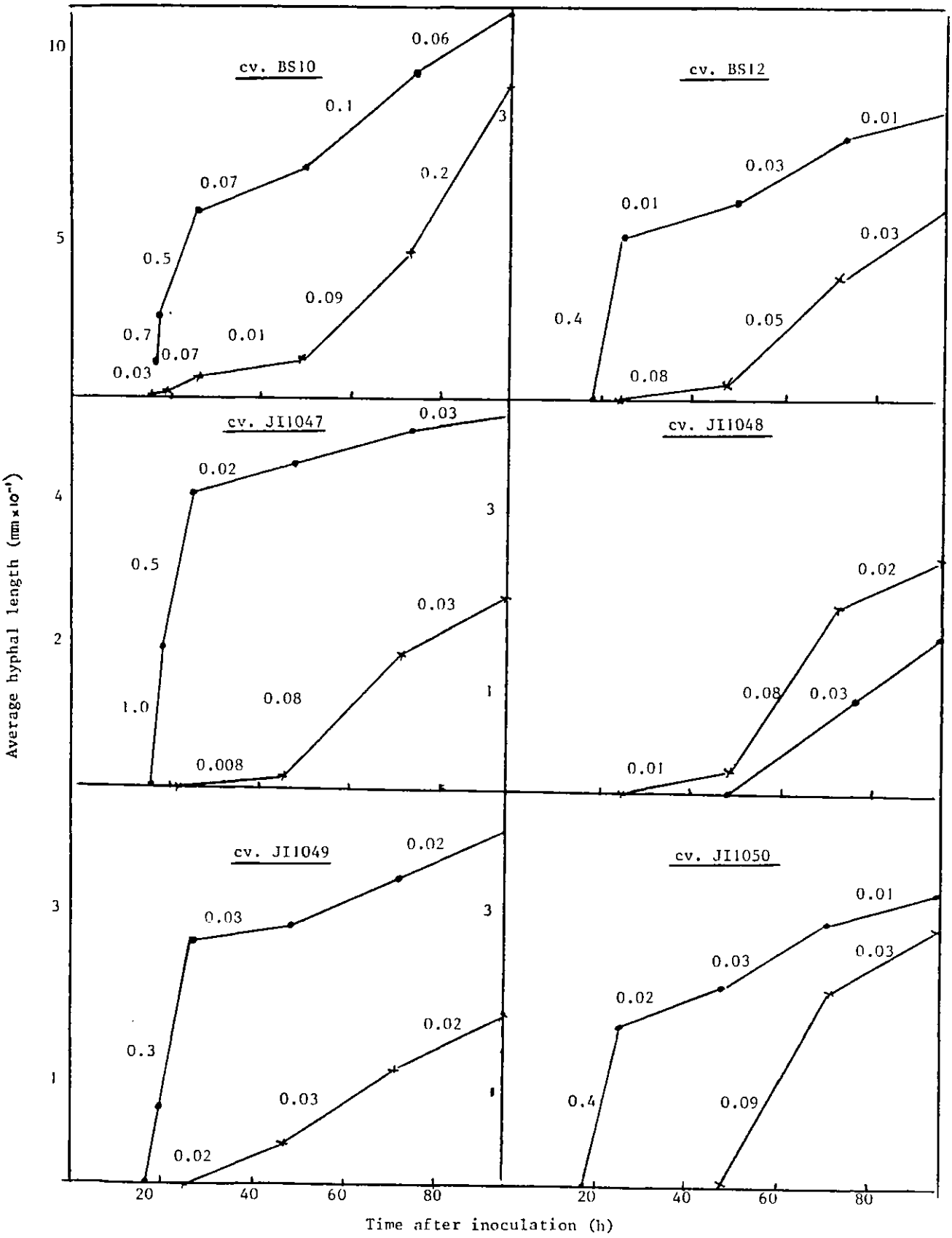
x-x : 15-day plants  o-o : 22-day plants

Table 46 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia) and statistical analysis comparing the effect of host plant age on the average hyphal length produced per germinated conidium on the oldest leaves of plants following "evening"- inoculation. Samples were taken 17, 19, 24, 48, 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| CULTIVAR | Sampling time (h) | 15-DAY PLANTS Micro-colonies (%) | Colonies (%) | Average hyphal length (mm x $10^{-1}$) | 22-DAY PLANTS Micro-colonies (%) | Colonies (%) | Average hyphal length (mm x $10^{-1}$) | Significance of differences in lengths of hyphae P |
|---|---|---|---|---|---|---|---|---|
| BS10 | 17 | 36.5 | - | 0.2 | 64.7 | - | 1.1 | 0.001 |
| | 19 | 38.0 | - | 0.26 | 63.6 | - | 2.4 | 0.001 |
| | 24 | 58.0 | - | 0.59 | - | 68.2 | 5.1 | 0.001 |
| | 48 | 91.0 | - | 0.9 | - | 75.1 | 6.8 | 0.001 |
| | 72 | - | 96.3 | 4.1 | - | 90.1 | 9.3 | 0.01 |
| | 96 | - | 97.2 | 8.2 | - | 94.1 | 10.7 | 0.001 |
| BS12 | 17 | 0 | - | 0 | 0 | - | 0 | - |
| | 19 | 0 | - | 0 | 0 | - | 0 | - |
| | 24 | 0 | - | 0 | 6.1 | - | 1.8 | 0.001 |
| | 48 | 1.6 | - | 0.2 | 6.3 | - | 2.1 | 0.001 |
| | 72 | - | 6.8 | 1.4 | - | 10.4 | 2.8 | 0.001 |
| | 96 | - | 9.2 | 2.1 | - | 15.8 | 3.1 | 0.001 |
| JI1047 | 17 | 0 | - | 0 | 0 | - | 0 | - |
| | 19 | 0 | - | 0 | 6.7 | - | 2.0 | 0.001 |
| | 24 | 0 | - | 0 | 11.1 | - | 4.3 | 0.001 |
| | 48 | 5.9 | - | 0.2 | 22.9 | - | 4.8 | 0.001 |
| | 72 | 8.6 | - | 2.0 | - | 28.9 | 5.2 | 0.001 |
| | 96 | - | 8.8 | 2.7 | - | 34.7 | 5.9 | 0.001 |
| JI1048 | 17 | 0 | - | 0 | 0 | - | 0 | - |
| | 19 | 0 | - | 0 | 0 | - | 0 | - |
| | 24 | 0 | - | 0 | 0 | - | 0 | - |
| | 48 | 3.5 | - | 0.3 | 0 | - | 0 | 0.001 |
| | 72 | 15.8 | - | 2.1 | 5.9 | - | 0.9 | 0.001 |
| | 96 | - | 25.1 | 2.6 | 6.7 | - | 1.8 | 0.001 |
| JI1049 | 17 | 0 | - | 0 | 22.2 | - | 0.3 | 0.001 |
| | 19 | 0 | - | 0 | 25.0 | - | 0.9 | 0.001 |
| | 24 | 0 | - | 0 | 20.1 | - | 2.7 | 0.001 |
| | 48 | 3.0 | - | 0.5 | 23.6 | - | 2.9 | 0.001 |
| | 72 | 20.2 | - | 1.3 | - | 20.7 | 3.4 | 0.001 |
| | 96 | 32.8 | - | 1.9 | - | 20.9 | 3.9 | 0.001 |
| JI1050 | 17 | 0 | - | 0 | 0 | - | 0 | - |
| | 19 | 0 | - | 0 | 0 | - | 0 | - |
| | 24 | 0 | - | 0 | 11.7 | - | 1.8 | 0.001 |
| | 48 | 0 | - | 0 | 10.5 | - | 2.2 | 0.001 |
| | 72 | 4.1 | - | 2.2 | - | 15.7 | 2.9 | 0.01 |
| | 96 | - | 9.6 | 2.8 | - | 20.8 | 3.2 | 0.01 |

Fig. 16

Comparisons of average hyphal lengths in colonies of E. pisi on six 15- and 22- day cultivars of pea following "evening"- inoculation.




Numbers on the graphs indicate the extension rates during each period

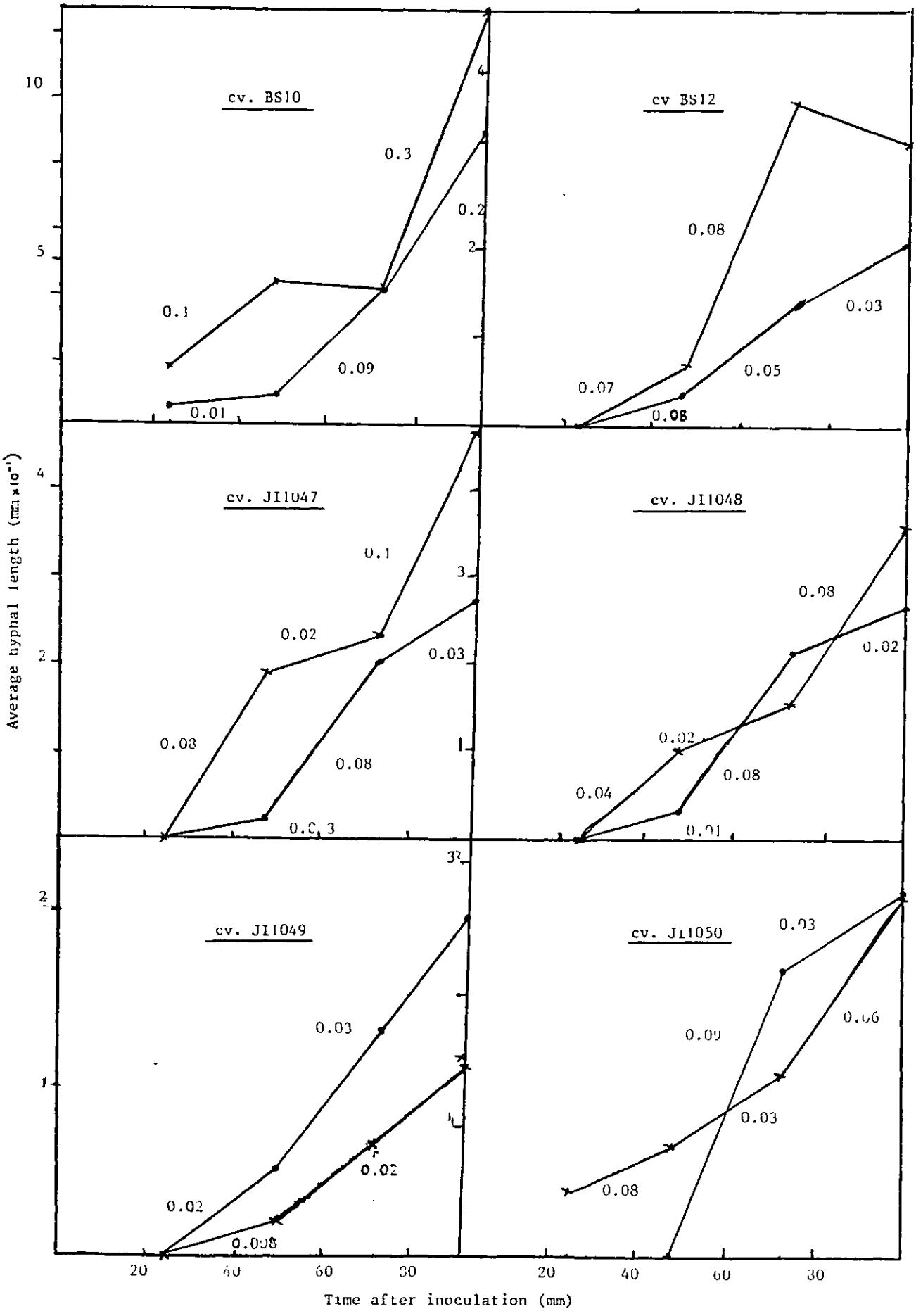
x-x : 15-day plants  o-o : 22-day plants

Table 47 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia) and statistical analysis of the effect of time of inoculation on the average hyphal length produced per germinated conidium on the oldest leaves of 15- day plants. Samples were taken 24, 48, 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| | | "MORNING"-INOCULATION | | | "EVENING"-INOCULATION | | | |
|---|---|---|---|---|---|---|---|---|
| CULTIVAR | Sampling time (h) | Micro-colonies (%) | Colonies (%) | Average hyphal length ($mm \times 10^{-1}$) | Micro-colonies (%) | Colonies (%) | Average hyphal length ($mm \times 10^{-1}$) | Significance of differences in lengths of hyphae P |
| BS10 | 24 | 81.2 | - | 1.8 | 58.0 | - | 0.59 | 0.001 |
| | 48 | - | 95.6 | 4.2 | 91.0 | - | 0.9 | 0.001 |
| | 72 | - | 95.5 | 4.1 | - | 96.3 | 4.1 | - |
| | 96 | - | 96.5 | 12.5 | - | 97.2 | 8.2 | 0.001 |
| BS12 | 24 | 0 | - | 0 | 0 | - | 0 | - |
| | 48 | 20.0 | - | 1.7 | 1.6 | - | 0.2 | 0.001 |
| | 72 | - | 62.0 | 3.7 | - | 6.8 | 1.4 | 0.001 |
| | 96 | - | 60.0 | 3.1 | - | 9.2 | 2.1 | 0.001 |
| JI1047 | 24 | 0 | - | 0 | 0 | - | 0 | - |
| | 48 | 10.0 | - | 1.9 | 5.9 | - | 0.2 | 0.001 |
| | 72 | 24.6 | - | 2.3 | 8.6 | - | 2.0 | 0.01 |
| | 96 | - | 28.2 | 4.9 | - | 8.8 | 2.7 | 0.001 |
| JI1048 | 24 | 0 | - | 0 | 0 | - | 0 | - |
| | 48 | 0.8 | - | 1.0 | 3.5 | - | 0.3 | 0.001 |
| | 72 | - | 6.2 | 1.5 | 15.8 | - | 2.1 | 0.01 |
| | 96 | - | 8.5 | 3.5 | - | 25.1 | 2.6 | 0.001 |
| JI1049 | 24 | 0 | - | 0 | 0 | - | 0 | - |
| | 48 | 10.7 | - | 0.2 | 3.0 | - | 0.5 | 0.01 |
| | 72 | 10.6 | - | 0.6 | 20.2 | - | 1.3 | 0.001 |
| | 96 | 40.0 | - | 1.1 | 32.8 | - | 1.9 | 0.01 |
| JI1050 | 24 | 8.0 | - | 0.5 | 0 | - | 0 | 0.001 |
| | 48 | 14.5 | - | 0.7 | 0 | - | 0 | 0.001 |
| | 72 | 15.2 | - | 1.3 | 4.1 | - | 2.2 | 0.001 |
| | 96 | - | 14.5 | 2.7 | - | 9.6 | 2.8 | 0.001 |

Fig. 17

Comparisons of average hyphal lengths in colonies of E. pisi on six 15- day cultivars of pea following "morning"- and "evening"- inoculation.




Numbers on the graphs indicate the extension rates during each period

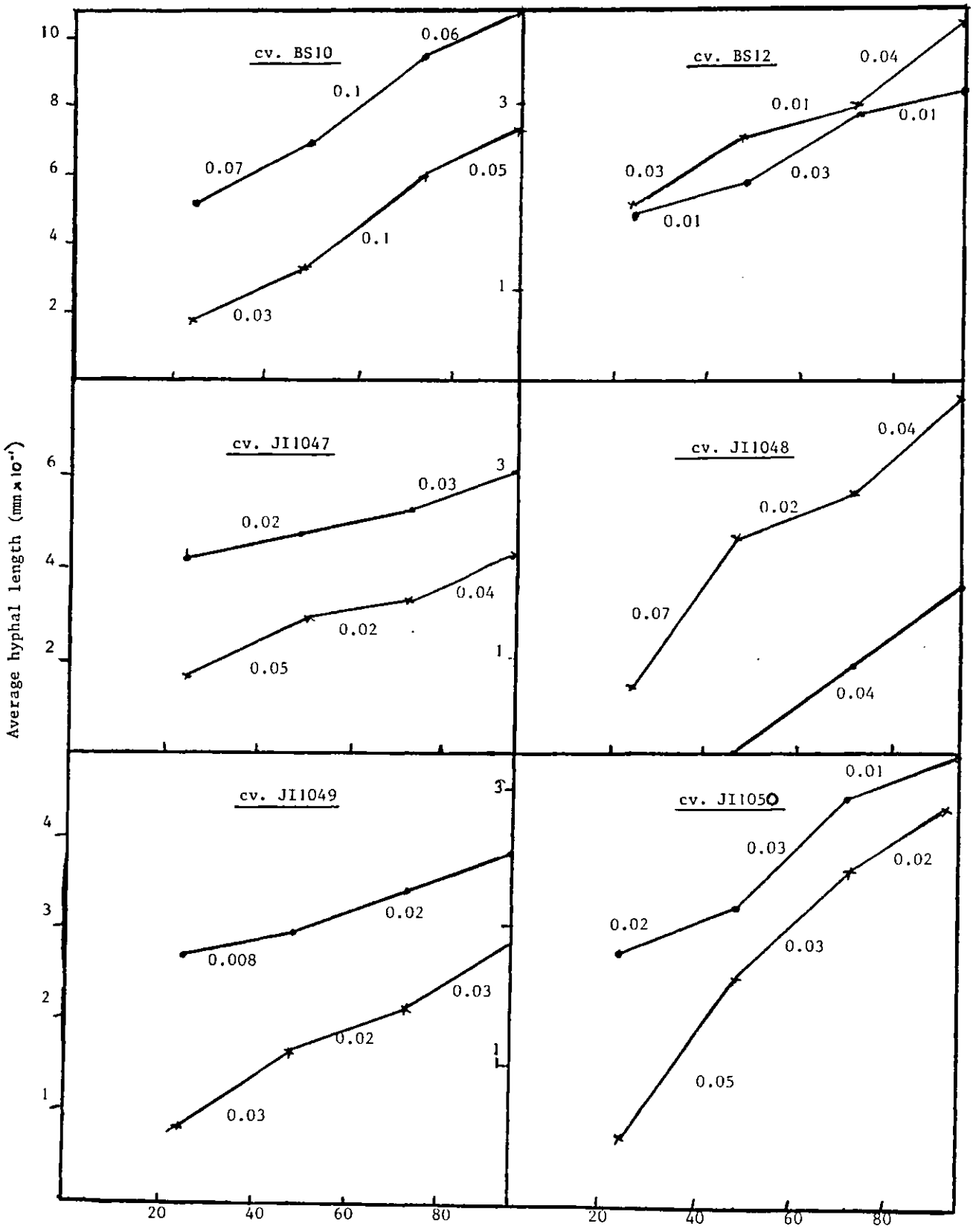
x-x : "morning"- inoculation  o-o : "evening"-inoculation

Table 48 Conidia producing micro-colonies and colonies (expressed as a % of germinated conidia) and statistical analysis comparing the effect of time of inoculation on average hyphal length produced per germinated conidium on the oldest leaves of 22- day plants. Samples were taken 24, 48, 72 and 96 h after inoculation and probability values (p) obtained from Student's t test.

| CULTIVAR | "MORNING-INOCULATION" | | | | "EVENING"-INOCULATION | | | Significance of differences in length of hyphae |
|---|---|---|---|---|---|---|---|---|
| | Sampling time (h) | Micro-colonies (%) | Colonies (%) | Average hyphal length (mm×10⁻³) | Micro-colonies (%) | Colonies (%) | Average hyphal length (mm×10⁻³) | P |
| BS10 | 24 | 92.0 | - | 1.9 | - | 68.2 | 5.1 | 0.001 |
| | 48 | - | 97.6 | 2.7 | - | 75.1 | 6.8 | 0.001 |
| | 72 | - | 97.8 | 3.9 | - | 90.1 | 9.3 | 0.001 |
| | 96 | - | 97.2 | 7.1 | - | 94.1 | 10.7 | 0.001 |
| BS12 | 24 | 1.4 | - | 2.0 | 6.1 | - | 1.8 | 0.1 |
| | 48 | - | 33.2 | 2.6 | 6.3 | - | 2.1 | 0.1 |
| | 72 | - | 65.0 | 2.9 | - | 10.3 | 2.8 | 0.1 |
| | 96 | - | 64.7 | 3.8 | - | 15.8 | 3.1 | 0.5 |
| JI1047 | 24 | 3.2 | - | 1.6 | 11.1 | - | 4.3 | 0.001 |
| | 48 | - | 8.5 | 2.9 | 22.9 | - | 4.8 | 0.001 |
| | 72 | - | 8.8 | 3.3 | - | 28.9 | 5.2 | 0.001 |
| | 96 | - | 10.4 | 4.2 | - | 34.7 | 5.9 | 0.001 |
| JI1048 | 24 | 1.7 | - | 0.7 | 0 | - | 0 | 0.001 |
| | 48 | 22.2 | - | 2.3 | 0 | - | 0 | 0.001 |
| | 72 | - | 27.3 | 2.8 | 5.9 | - | 0.9 | 0.001 |
| | 96 | - | 29.3 | 3.8 | 6.7 | - | 1.8 | 0.001 |
| JI1049 | 24 | 17.6 | - | 0.8 | 20.1 | - | 2.7 | 0.001 |
| | 48 | 61.5 | - | 1.6 | 23.6 | - | 2.9 | 0.001 |
| | 72 | 60.8 | - | 2.1 | - | 20.7 | 3.4 | 0.001 |
| | 96 | - | 67.8 | 2.8 | - | 20.9 | 3.9 | 0.001 |
| JI1050 | 24 | 18.8 | - | 0.5 | 11.7 | - | 1.8 | 0.001 |
| | 48 | 18.3 | - | 1.7 | 10.5 | - | 2.2 | 0.001 |
| | 72 | - | 15.3 | 2.4 | - | 15.7 | 2.9 | 0.01 |
| | 96 | - | 20.4 | 2.8 | - | 20.8 | 3.2 | 0.01 |

Fig. 18

Comparisons of average hyphal lengths in colonies of E. pisi on six 22-day cultivars of pea following "morning"- and "evening"- inoculation.




Numbers on the graphs indicate the extension rates during each period

x-x : "morning"-inoculation  o-o : "evening-inoculation

e Production of secondary appressoria and secondary haustoria

1 Introduction

Results from the investigation of the average hyphal length produced by each conidium (CIIId 3.1, .2, .3, .4) showed that the values recorded on the susceptible cultivar were higher than on all the resistant cultivars irrespective of host plant age or time of inoculation. This investigation was carried out to find out if the extension of colonies was correlated with the numbers of secondary haustoria. These arise from secondary appressoria which are produced along the length of secondary hyphae and lack the lobed structure characteristic of primary ones. Secondary haustoria are smaller than primary ones.

2 Experimental

After "morning"- inoculation (BI 5.1), 22-day old plants were incubated in a Fison's Fitotron, 60093/THTL, with 16 h light and 8 h dark periods. Samples were taken after 30 and 48 h, with five replicates for each of the six test cultivars. The surface fungal structures were prepared for observation on Necoloidine films (BII 3.2) and stained on lactophenol cotton blue (BII 1.2). One epidermal strip was removed from each leaf and stained in acid fuchsin (BII 1.4); strips bearing only conidia with secondary hyphae were used, as the inclusion of conidia producing only primary appressoria would have created confusion when counting corresponding primary and

secondary haustoria. Furthermore, it was necessary for the conidia with secondary hyphae to be well spaced so that no overlap of hyphae in order to assign the correct secondary haustoria to their respective micro-colony. For each micro-colony or colony counts were made of the number of secondary appressoria on the section of the Necoloidine film corresponding with the stripped epidermes where secondary haustoria were counted. The average values are recorded in Table 49.

3 Results

Secondary appressoria were not formed on any cultivar until at least two secondary hyphae had been produced by a conidium. One secondary appressorium was produced on micro-colonies with two secondary hyphae, up to three secondary appressoria when three secondary hyphae were present and up to four with four secondary hyphae. When the growths progressed to colonies (with at least four branching secondary hyphae) more than four secondary appressoria were usually produced. Secondary appressoria were recorded on BS10, BS12 and JI1047 by 30 h and on all the cultivars by 48 h (Table 49). By 30 h, micro-colonies with three secondary hyphae produced an average of three secondary appressoria on cultivars BS10 and BS12 but only one on JI1047, JI1048, JI1049 and JI1050. At this time micro-colonies with four secondary hyphae produced four secondary appressoria on cultivar BS10 whereas on the other cultivars none to two were recorded. By 48 h, when colonies had developed on cultivars BS10, BS12 and JI1047, they had produced an average of eight on BS10, three on

BS12, two on JI1047 and one or more on the other cultivars.

Secondary haustoria were not produced on cultivars JI1047, JI1048, JI1049 and JI1050. More were recorded on BS10 than on BS12. On cultivar BS10 two secondary haustoria were produced at 30 h by micro-colonies with three or four secondary appressoria but on BS12 only one was recorded on each micro-colony with three secondary appressoria. By 48 h, five secondary haustoria had been produced by each colony on BS10 but only two on BS12.

Table 49 Average numbers of secondary appresoria (app.) and haustoria (haust.) produced by colonies with 1-4 secondary hyphae on the oldest leaves of (six 22- day cutlivars following "morning"- inoculation. Samples were taken 30 and 48 h after inoculation.

| | | NUMBER OF SECONDARY HYPHAE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ONE | | TWO | | THREE | | FOUR | |
| CULTIVAR | Sampling time (h) | 2° app. | 2° haust. | 2° app. | 2° haust. | 2° app. | 2° haust. | 2° app. | 2° haust. |
| BS10 | 30 | 0 | 0 | 1 | 0 | 3 | 2 | 4 | 2 |
| | 48 | 0 | 0 | 1 | 1 | 3 | 2 | 8 | 5 |
| BS12 | 30 | 0 | 0 | 1 | 0 | 3 | 1 | 0 | 0 |
| | 48 | 0 | 0 | 1 | 0 | 3 | 1 | 3 | 2 |
| JI1047 | 30 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 0 |
| JI1048 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| JI1049 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| JI1050 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

## f Sporulation

### 1 Introduction

The purpose of this investigation was to discover if sporulation of colonies occurred on the resistant cultivars, if so, the proportion of colonies sporulating and whether it occurred later on these, than on the susceptible, cultivars.

### 2 Experimental

"Morning"- inoculation (BI 5.1) of 22-day old plants was carried out. Samples were taken 5, 6, 7 and 10 days after inoculation using give of the oldest leaves from each of the six test cultivars. Surface fungal structures were prepared for observation on Necoloidine films (BII 3.2), stained in lactophenol cotton blue (BII 1.2) and examined for the presence of colonies. Counts were made of sporulating colonies and these data are expressed as a percentage of total colonies.

### 3 Results

Sporulating colonies were seen on cultivar BS10 5 days after inoculation but not until 7 days on BS12, JI1048 and JI1049. No sporulation was recorded on the other cultivars during the 10 day period. By 7 days 97.4% of conidia producing colonies on cultivar BS10 exhibited sporulation but the values were much lower on BS12 (2.8%), JI1048 (7.6%) and JI1049 (5.4%).

# IV INVESTIGATION OF THE EFFECT OF CULTIVAR ON STRUCTURES ASSOCIATED WITH HAUSTORIA

## a Fluorescence microscopy

### 1 Introduction

These investigations were carried out in order to find sub-cellular features associated with the reduced frequency of primary and absence of secondary haustoria (Cb 2.3) and reduced numbers and growth rates of secondary hyphae on resistant cultivars (CIII c and d). The possibility that lack of penetration of epidermal cells or failure to produce haustoria following penetration was investigated using aniline ablue. This fluoresces with callose deposits (Eschrich and Currier, 1964) produced at contact between primary germ tubes, appressoria or secondary hyphae and the epidermis or during penetration of the epidermis by appresoria (Sargent et al, 1973, Stanbridge et al, 1971). In addition, the effect of cultivar on host-haustorial interfacial structures was assessed using Calcofluor white M2R New (American Cyanamid Co.) (Hughes and McCully, 1975) and 4-acetamido-4'-isothiocyanato stilbene -2, 2' disulphonic acid (SITS) (Maddy, 1964). Calcofluor causes fluorescence of β -linked polysaccharides (Maeda and Ishida, 1967) in the extra-haustorial membrane and matrix of haustorial complexes isolated from pea (Gil, 1976). SITS causes fluorescence of anion-binding sites of plasma membranes (Maddy, 1964) including the extra-haustorial membrane of haustorial complexes isolated from pea (Spencer-Phillips and Gay, 1980).

It is thought that Calcofluor ( Gunning and Hughes 1976 ) and SITS (Maddy, 1964) cannot pass through the plasmalemma of plant cells. Thus these experiments were carried out with epidermes stripped from leaves. Stripping damages the epidermal cells so that haustorial complexes are isolated from the leaf tissue but held in the epidermal wall by their necks. In this condition the stains should have access to the haustorial complexes and this was verified after each examination for fluorescence by mounting the strips in neutral red which accumulates in the vacuoles of any undamaged cells (Levitt, 1969). Lack of vacuolar staining, indicating damaged vacuoles, was taken to show that the wall lining region of the epidermal plasmalemma was broken and thus the stain had access to the extra-haustorial membrane.

## 2 Experimental

### 2.1 Aniline blue:callose fluorescence.

After "morning"-inoculation, 22-day old plants were incubated in a Fisons Fitotron (BI 2) with 16 h light and 8 h dark periods. The oldest leaves were sampled after 48 h, with five replicates in each instance. Surface fungal structures were prepared for observation on Necoloidine films (BII 3.2) and stained in lactophenol cotton blue (BII 1.2). After taking epidermal strips and drawing their areas onto leaf outlines recorded on graph paper as described above (Cb 2.2), the corresponding areas were recorded on the coverslip over each Necoloidine film. Counts were made of conidia producing a germ tube only and those with mature appresoria within these areas and the

data, together with the total germination, are recorded in Table 50. The epidermal strips were stained in aniline blue (BII 1.9) and the fluorochromatic reaction was viewed using E3 exciter and Sp3 barrier filters fitted to a Reichert Zetopan microscope with a UV source (HBO-200). The stained epidermes were examined immediately after staining to avoid fading of the fluorescence. Counts were made of fluorescing sites on epidermal walls and of haustoria within the strips. These data, expressed as a percentage of germinated conidia and the haustoria also as a percentage of fluorescing sites, are also recorded in Table 50.

2.2 Calcofluor : fluorescence of β -linked polysaccharides.

The procedure described above (CIV a 2.1) was repeated but using Calcofluor white M2R New (BII 1.7) instead of aniline blue. The fluorochromatic reaction was viewed using E2 exciter and Sp3 barrier filters. Epidermal strips from cultivar BS10 were examined on the same slide as those from each resistant cultivar so that the brightness of the fluorescence could be compared. The stained epidermes were examined immediately after staining to avoid fading of the fluorescence. Each strip was stained in neutral red (BII 1.10) after examining for fluorescence and data obtained only from damaged epidermes were used. Counts were made of haustorial complexes seen by bright field microscopy and of those fluorescing in ultraviolet light. The intensity of the fluorescence was classed as "bright" or "dull", "bright" fluorescence being equivalent

to that observed on the susceptible cultivar, BS10. Note was made of the site of fluorescence in the haustorial complexes. The numbers of haustorial complexes seen by bright field microscopy and the percentage of these fluorescing in ultraviolet light and recorded in Table 51. The numbers of haustoria exhibiting "bright" and "dull" fluorescence of the extra-haustorial membrane only and of both extra-haustorial membrane and matrix, expressed as a percentage of total fluorescing haustorial complexes are also recorded in Table 51.

2.3 SITS - fluorescence of anion-binding sites.

The experiment described above (CIV a 2.1) was repeated using 4-acetamido - 4'-isothiocyanto-stilbene-2,2'-disulphonic acid (SITS) followed by examination in ultraviolet light with a Reichert Jung light microscope and an HBO-50 mercury vapour lamp Rhodamine (exciter 546 nm and barrier OG590 filters ) and FITC, Acridine Orange (exciter BG12 and KV418 and barrier OG515 filters) filters were used. The results, recorded in Table 52, are expressed as above (CIV a 2.2).

3 Results

3.1 Comparison of numbers of sites of callose deposition fluorescing in aniline blue and of haustoria in susceptible and resistant cultivars.

As recorded above (CIII a 3.3) the frequencies of germination and formation of appresoria were lower on the resistant cultivars than on BS10 (Table 50). The number of sites fluorescing in aniline blue was also lower on the resistant cultivars but the differences between susceptible

and resistant cultivars were not correlated with the differences in germination frequencies on the different cultivars. The proportions of germinated conidia were 15-20% lower on cultivars BS12, JI1047, JI1048 and JI1049 and 7% lower on JI1050 than on BS10, whereas the difference in the numbers of fluorescing sites on susceptible and resistant cultivars was much greater (BS12-32%, JI1047-34%, JI1048-58%, JI1049-62% and JI1050-54% lower than on cultivar BS10). Also, as recorded above (CIII a 3.3), higher proportions of appressoria gave rise to haustoria on cultivar BS10 than on the resistant cultivars and large numbers of fluorescing sites had haustoria beneath them in this cultivar. Only on cultivar JI1049 did all the fluorescing sites have associated haustoria. Approximately 90% of fluorescent sites in cultivar BS10 had haustoria whereas in all the resistant cultivars except JI1049 the proportions were lower (BS12;64.8%, JI1047-69.1%, JI1048-15.9% and JI1050-55.2%).

3.2 Comparison of the fluorescence of haustorial complexes mounted in Calcofluor and examined in ultraviolet light on susceptible and resistant cultivars.

All the haustorial complexes seen on cultivar BS10 by bright field microscopy exhibited fluorescence of the extra-haustorial membrane only (Table 51). The fluorescence of all the haustoria was "bright" immediately after staining, fading after approximately 1 min and eventually disappearing. All the haustoria seen on cultivars BS12, JI1047 and JI1048 also fluoresced but only a small proportion did so on JI1049 (11.1%) and JI1050 (4.3%). On

cultivars JI1049 and JI1050 the fluorescence was confined to the extra-haustorial membrane but some of the haustoria on the other resistant cultivars also exhibited fluorescence of both the extra-haustorial membrane and matrix (BS12-50.0%, JI1047-7.6%, and JI1048-12.6%). Only "bright" fluorescence of haustorial complexes was recorded on cultivars BS12 and JI1050 but it faded faster than on BS10; on cultivars JI1047 and JI1049 almost all fluorescing haustorial complexes exhibited "dull" fluorescence and on JI1048 more than half did so.

3.3 Comparison of the fluorescence of haustorial complexes mounted in SITS and examined in ultra-violet light on susceptible and resistant cultivars.

All the haustorial complexes seen on cultivar BS10 by bright field microscopy exhibited "bright" fluorescence immediately after staining which faded after about 1 min and careful focussing showed that this all fluorescence in the other cultivars was confined to the extra-haustorial membrane (Table 52). Only about half of the extra-haustorial membranes exhibited fluoresence on cultivars JI1048, JI1049 and JI1050 and a slightly lower proportion did so on BS12. "Bright" fluoresence was exhibited by approximately 19% of fluorescing extra-haustorial membranes on cultivars JI1047, JI1048 and JI1049, by about 30% on BS12 and by 43% on JI1050, fading faster than on BS10; the other extra-haustorial membranes had "dull" fluorescence.

Table 50 Aniline blue fluorescence sites and associated haustorial complexes in stripped leaf epidermis, 48 h after "morning"-inoculation of 22- day plants of pea cultivars.

| CULTIVAR | Germination (% total conidia) | Germ Tube only (% germinated conidia) | Mature appressorium (% germinated conidia) | Sites fluorescing with aniline blue (% germinated conidia) | Haustoria (% germinated conidia) | Haustoria (% fluorescent sites) |
|---|---|---|---|---|---|---|
| BS10 | 70.0 | 24.5 | 75.5 | 65.0 | 53.9 | 89.8 |
| BS12 | 55.8 | 40.6 | 59.4 | 33.0 | 21.4 | 64.8 |
| JI1047 | 50.7 | 47.7 | 52.3 | 29.8 | 20.6 | 69.1 |
| JI1048 | 53.2 | 43.1 | 56.9 | 6.9 | 1.1 | 15.9 |
| JI1049 | 52.2 | 57.9 | 49.1 | 4.8 | 4.8 | 100 |
| JI1050 | 62.9 | 32.1 | 67.9 | 9.6 | 5.3 | 55.2 |

Table 51 Numbers of hautorial complexes seen by bright field microscopy (BFM) and the proportions fluorescing with Calcofluor in ultra violet light (CF) in epidermis stripped from the oldest leaves of 22- day plants following "morning"- inoculation. Samples were taken 48 h after inoculation. Each resistant cultivar was examined with a standard from BS10.

| CULTIVAR | FREQUENCY OF HAUSTORIA | | | | | |
|---|---|---|---|---|---|---|
| | | ULTRA VIOLET (% fluorescing haustoria) | | | | |
| | | | "Bright" fluorescence | | "Dull" fluorescence | |
| | No. haust. by BFM | Haustoria CF (% bright field) | Eh mem only | Eh mem and Eh mat | Eh mem only | Eh mem and Eh mat |
| BS10 | 52 | 100 | 100 | 0 | 0 | 0 |
| BS12 | 21 | 100 | 50.0 | 50.0 | 0 | 0 |
| BS10 | 61 | 100 | 100 | 0 | 0 | 0 |
| JI1047 | 32 | 100 | 7.6 | 0 | 84.7 | 7.6 |
| BS10 | 48 | 100 | 100 | 0 | 0 | 0 |
| JI1048 | 18 | 100 | 37.5 | 0 | 49.9 | 12.6 |
| BS10 | 41 | 100 | 100 | 0 | 0 | 0 |
| JI1049 | 27 | 11.1 | 0 | 0 | 100 | 0 |
| BS10 | 54 | 100 | 100 | 0 | 0 | 0 |
| JI1050 | 30 | 43 | 100 | 0 | 0 | 0 |

* Eh mem = Extra-hasutorial membrane
Eh mat = Extra-haustorial matrix

Table 52 Numbers of hautorial complexes seen by bright field microscopy (BFM) and the proportions fluorescing with SITS in ultra violet light (SITS F) in epidermes stripped from the oldest leaves of 22- day plants following "morning"- inoculation. Samples were taken 48 h after inoculation. Each resistant cultivar was examined with a standard from BS10.

| CULTIVAR | No. haust. by BFM | FREQUENCY OF HAUSTORIA ULTRA VIOLET (% fluorescing haustoria) Haustoria SITS F (% bright field) | "Bright" fluorescence Eh mem only | "Bright" fluorescence Eh mem and Eh mat | "Dull" fluorescence Eh mem only | "Dull" fluorescence Eh mem and Eh mat |
|---|---|---|---|---|---|---|
| BS10 | 60 | 100 | 100 | 0 | 0 | 0 |
| BS12 | 18 | 44.4 | 29.6 | 0 | 70.4 | 0 |
| BS10 | 57 | 100 | 100 | 0 | 0 | 0 |
| JI1047 | 28 | 57.7 | 18.9 | 0 | 81.1 | 0 |
| BS10 | 52 | 100 | 100 | 0 | 0 | 0 |
| JI1048 | 20 | 59.1 | 15.7 | 0 | 84.3 | 0 |
| BS10 | 48 | 100 | 100 | 0 | 0 | 0 |
| JI1049 | 21 | 58.1 | 19.3 | 0 | 80.7 | 0 |
| BS10 | 57 | 100 | 100 | 0 | 0 | 0 |
| JI1050 | 27 | 61.9 | 43.2 | 0 | 56.8 | 0 |

* Eh - mem = Extra-haustorial membrane Eh - mat = Extra-haustorial matrix

b Transmission Electron Microscopy

1 Introduction

The purpose of these experiments was to make a comparative study of the ultrastructure of haustorial complexes in the susceptible and resistant cultivars. This was necessary because the results of the investigation of the effect of cultivar on the haustorial complex by fluorescence microscopy (CIVa 3.2, 3.3) indicated that the extra-haustorial membranes in the resistant cultivars were different from those in BS10.

2 Experimental

2.1 Preparation of whole leaf pieces

Following "morning"- inoculation of 22- day plants, the six test cultivars were incubated in a growth room with a 16 h daylength. Leaf samples were taken 24 and 48 h after inoculation and pieces of leaf bearing mature appressoria or secondary hyphae, located by light microscopy, were cut out and prepared for electron microscopy, as described above (BIV 2.1). The experiment was repeated four times.

2.2 Preparation of excised epidermes

Inoculated plants were incubated as described above (4b 2.1). Samples were taken 24, 30 and 48 h after inoculation and prepared for electron microscopy as described above (BIV 2.2), after punching out discs bearing mature appressoria and secondary hyphae. The experiment was repeated six times. This procedure was also carried out using uninfected leaves from the susceptible cultivar, Onward, with and without prefixation of the samples before excision, in order to assess the damage caused to the cyto-

plasm by the cutting technique.

2.3 Preparation of epidermes exposed by grinding

Inoculated plants were incubated in a Fisons Fitotron, 600G3/THTL, with a 16 h daylength. Samples were taken 96 h after inoculation and the sections bearing micro-colonies were cut out and prepared for electron microscopy as described above (BIV 2.3).

3 Results

3.1 Preparation of whole leaf pieces

Haustoria were seen in sections cut from cultivar BS10 but none was located in the resistant cultivars. It was decided that this technique for locating haustoria was unsuitable for use with plants containing low numbers of haustoria as there was a low probability of locating the latter without examining the epidermal cell contents. The technique of epidermis excision, which facilitates examination of the epidermes, was selected for use in future experiments.

3.2 Preparation of excised epidermes

This technique was unsatisfactory because it caused general disorganisation of the cytoplasmic organelles, even when prefixation of the leaf discs was carried out before excision. Furthermore, despite the precautions taken to avoid undulation of the epidermes during polymerization, the samples did not lie completely flat in the resin. This made both trimming and sectioning extremely difficult. Occasionally some resin came away from the polymerized sample during sectioning. Also, it was difficult to judge how deep in the resin the epidermes were lying which hindered

sectioning. Location of haustoria by light microscopy after polymerization was difficult, not only because of undulations in the samples, but also because of the thickness of the resin blocks. Although resin discs of minimal thickness were prepared, they were still too thick to allow high power resolution of structures which were possibly haustoria, thus impairing positive identification.

Haustoria were located in sections cut from cultivars BS10 but none was seen in the resistant cultivars. On several occasions the structures which had been identified as haustoria in the resistant cultivars by light microscopy were seen to be nuclei exhibiting an unusually high density of chromatin. Such nuclei were not seen in cultivar BS10.

In order to facilitate identification of haustoria this technique was rejected in preference to the grinding technique (BIV 2.3).

3.3 Preparation of epidermes exposed by grinding

Although high resolution light microscopy was possible with the thin resin blocks obtained by this technique, positive identification of haustoria in the resistant cultivars was difficult because of their small size. Furthermore, the dense accumulations of chromatin in the nuclei of epidermal cells in the resistant cultivars appeared similar to haustorial lobes, and were, consequently, wrongly identified. No such modification occurred in the nuclei of epidermes from inoculated BS10. No haustoria were found in the resistant cultivars using this method even though samples bearing micro-colonies had been prepared.

## D DISCUSSION

The results of this investigation showed that development of Erysiphe pisi on pea was curtailed and delayed at the following stages of the infection process on the five resistant test cultivars in comparisons with a susceptible variety : germination of conidia, initiation and maturation of appressoria and formation of haustoria; subsequent to the latter developmental stage being affected, the production of secondary hyphae and development and sporulation of colonies were influenced. Thus, it is proposed that resistance of the cultivars to infection is due to their influence at one or more stages of a developmental sequence normal to the pathogen.

The effect of cultivar on each developmental stage is discussed separately as is the effect of time of inoculation and host plant age which were also found to affect the above mentioned developmental stages.

I Germination of conidia

1 Effect of cultivar

The results of the first investigation of the effect of cultivar on conidial germination (CIIa 1.3.1.2), using 22-day plants, showed that, in the early stages of the primary infection process (2-6 h after inoculation), germination frequencies were lower on all the resistant cultivars than on BS10; in the second, more detailed investigation (CIIa 2.3), covering the period 1-8 h after inoculation, these frequencies were lower only on cultivars BS12, JI1047, JI1048 and JI1050. Furthermore, the production of primary germ tubes was delayed on cultivars BS12, JI1047 and JI1049 in the first investigation and only on JI1049 in the second. Elongation of primary germ tubes was delayed on cultivar JI1047 in the second investigation.

Penetration of the host cells has not occurred at these early stages and, therefore, the results suggest that chemicals, inherent in or naturally produced by the leaf, on fungal spore germination and germ tube growth act either as inhibitors or stimulants. Their importance has been discussed in various reviews and articles (Allen, 1976, Blakeman, 1971, Blakeman and Atkinson, 1981, Godfrey, 1976, Martin 1964, Martin and Juniper, 1970, Tarr, 1972, Walker and Stahmann, 1955, Wood, 1967). The reduction in germination frequencies on the resistant cultivars may have been caused by an inhibitor which was either a constituent of the cuticle or present in leaf exudates. As only a proportion of the conidia was affected, it is possible that the inoculum comprised a heterogeneous conidial population of which a part was susceptible to the effects of the inhibitor.

Alternatively, the presence of only a limited quantity of an inhibitor would account for the partial inhibition of germination in the conidial population.

The role of the cuticle in resistance to plant pathogens has been reviewed by Martin (1964) and discussed by Martin and Juniper (1970). The cuticle consists of a layer of cutin impregnated with waxy material. The surface waxy layer usually consists of two fractions : a hard or true wax consisting of long-chain paraffinic compounds and a soft wax or oil. The true wax component is usually deposited on the surface of the cuticle and is known as epicuticular wax, whereas oil and acid fractions are usually embedded in the cutin. As the epicuticular wax presents the first potential barrier to fungal infection, the effect of its components on spore germination has been extensively studied. However, it is not always easy to assess the importance of an antifungal chemical in the epicuticular wax as a resistance mechanism. The germination of conidia of Podosphaera leucotricha was suppressed by wax extracted from resistant apple leaves and deposited on healthy leaves. A phenolic component of the wax also suppressed germination but the most inhibitory effect was exerted by an ether-soluble acid fraction extracted from the wax using dilute potash (Martin, Batt and Burchill, 1957). This fraction also inhibited the formation of lesions of Botrytis cinerea on broad bean leaves. However, this evidence is not conclusive as the acidic fraction has been found in the same quantities in the wax from susceptible cultivars. Some components of the wax from tea leaves have been shown to completely inhibit germ tube production and elongation by

Pestalotia theae in vitro, whereas some fractions had a stimulatory effect (Venkata Ram, 1962). Germination and germ tube elongation by Monilinia fruticola and Stemphylium sarcinaeforme were inhibited by a chloroform-soluble crude wax fraction from Ginkgo biloba leaves, with greater inhibition being caused by the n-heptane soluble true wax craction than by the methanol-soluble cuticle oil (Johnston and Sproston, 1965). Martin et al (1966) showed that germination of Gleosporium limetticola spores, the cause of the withertip disease, was reduced by extracts containing wax components from citrus lime. Blakeman and Sztenjberg (1973) recorded 40-70% inhibition of conidial germination in vitro of Botrytis cinerea on chloroform-extracted wax from the leaves of red beetroot, the degree of inhibition depending on the thickness of the wax deposit and the initial extraction time (1-60 s) i.e., on the quantity of wax. Furthermore, continuous spraying of beetroot leaves with water to remove wax or treatment with chemicals which inhibit wax biosynthesis resulted in increased germination frequencies. Yeaman (1976) found that germination of Botrytis cinerea conidia in vitro was suppressed by waxes extracted from chrysanthemum leaves. Waxes extracted from leaves of chrysanthemum, broad bean, beetroot, birch, lettuce and tomato, also caused inhibition of conidial germination of Botrytis cinerea in vitro (Blakeman and Atkinson, 1976). Although the above mentioned examples indicate that fungistatic chemicals may be present in the leaf waxes of resistant plants, it has been suggested that the antifungal activity demonstrated by extracts obtained using organic

solvents may be due to other chemicals co-extracted with the wax (Blakeman and Atkinson, 1981). Blakeman and Atkinson, (1976), for example, showed that sporostatic activity associated with wax extracts of chrysanthemum leaves was confined to the more polar components; water-soluble polar substances are not usually regarded as wax constitutents. Detailed analysis of leaf waxes and of antifungal compounds extracted from them is necessary before conclusions can be drawn about the source of the inhibitory agents.

It is uncertain, from investigations made to date, whether the epicuticular waxes of pea possess antifungal chemicals. Chemical analysis of waxes of pea leaves has shown that they contain n-alkanes ($C_{27}$-$C_{35}$, mainly $C_{31}$), aldehydes and esters, primary ($C_{16}$-$C_{32}$, mainly $C_{26}$ and $C_{28}$) and secondary alcohols (Holloway, 1967). It seems possible that inhibitors of primary germ tube production are present in the epicuticular waxes of cultivars JI1047, JI1048, JI1049 and JI1050, as their morphology, as seen by scanning electron microscopy (CIIa 4.3) is different from that on BS10. Kreger (1949) postulated that the physical structure and arrangement of waxes on leaf cuticles is correlated with their chemical composition. This claim is supported by Hallam (1970), who suggested that the morphology of waxes is a function of their chemical constituents rather than of the mode of extrusion. It is, therefore, likely that the waxes on the surface of the above mentioned resistant cultivars contain sporostatic chemicals absent from BS10. A good example of the relationship between wax morphology and chemistry is that of subglaucous or glossy-leaved mutants of plants whose wild-type exhibits glaucous leaves. Glaucous

leaf surfaces have a waxy bloom, as opposed to a glossy appearance, due to reflection and scattering of light on the surface by waxy deposits whose dimensions are close to or just above the wavelength of light. The waxes on the leaf surfaces of normal glaucous plants and genetically different mutants were studied by Hall et al (1965) by scanning electron microscopy. They found morphological differences between the glaucous wild-type (cultivar Kelvedon Monarch) and three mutants. The waxes on the glaucous plant comprised rods and irregularly shaped platelets distributed in a random fashion. Of the two subglaucous mutants, one (with the recessive mutant allele wa) exhibited a mixture of very small flat platelets and some which were larger than those on the normal plant, and the other (recessive mutant allele wb) had large platelets. The glossy or "green" mutant (recessive mutant alleles wb and wlo) had wax consisting of small spherical granules and a few rods but without platelets. From these and investigations of waxes on normal plants and mutants of cauliflower, Eucalyptus urnigera and Poa colensai, Hall et al concluded that glaucous leaves exhibit : (1) wax deposits that grow outwards from the leaf surface, (2) random orientation of the wax and (3) a leaf surface that is densely covered with exuded deposits; waxes on subglaucous and glossy surfaces are (1) mainly of a form that lies flat on the cuticle i.e., the top of each deposit is almost parallel to the cuticle surface, (2) sometimes less plentiful than on a glaucous surface or (3) orientated in a well defined manner. The leaves of cultivar BS10 have a very pronounced bloom,

cultivars JI1047, JI1048 and JI1050 exhibiting this to a lesser extent. Furthermore, the morphology of the waxes on the resistant cultivars approaches that described by Hall et al for the mutants of pea in exhibiting a reduction in projections and a more ordered arrangement of the waxes. It is, therefore, likely that this difference in physical structure from that seen on BS10 is correlated with a difference in chemical composition. This was found to be the case with epicuticular waxes on four mutants of pea (wa, wb, was, wsp) whose chemicals constituents were compared with the wild-type by Macey and Barber (1970a). The wax on the glaucous leaves contained 50% n-alkanes whereas the level was much lower in the mutants. 98% of the alkanes on the wild-type was n-hentriaceantane ($C_{31}$) whereas in wb, was and wsp, although it was still the main alkane constituent, its level was reduced; in mutant was the main constituent was nonacosane. There was an increase in the quantities of esters, aldehydes and primary alcohols in the mutants but levels of secondary alcohols were much reduced. The general trend was, therefore, a reduction in the more hydrophobic components in the waxes on the mutant leaves. Chemical analysis of normal and mutant waxes of brussels sprout (Baker, 1972, 1974) and cauliflower (Hall et al, 1965, Macey and Barber, 1970b) also showed a decrease in the total amount of wax and an increase its hydrophilic components on mutant leaves. Martin and Juniper (1970) suggested that the more hydrophilic components of leaf waxes (especially fatty acids and primary alcohols) are more likely to exert fungistatic action than the hydrophilic compounds (alkanes, ketones, esters and secondary

alcohols. Furthermore, Blakeman and Atkinson (1976) found that ether-soluble extracts, from a variety of plants, which inhibited spore germination contained a large number of polar components. It, therefore, seems possible that a similar variety in wax constituents to that described above on pea, brussels sprout and cauliflower, occurred on cultivars JI1047, JI1048, JI1049 and JI1050. Increasing levels of hydrophilic components in the waxes of these resistant cultivars may have been responsible for the inhibition of conidial germination of Erysiphe pisi.

Inhibition of germination may have been caused by chemicals present in the cutinaceous component of the cuticle. Cutin, the main component of the cuticle, is a polymer comprising a mixture of esterified fatty acids, the major constituents being hydroxy-and epoxy-fatty acids (either $C_{16}$ of $C_{18}$ usually predominate) and the minor constituents being mono- and dicarboxylic fatty acids and phenolics (Martin and Juniper, 1970). The best documented example of chemical resistance exerted by preformed substances present in the cutin is that of resistance exerted by the pigmented thin dry dead outer scale leaves of onion to Colletotrichum circinans, the cause of the smudge disease (Walker, 1923, Walker and Lindegren, 1924). Colourless water-soluble phenols, catechol and protocatechuic acid have been isolated from dead pigmented scales (Link et al 1929, Link and Walker, 1933). Walker et al (1929) and Angell et al (1930) showed that spores did not germination in drops of water on the dead pigmented outer scales, only on dry colourless outer scales. This is due to the sporostatic effect of the above mentioned phenols which diffuse into the infection drop from the leaf

surface. These compounds are either absent from dead colourless and living coloured scales or are in a form which does not diffuse into the infection drop. Furthermore, these compounds cause lysis of the spores, particularly if they germinate The results of these investigations have been summarised by Walker and Stahmann (1955). The peel of potato, carrot and turnip have been found to contain sporostatic components (Kuć et al, 1955). Germination of Helminthosporium carbonum, Ceratostomella ulmi and Fusarium oxysporum f. lycopersica was inhibited on the peel of the three vegetables. The inhibitory material seems to be localised at the site of inoculation since extracts of tissue taken 5 mm from the inoculated surface showed no inhibitory effects on the growth of the fungi. These results suggest that inhibitors were produced in response to chemical activity by the fungal spores. Roberts et al (1960) showed that cutin acids isolated from the cuticle of apple and rose leaves inhibited the germination of powdery mildew conidia. The effect of the cutin acids was not specific as acids from apple leaves reduced conidial germination of rose powdery mildew, Sphaerotheca pannosa. However, there is no evidence that these inhibitors are released in an active form during infection by the fungus. Also, the extracts used in the in vitro germination tests may have been modified during extraction. In general, the question of whether the plant cuticle plays a significant role in protection against disease is as yet unresolved.

Inhibitors of fungal spore germination have been shown to be present in leaf exudates, produced independently of

the pathogen. The leaf sap of apple leaves from varieties resistant to Venturia inequalis was found to inhibit conidial germination (Johnstone, 1931, Wiltshire, 1915). Exudation of the inhibitors to the leaf surface in aqueous solution may be the cause of resistance; Gilliver (1947) obtained similar results with this fungus and almost 500 plants from various families. Kovacs (1955) and Kovacs and Szeoke (1956) showed that washings from the leaves of poplar and some herbaceous plants contained inhibitors at concentrations high enough to inhibit spore germination of Botrytis cinerea. Topps and Wain (1957) made ether extracts from leaf washings of thirteen tree species and ivy. The extracts from all the washings except those obtained from laburnum, sycamore and ivy were inhibitory to germination and germ tube elongation. Strongest inhibition was exerted by extracts from elder and privet. The washings, themselves, were too dilute to inhibit spore germination which indicates that inhibition is correlated with the concentration of the suppressant. Disease resistance of oak to Microsphaera alphitoides was associated with inhibition of germination of conidia on leaves of several species (Edwards, 1979). He found that the inhibition was caused by several unidentified phenolic compounds obtained from leaf washings; no inhibitory action was exerted by chloroform extracts of whole leaf wax nor by separated components of the wax. Of the known antifungal substances which are active on leaf surfaces, the most frequently occurring are phenols and terpenoids; few workers have found other groups of substances such as organic acids, polyacetylenes and alkaloids to be instrumental in the

inhibition of germination.

The phenomenon of stimulation of conidial germination must also be considered. It is possible that germination was chemically stimulated on cultivar BS10 but not on the resistant cultivars Brown (1922) found an increase in electrical conductivity of water drops placed on leaf surfaces and concluded that exudation of materials from within the leaf to the surface had occurred. Growth of Botrytis cinerea was enhanced in these drops, showing that stimulatory chemicals were present. Since then many workers have reported that germination is stimulated by leaf exudates. The influence of the leaf surface on conidial germination of powdery mildews has caused controversy amongst researchers. The question of whether powdery mildew conidia require a stimulant to bring about primary germ tube production has been discussed by Wheeler (1981). Germination has been found to be higher on host leaves than on glass slides and this led to the suggestion that germination is stimulated by chemicals on the leaf surface (Yarwood and Hazen, 1944). Low germination frequencies on glass were exhibited by the following powdery mildews : Erysiphe cichoracearum (Yarwood, 1936, Erysiphe graminis (Brodie, 1945, Cherewick, 1944, Grainger, 1947), Erysiphe ranunculi (Brodie and Neufield, 1942), Erysiphe trifolii (Clayton, 1942), Leveillula taurica (Nour, 1958), Podosphaera leucotricha (Berwith, 1936) and Sphaerotheca fuliginea (Hashioka, 1937). However, equally good germination on glass as on leaves was obtained with Erysiphe graminis (Graf-Marin, 1934, Manners and Hossain, 1963, Zaracovitis, 1964), Erysiphe trifolii (Yarwood, 1936),

Leveillula taurica (Nour, 1958) and Sphaerotheca pannosa (Hammarlund, 1925). However, the above mentioned fungi were not investigated in comparative experiments i.e., they were only carried out using glass. Comparisons of germination on glass and leaves within one experiment have only been carried out with six powdery mildews and the results some contradictions. As germination of conidia is influenced by environmental conditions as well as by the chemical environment it is possible that the results of these experiments were influenced by the former as well as by the presence or absence of a stimulant. It has not been possible, to date, to prove conclusively that powdery mildew conidial germination ischemically stimulated

It is not possible to state conclusively which of the above mentioned mechanisms caused the reduction in germination frequencies on the resistant cultivars without further investigations. Inhibitory chemicals may be present in the epicuticular waxes of cultivars JI1047, JI1048 and JI1050. Germination tests on isolated waxes from these and cultivar BS10 are necessary but there is a risk that other cuticular components might be co-extracted. Work done by Martin (personal communication) that recorded almost 100% germination of Erysiphe pisi conidia on isolated waxes, extracted with $CCl_4$, from the susceptible pea cultivar. Onward, indicated that all required stimulants were present in the wax extracts. However, she obtained equally high germination frequencies on glass distilled water which conversely, implies that stimulants are not required. This supports the theory of chemical inhibition by the resistant

cultivars.

The discrepancy between germination frequencies recorded in the early stages of the primary infection process, i.e. lower values in the first investigation (CIIa 1.3.1.2.) than in the second (CIIa 2.3), must be explained. The plants used in the first experiment were grown in a greenhouse where fluctuations in temperature, light or RH may have occurred, whereas the other batch was grown in a growth cabinet under strictly controlled environmental conditions. Fluctuations in environmental conditions may have affected wax development possibly affecting its chemical constituents. Martin and Juniper (1970) found that peas grown in darkness had hardly any wax but the levels increased when the plants were kept in the light for increasing periods. Hallam (1970) found that Eucalyptus plants, grown at low light intensities, produced less wax and that morphological modifications could occur. He suggested that this might be a reason why seedlings grown at low light intensities are more susceptible to fungal infection.

The maximum germination frequencies recorded in the detailed investigation of germination between 22 and 240 h (CIIa 3.3) were lower on the above, mentioned resistant cultivars than on BS10 and showed little increase after 22 h on JI1047, JI1048 and JI1049 and after 92 h on JI1050. The fact that the values did not eventually reach the maximum recorded on cultivar BS10 implies that the degree of inhibition was correlated with the concentration of the suppressant, it also indicates that inhibition was not caused by a delay in the production of a stimulant. Further-

more, if the constituents of the waxes on these resistant cultivars are more hydrophilic, as suggested above or if less wax is present, the passage of aqueous exudates containing stimulants would be more likely than on BS10; the passage of solutions with inhibitors would, therefore, also be facilitated. However, as there seem to be limitations on the concentration of inhibitor available to the conidia it is most likely that they are already present in the wax; if ready passage was available to the inhibitor from within the leaf higher levels of inhibition would probably have occurred. The inhibition of germination on cultivar BS12 is more difficult to explain as the wax on this and on cultivar BS10 appears to be identical. There may, however, be an inhibitor present in the wax.

If, however, the assumption that the waxes on cultivars JI1047, JI1048, JI1049 and JI1050 are more hydrophilic than those on BS10 is wrong, then the stimulation of germination may be considered. Sufficient concentrations of stimulants to result in the same germination frequencies as on BS10 might not be produced by the resistant cultivars.

The delay in primary germ tube production recorded on cultivar JI1049 in both investigations may have been similarly due to a constituent of the wax or a leaf exudate. This occurred on BS12 and JI1047 only in the first experiment so it is possible that other factors were responsible for the delay on these cultivars. An inhibitor of germ tube elongation might have been present on cultivar JI1047. Leachates from lilac leaves have been shown to inhibit germ tube growth of Alternaria alternata and Botrytis cinerea. At

least three different phenolic compounds were detected in the leachates (Godfrey and Clements, 1978). Also, unidentified fractions from leaf extracts of a variety of plants were found to inhibit germ tube elongation by Colletotrichum acutatum (Parbery and Blakeman, 1978). However, as elongation of primary germ tubes was only delayed on cultivar JI1047 it may have been caused by a volatile inhibitor or due to delayed production of the necessary stimulant.

2 Effect of host plant age

Host plant age was found to affect germination on some of the cultivars (BS10, BS12 and JI1049) in the early stages (0-6 h) of the primary infection process, lower frequencies occurring on 15 - day plants. Variations in germination frequencies occurred on the other cultivars but no clear trends were apparent. The values recorded in the three age groups (15-, 22- and 33- day plants) became similar on all the cultivars by 24 h after inoculation and were similar on 15- and 22- day plants by this time or earlier in experiments primarily designed to investigate other aspects of development. The general trend was, therefore, a delay in germination of some conidia in the early stages of infection on 15- day plants. The variations which occurred may have been due to environmental variations

It is unlikely that a delay in the production of a stimulant was responsible for the inhibition of germination on the younger plants in the early stages of the infection process as conidia of Erysiphe pisi do not seem to require an external stimulant for germination. This is supported by the observation that about 80% germination was obtained

on glass and distilled water (Martin, personal communication). Inhbitors of germination may have been present in the waxes of 15- day plants or exuded by the leaf. Scanning electron microscopy did not show any morphological difference in the waxes on 15- and 22- day pea cultivars. Juniper (1960a, b) also reported that the very young leaves of pea have prominent wax deposits almost identical to those on mature leaves. However. inhibition of germination may have been caused by sub-microscopical featuresof the waxes or by some of the chemical constituents. Kolattakudy (1970), who analysed pea leaf waxes suggested that their composition may change as the leaves grow. Jeffree et al (1976) also stated that waxes may undergo chemical modifications during their development and ageing

## II Formation of appressoria

### 1 Effect of cultivar

It has been shown that appressorium development does not proceed normally on the resistant cultivars and this suggests that these plants possess features which are not conducive to or interfere with development. Investigations of appressorium initiation and maturation showed that these developmental stages were affected on all the resistant cultivars in the first 8 h of the infection process. Inhibition of appressorium initiation occurred on three of the resistant cultivars. The detailed investigation (CIIIa 2.3) showed that initiation of appressoria was delayed by 1 h on cultivars BS12 and JI1048; even after initiation had begun, a higher proportion of conidia. on these cultivars and on JI1050, remained at the primary germ tube stage than on BS10 throughout the 8 h observational period. Inhibition of the maturation of appressorial initials occurred on cultivars JI1047 and JI1049. However, both these inhibitory effects merely caused a delay in achieving maximum incidence of mature appressoria and in the later stages of the infection process (by 24 h) they were not apparent.

In their review on appressoria, Emmett and Parbery (1975) suggested that appressorium formation is partly governed by the genotype of the spore and that a spore population may be heterogeneous in its requirements for the development of this morphological stage i.e. part of the spore population may be very specific in its requirements and highly sensitive to fluctuations in the physical

and chemical environment, whereas part may be more tolerant of such variations. Singh (1973) postulated that requirements for appressorium formation are predetermined by the conditions under which the spores develop. The generally accepted view, put forward by Emmett and Parbery (1975) is that development of appressoria is governed by both physical and chemical stimuli although one or other may predominate.

Busgen (1893) postulated that appresoria are initiated when a germ tube or hyphal apex contacts a solid surface. This view has been supported by the findings of other workers that appressoria can develop on surfaces other than that of the plant. Germination experiments carried out by Dickinson (1949a, 1964) with rust fungi on paraffin wax-collodion membranes led to the conclusion that initiation of appresoria is a result of a contact stimulus. The importance of surface texture on the development of appressoria was also investigated by van Burgh (1950), who obtained a higher frequency of appresorium formation by Colletotrichum phomoides on cellophane than on glass and suggested that the former resembles the plant surface more closely. It, thus, seems that, although appressoria will develop when contact is made between part of a germ tube and a solid surface, the physical nature of that surface influences subsequent development. The angle between the hypha and the plant surface may determine the extent to which a germ tube extends over the surface before contact is made. It has been suggested that with a low angle of approach extensive growth may occur, even

away from the surface, whereas with a high angle, penetration is more likely than surface growth (Leach, 1923). Stakmann (1915) found that appressoria are initiated by uredospores of Puccinia graminis in depressions between epidermal cells, where the angle of approach is probably higher. If this hypothesis is true, the morphology of the epicuticular waxes may strongly influence development of appressoria. Yang (1971) found that appressoria produced by Erysiphe graminis on agar media, synthetic membranes and a variety of plant surfaces were abnormal, differing in size and shape from those produced on intact leaves of barley and wheat. Malformed appresoria were formed on : 1) the surface of epidermal strips formerly adjacent to the mesophyll, 2) on mesophyll cells exposed by removal of epidermal strips, 3) on the undersurface of cuticles isolated enzymatically, 4) on reconstituted cuticles after extraction of waxes and 5) on plants with eceriferum mutations. These observations indicate that wax is essential for normal appressorium formation by Erysiphe graminis. As Yang also found malformed appressoria on reconstituted wax layers, it seems likely that alterations in the spatial distribution of the waxes due to extraction, recrystallization or both were also important, or that wax alone did not provide all the requirements for appressorium formation. Conclusions from Yang 's work have been summarised by Ellingboe (1972). Further investigations (Yang and Ellingboe, 1972) provided more evidence that appressorium formation depended on the wax as well as the cuticle. Very low frequencies of

appressorium formation were recorded if wax or cuticle were used alone i.e. on the side of the cuticle adjacent to the epidermal cell wall, on dewaxed isolated cuticles and on reconstructed wax layers. Furthermore, differences in the proportions of normal and malformed appressoria on cuticles isolated from abaxial and adaxial leaf surfaces indicated that the epicuticular waxes were the most influential factor. The observation that appressoria were produced on isolated cuticles indicates that their formation does not depend on chemicals exuded from the epidermis in response to the proximity of the spores. Investigations using eceriferum mutants showed that, as well as producing malformed appressoria, Erysiphe graminis conidia produced appressoria which, although appearing normal, did not give rise to secondary hyphae, implying that haustoria were either not produced or were malformed, as the production of elongating secondary hyphae is indicative of the establishment of a compatible functional relationship between the haustorium and the host cell (Slesinski and Ellingboe, 1969). However, this claim of surface specificity and particularly that wax configurations were a determining factor was challenged by Staub et al (1974), who found that appressorium formation by Erysiphe graminis conidia was not inhibited on cucumber, despite the lack of epicuticular waxes on the non-host. Also, Zaracovitis (1966) obtained high frequencies by appressorium formation by Erysiphe graminis conidia on glass slides under certain strictly defined environmental conditions.

It has been postulated that chemical stimuli also

govern the formation of appressoria in other plant-pathogen interactions. Hurd-Karrer and Rodenhiser (1947) recorded rust appressoria on a nutrient agar medium containing glucose, phosphates and other nutrients but not on water agar. Appressoria of Puccinia triticina and Puccinia striiformis were formed on artificial membranes only if material from the leaf of a susceptible cultivar was incorporated (Dickinson, 1964). Phenols extracted from wheat leaves induced appressorium formation by Puccinia graminis on agar when applied with volatile fractions from the leaves (Grambow, 1977, Grambow and Riedel, 1977, Grambow and Grambow, 1978). Appressorium formation by Alternaria porri is increased by benzene and benzene-ether extracts from onion cuticle, but a similar effect was not found with non-pathogens (Akai et al, 1967). Grover (1971) showed that Colletotrichum piperatum did not produce appressoria in water on glass slides but did so in the presence of sucrose, fructose and thiamine. These substances were found on the surface of red pepper fruits where appressoria were formed. However, amino acids and amides, also present in host exudates, were inhibitory and it was suggested that appressorium formation is dependent on the levels of stimulants and inhibitors in the infection drop.

Little work has been done to investigate the factors influencing appressorium formation by Erysiphe pisi. Martin (personal communication) investigated the development of Erysiphe pisi conidia on cuticular wax, extracted with $CCl_4$, on the dewaxed leaf surface and on drops of glass distilled water in a damp chamber (approximately 100% RH).

Martin (personal communication) found that less than 1% of the conidia produced appressoria on the wax or the water and none on the dewaxed leaf surface. These results indicate that epicuticular waxes or cuticle, alone, do not provide the stimulus required for appressorium formation. The high relative humidity, however, may have inhibited appressorium formation. Webb (ARC Report, 1979) showed that no appressoria developed on detached pea leaves (maintained on water agar containing 1 mg $l^{-1}$ benzylaminopurine) whose mesophyll had been inoculated with Erysiphe pisi after removal of the abaxial epidermis, nor on mesophyll overlayed with enzymatically isolated cuticle. This indicated that epidermal cells were required for the formation of mature appressoria. The requirement for a specific interface was also indicated by the failure of conidia to produce appressoria on callus cultured from pea leaves or peticles, even after overlaying with enzymatically isolated cuticle (Webb and Gay, 1980). The observations showed that Erysiphe pisi conidia require the presence of epicuticular waxes as well as cuticle and epidermis for the production of mature appressoria and indicate that a specific surface is necessary for initiation. It has been suggested that the hardness of a plant surface can influence appressorium formation (Emmett and Parbery, 1975). Van Burgh (1950) found that increased frequencies of appressorium production by Colletotrichum gloesporoides were correlated with increasing surface hardness. If this is so, then the epicuticular waxes on the pea leaf might provide the rigid surface required for Erysiphe pisi

development. However, it may be argued that initiation cannot occur in the absence of the epidermis as was shown by the failure in appressorium formation on isolated cuticle and on mesophyll overlayed with wax and cuticle. The requirement, is, therefore, for a specific type of living cell producing stimulatory chemicals that are exuded to the leaf surface in solution. It has been stated that waxes comprising high proportions of hydrophilic components allow passage of aqueous exudates to the leaf surface more readily than those containing mainly hydrophobic components (Martin, 1964). However, it was suggested, earlier in the discussion (DI 1 ), that the waxes on cultivars JI1048 and JI1050 were probably less hydrophobic than those on BS10 and, therefore, passage of water soluble molecules to the leaf surface is facilitated in these resistant cultivars. The morphology of the waxes on cultivar JI1047 appears to be the same as on JI1048 and JI1050 but no delay in appressorium initiation was observed on it. Furthermore, the waxes on cultivars BS10 and BS12 appear to be morphologically similar but no delay in initiation occurred on BS10. It, therefore, seems unlikely that inhibition of appressorium initiation on cultivars BS12, JI1048 and JI1050 was due to a delay in the necessary stimulatory chemicals reaching the conidia on the leaf surface because of the nature of the cuticular waxes. However, qualitative and quantitative analysis of the waxes on cultivars BS10, BS12, JI1048 and JI1050 are necessary to substantiate this claim. Inhibitors of appressorium initiation may have been produced by cultivars BS12,

JI1048 and JI1050 The penetration of substances by epidermal cells in the very early stages of the primary infection process of Erysiphe graminis on barley has been deomonstrated by Kunoh and Ishizaki (in Kunoh, 1981) These workers deomonstrated an exchange of substances between the host and pathogen by means of the movement of fluorescent dyes from the host cells to the conidia during, or soon after cytoplasmic aggregate formation, followed by movement into the appressoria and eventually into the papilla and epidermal cell walls Thus, it seems likely that a similar movement of inhibitors occurred between the epidermal cells of the resistant cultivars and the conidia on the plant surface

Maturation of appressoria appears to be controlled by a different mechanism from initiation. Although the waxes on cultivars JI1047, JI1048 and JI1050 appeared identical appressorium maturation was only inhibited on JI1047. Furthermore the waxes on cultivars JI1047 and JI1049 are different, but inhibition occurred on both. These observations indicate that wax morphology does not affect maturation of Erysiphe pisi appressoria. This conclusion is supported by the observation that the primary germ tubes on these cultivars were not abnormally long, which suggests that they had reached sites conducive to the initiation of appressoria. In contrast to the control of appressorium initiation there is no further evidence for any specific factor responsible for maturation

2 Effect of host plant age

Formation of appressoria was affected by host plant

age on all six test cultivars in the early stages (0-6h) of the primary infection process Appressorium formation was delayed on some 15- day plants (cultivars BS10, BS12, JI1047 and JI1048) and lower frequencies were recorded on all 15- day cultivars However, by 24 h after inoculation the values became similar in all age groups (15-, 22- and 33- day plants) and were also similar on 15- and 22- day plants by this time or earlier in experiments primarily designed to investigate other aspects of infection.

Appressorium formation by conidia of Erysiphe pisi depends on the presence of the specific interface of the cuticle and epidermis (discussed above, DII1). This suggests that appressorium formation is stimulated by substances produced by the epidermal cells and it seems most likely that these are normal constituents of the cuticle and most probably in the waxes.

The inhibition of appressorium formation on 15- day plants may have been caused by inhibitors or reduced quantities of stimulants reaching the conidia on the leaf surface. However, as appressorium formation frequencies became similar in all age groups by 24 h after inoculations it seems likely that stimulants were involved, their production or diffusion was only delayed Alternatively, although no morphological differences were apparent on 15- and 22- day plants, appressorium formation may have been adversely affected by sub-microscopical features of the waxes or diffusible substances present in them.

3 Effect of time of inoculation

Time of inoculation was found to affect appressorium formation on all six test cultivars. This effect was

manifested in a delay in appressorium formation on some cultivars (BS10 BS12 and JI1048) and a reduction in its frequency on all the cultivars in the early stages (0-6h) of the primary infection process after "morning"- inoculation. The values recorded at 24 h after inoculation were similar after both inoculation times. These results suggest that appressorium formation was inhibited by a light period. The conidia of Erysiphe pisi, thus show similar requirements for appressorium formation as those of Erysiphe graminis, which is inhibited by high light intensity (Masri and Ellingboe, 1963, 1966a). These workers found that maximal frequencies of appressorium initiation were obtained within 5 h when wheat plants were incubated in darkness or under low light (50ft-c), beginning 2 h after inoculation Appressorium maturation in Erysiphe graminis conidia was inhibited by high light (2800ft-c) and by darkness and became synchronized when low light (240ft-c) was applied 2 h after inoculation In the present investigation, synchronization of appressorium initiation was observed after "evening"- inoculation by Erysiphe pisi on the cultivars (BS10 JI1047 and JI1049) where resistance did not affect this developmental stage. However, it is uncertain whether the light conditions operating after "evening"- inoculation were optimal for appressorium maturation in Erysiphe pisi conidia.

III Penetration

Initial stages of penetration were difficult to assess by direct observation so the presence of callose, which fluoresces with aniline blue, and haustoria at the initial and older stages were taken as indices that penetration had occurred. Currier and Struges (1955) found that the walls of wounded onion scale epidermis showed only a small amount of callose, whereas the underlying parenchyma gave a more intense reaction, the enchanced fluorescence with aniline blue being due to a greater quantity of callose. Its most commonly recognised function is that of sealing and plugging a wound in plant tissue (Currier and Struges, 1955). Several workers have shown fluorescence of aniline blue with callose, which is believed to be the most commonly identified chemical constituent in papillae (Aist, 1976), in association with fungal penetration (Aist and Williams, 1971, Sargent et al, 1973, Sherwood and Vance, 1976, Stanbridge et al, 1971). However, there are also claims that papillae are produced before penetration of the epidermal wall (Aist and Israel, 1975, Edwards and Allen, 1970, McKeen and Rimmer, 1973, Stanbridge et al, 1971). Thus, haustorium initiation is the only certain criterion denoting penetration, but clearly, it is not comprehensive.

The production of haustoria by Erysiphe pisi was affected by all the resistant cultivars, resulting in smaller numbers than in the susceptible cultivar, BS10, (Tables 23 and 24). It is unlikely that haustoria went undetected by virtue of being necrotic as no necrotic haustoria were seen in any of the resistant cultivars. It is

concluded that development of some haustoria was prevented during the period between penetration peg production and contact of the latter with the plasma membrane of the epidermal cell. Penetration is discussed below with respect to (a) cuticle penetration, (b) wall penetration and (c) papilla formation and penetration, if formed, as it is uncertain at which of these stages fungal development was arrested.

a. Cuticle penetration

Reduced numbers of haustoria in the resistant cultivars may have been due to failure to breach the cuticle as a result of abnormal thickness or hardness of the latter. Penetration of the cuticle of pea by Erysiphe pisi is believed to occur by mechanical pressure (Gil, 1976). He found that the pea leaf cuticle, which appeared to be unmodified by contact with the penetration peg, occassionally outlined the cell wall of the latter. This observation led him to conclude that the cuticle was breached and displaced, rather than degraded enzymically, by mechanical pressure. However, the penetration of the cuticle by enzymic action cannot be ruled out. The role of the cuticle as a mechanical barrier to fungal invasion has also been questioned by Martin (1964), who concluded that it contributed little to the exclusion of fungal pathogens. However, instances of the prevention of fungal penetration by cuticles have been recorded. Resistance of strawberry varieties to Sphaerotheca macularis was correlated with cuticle thickness by Jhcoty and McKeen (1965), who described cuticles on the resistant cultivars seven times

thicker than on susceptible plants. Furthermore, Peries (1962) found that the quantity of cutin acids, which affect the hardness of the cuticle, was inversely proportional to the susceptibility of strawberry leaves. The lime leaf cuticle is believed to provide a mechanical barrier to invasion by Gleosporium limetticola (Roberts and Martin, 1963). Susceptibility of the leaf is lost when the quantity of cutin reaches a level of 0.1 mg/cm$^2$ of surface. Thus, penetration of the cuticle on the resistant pea leaves may have been due to increased hardness as a result of higher levels of cutin acids than on BS10. Chemical analysis of the cuticles of the six test cultivars and measurement of the cuticular thickness is necessary to test this point. However, it has been shown that infection structures of fungi can exert considerable pressure, as has been demonstrated by the ability of Botrytis cinerea to penetrate artificial membranes of paraffin wax, collodion and gold leaf (Brown and Harvey, 1927, Miyoshi, 1895). However, no penetration by Botrytis cinerea was observed after the artificial membranes exceeded a certain thickness or hardness. Where considerable force is required for penetration, an anchoring mechanism is necessary to provide a firm contact between the penetration peg and the plant surface, and that is believed to be one of the roles of the appressorium (Dickinson, 1960). Dickinson suggested that penetration without appressoria can only occur on a very soft plant surface or, possibly, with a germ tube penetrating a thin cuticle with a high angle of approach. Thus, some of the appressoria on the resistant cultivars may have been

unable to provide the archorage necessary for penetration. A modification of the appressorial wall occurs during contact with the leaf cuticle of susceptible pea cultivar, Onward, the contact region becoming thicker than the wall around the rest of the appressorium, characterized by a fibrillar or layered appearance, with some disorganisation, and, occassionally, discontinuity (Gil, 1976). This modification may have provided the rigidity required by the penetration for successful breaching of the cuticle and may not have been formed in the appressorial wall on the resistant cultivars.

b Wall penetration

It seems likely that some penetration pegs of Erysiphe pisi breached the cuticle of the resistant pea cultivars and began penetration of the cell wall but were unable to reach the host plasmalemma. Epidermal wall penetration by Erysiphe pisi is believed to occur by enzymic activity of the penetration peg (Gil, 1976). Enzymic degradation of the wall in the resistant pea cultivars may have been incomplete if the wall was excessively thick. Alternatively, the penetration peg may have been deficient in some enzymes necessary for degradation of the wall components if the latter was different in the resistant cultivars from that in BS10. Various types of cell wall modification have been recorded in response to fungal infection (Ride, 1978). Bateman (1964) suggested that the accumulation of calcium can make the pectate in the wall of bean hypocotyls more resistant to penetration by Rhizoctonia . However,

Hirata, (1958, 1967, 1971), Hirate and Togashi (1957) and Takamatsu et al (1979) found that some salts of calcium increased the initial development of primary infection by Erysiphe graminis on barley leaves, so the role of calcium in resistance to fungal ingress is questionable. The modification may be manifested in silicon deposition around potential penetration sites. Akai (1939) found that silicifed bulliform cells of rice leaves were more resistant to penetration by Pyricularia oryzae than non-silicified cells and concluded that silicon provides a mechanical barrier to fungal attack. Silicon accumulation around penetration sites of powdery mildews, manifested as a halo area after staining with cotton blue was believed to be a result of enzymic degradation of the cell wall (Kunoh and Ishizaki, 1975, 1976, Kunoh et al, 1975). However, Volk et al (1958) suggested that a combination of silicon with certain components of the cell wall resulted in a complex which was resistant to fungal enzymes. Similarly, detailed investigation of the halo produced in barley epidermis in response to Erysiphe graminis, Sargent and Gay, (1977) concluded that it is a site of silica deposition and lipidic accumulation which provides a seal against water loss, following contact of the pathogen with the host and is independent of penetration, the host cuticle and wall structure remaining intact. The possibility that silicon deposition around the point of contact between the penetration peg of Erysiphe pisi with the cuticle on the resistant pea cultivars prevented penetration of the wall should be investigated.

c Papilla formation and penetration

It is likely that penetration of the epidermal walls of the resistant cultivars by Erysiphe pisi penetration pegs was successful but breaching of a pre-formed papilla is incomplete. The papilla is defined as a mound of material laid down on the inner surface of the host cell wall either round the haustorial neck or opposite the penetration peg before wall penetration (Bushnell, 1972). Smaller numbers of sites fluorescing with aniline blue were observed on the epidermes of the resistant cultivars (approximately 30% on BS12 and JI1047, 5-10% on JI1048, JI1049 and JI1050) than on BS10, where 54% of germinated conidia were associated with fluorescent sites. The low numbers of fluorescent sites in the resistant cultivars indicate that penetration by many appressoria on the latter did not progress to the stage of initating papillae, but no definite estimate can be made of the number of failures as some of the fluorescent sites may have been caused by germ tubes (Stanbridge et al, 1971). It seems unlikely that papillae prevented the penetration pegs reaching the plasma membrane and producing haustoria in some of the resistant cultivars, as high proportions of fluorescent sites were associated with haustoria (BS12 and JI1047 - 65-70%, JI1049-100%, JI1050-55%). Smaller numbers of haustoria were associated with fluorescent sites in cultivar JI1048 (16%) but it is not possible to conclude that this was due to failure by the penetration pegs to breach the papillae, as fungal development may have ceased after entry into the host cell. The role of the papilla as a barrier to fungal ingress into the host cell has been widely investigated and failure to

penetrate papillae has been observed by many workers (Bushnell and Bergquist, 1975, Carver and Carr, 1977, Heath, 1972, Hyde and Colhain, 1975, Johnson et al, 1979, Kunoh, 1972, Lin and Edwards, 1974, Stanbridge et al, 1971). Zeyen and Bushnell (1979) postulated that the effectiveness of the papilla as a defence mechanism against fungi depends on the ability of the epidermal cell to complete its deposition before any appreciable progress in penetration has occurred. Aist et al (1979) found that challenge appressoria of Erysiphe graminis failed to penetrate preformed papillae in a compatible barley cultivar. Centrifugally induced papillae can also prevent fungal ingress (Waterman et al, 1978). Furthermore, Aist (1977) found that pre-inoculation induction of papillae in Kohlrabi root hairs by mechanical wounding resulted in prevention of penetration by Olpidium brassicae. However, the function of papillae in resistance has been questioned as they also occur in compatible host-parasite combinations and often fail to exclude the fungus (Aist and Israel, 1975, 1976, 1977a,b,c, Bracker and Littlefield, 1973), even if the papilla is initated before penetration peg production (Aist and Israel, 1976). Furthermore, penetration efficiency of Olpidium brassicae and Erysiphe graminis on their respective hosts was similar in cells exposed to heat shock (to delay the formation of cytoplasmic aggregates and subseqent papilla formation) and in untreated cells. This implies that the papillae were not involved in the prevention of fungal penetration (Aist and Israel, 1977c). The same general conclusion is reached from the present study of Erysiphe pisi.

IV Formation of haustoria

1. Effect of cultivar

Successful penetration of the epidermal cell wall and papilla by the infection peg of Erysiphe pisi is assumed to be followed by contact of the fungal wall with the plasma membrane of the epidermal cell; the plasma membrane, which is invaginated, increases in area as a result of swelling of the penetration peg which develops into the haustorial body (Gil, 1976). The greatly reduced numbers of haustoria observed in the resistant cultivars may have been a result of failure by the penetration peg to invaginate the host plasma membrane and form a rudimentary haustorium. Ellingboe (1968) suggested that this was the stage at which resistance operated in cultivars of wheat and barley, where small proportions of Erysiphe graminis penetration pegs gave rise to haustoria (Masri and Ellingboe, 1969b, McCoy and Ellingboe, 1966, Slesinski and Ellingboe, 1969b). Furthermore, he postulated that genes for resistance, which prevented haustorium formation in this way, are constitutive in function, as it seems unlikely that adaptive genes would have reacted fast enough to prevent haustorium initiation. However, it seems likely that an exchange by substances occurred between Erysiphe pisi conidia and the host cells of the resistant cultivars before the penetration peg had contacted the host plasma membrane and that early recognition by the host of the presence of the fungus resulted in the operation of adaptive genes preventing haustorium initiation. This is supported by the observation that an exchange of substances occurs between Erysiphe graminis conidia and host cells in the early stages of the primary infection process (Kunoh and Ishizaki in

Kunoh, 1981), discussed above (DII1).

Haustoria were distinguishable between 12 and 14 h in all the pea cultivars. At 17 h after inoculation, when measurements of size were first made, the haustorial complexes were smaller in all the resistant cultivars than in BS10 (Table 21). Abnormally small haustoria have been observed in other pathogen: non-host combinations. Johnson et al (1982) observed very small haustoria in cultivars of wheat, rye and oats infected with Erysiphe graminis f. sp. hordei at 24 h after inoculation. Small haustoria have also been recorded in incompatible cultivars of barley, wheat and oats inoculated with the respective forms of Erysiphe graminis (Carver and Carr, 1978a, Chelewick, 1944, Corner, 1935, Masri and Ellingboe, 1970 b). Observations of the development of the extra-haustorial membrane in the six pea cultivars indicated that the rate of increase of the surface area was lower in the resistant cultivars (Table 22a). Furthermore, little enlargement occurred in cultivar JI1048 after 18 h. The accomodation of the growing haustorial body in pea is believed to occur by active synthesis of new extra-haustorial membrane rather than by mere stretching of the invaginated host plasma membrane (Gil, 1976) From results of freeze fracture, negative staining and sectioning techniques, using a susceptible pea cultivar, Onward, infected with Erysiphe pisi, Gil (1976) concluded that growth of the extra-haustorial membrane and consequent enlargement of the matrix occurs by fusion of secretary vesicles, originating in the Golgi apparatus of the host cell, with the extra-haustorial

membrane, the vesicle contents contributing to the matrix. Gil (1976) suggested that the production of a metabolite by the haustorium stimulated increased Golgi activity around the haustorial complex. The reduced surface area of the extra-haustorial membranes in the resistant pea cultivars may, thus, have resulted from failure by the infected cells to respond to chemical stimulus from the fungus. Alternatively, large quantities of stimulatory metabolites may have reached the host cytoplasm resulting in reduced production of secretary vesicles Stavely et al (1969) recorded the absence of Golgi apparatus proliferation in resistant cultivars of clover infected with Erysiphe polygoni and attributed this to failure by the fungus to stimulate this host activity. Consequently the extra-haustorial membrane failed to develop a network of invaginations characteristic in susceptible cultivars

The reduced levels of calcium observed in the resistant pea cultivars (Chard, personal communication) may have been responsible for the reduced surface area of the extra-haustorial membranes recorded in these cultivars. She noted that the fluorescence of a calcium-binding compound, introduced into the extra-haustorial membranes produced in susceptible pea cultivar, Onward, was brighter than that in the five resistant cultivars used in the present investigation. Since Douglas (1974) showed that calcium ions enhance the fusion of secreting vesicles with the plasmalemma, Chard's observations imply that there may have been less frequent fusion of Golgi vesicles with the extra-haustorial membrane. This is in accord with the results of Hirata

(1958, 1967 , 1971 ), Hirata and Togashi (1957) and Takamatsu et al (1978), who showed that calcium ions increased haustorium incidence of Erysiphe graminis.

Extra-haustorial membranes formed in the resistant pea cultivars varied in their fluorescence with Calcofluor and, therefore, they are clearly different from those in the susceptible host, Onward, described by Gil (1976). In his investigation of the structure of the extra-haustorial membrane in susceptible pea cultivar, Onward, Gil (1976) recorded intense fluorescence with Calcofluor, which indicated the presence β-linked polysaccharides (Maeda and Ishida, 1967). His experiments led to the conclusion that the membrane contained β-linkages in amorphous polysaccharides. All the extra-haustorial membranes in cultivar BS10 exhibited bright fluorescence but no fluorescence of the matrix was observed. The extra-haustorial membranes in resistant cultivars BS12, JI1047 and JI1048 always fluoresced with Calcofluor, whereas only a low proportion did so in the other cultivars (JI1049-11.1%, JI1050-43%). In some resistant cultivars some of the fluorescing extra-haustorial membranes exhibited only dull fluorescence (JI1047-92.4% JI1048-62.5% JI1049-0%). Fluorescence of the matrix was observed in some resistant cultivars (BS12, JI1047 and JI1048). As Calcofluor does not pass through plasma membranes (Gunning and Hughes, 1976), fluorescence of the extra-haustorial matrix may indicate an alteration in permeability of some extra-haustorial membranes in these cultivars Alternatively, they may be more susceptible to mechanical damage caused during the epidermal stripping procedure.

The modified fluoresence of some extra-haustorial membranes in the resistant cultivars may be the result of absence of or reduced quantities of β-polysaccharides. It also seems likely that the β-polysaccharides were masked in the extra-haustorial membranes of these cultivars The possible absence or reduction of β-polysaccharides in the extra-haustorial membrane may be the result of reduced thickness of the extra-haustorial membrane in the resistant cultivars. Gil (1976) found that the extra-haustorial membranes of Erysiphe pisi are over twice as thick as the normal host plasma membrane. He attributed this thickness to the presence of increased contents of polysaccharides. Treatment of isolated haustorial complexes with hydrolytic enzymes resulted in a reduction of the thickness from 20-25 nm to 15 nm. Following this enzymic treatment the fluoresence of the extra-haustorial membrane with Calcofluor was also reduced, further indicating that the increased thickness in Erysiphe pisi infections is caused by addition of β-polysaccharides. The carbohydrates found in the extra-haustorial membrane of Erysiphe pisi are claimed to be of host origin (Gil, 1976). The results obtained in the present investigation, thus, suggest a failure by the host cells of resistant cultivars JI1047, JI1048 and JI1049 to respond to stimuli from the fungus and produce at least some of the carbohydrates necessary for thickening of the extra-haustorial membrane. Some cells in these cultivars may have produced reduced quantities of such carbohydrates which might account for the dull fluoresence of some of the extra-haustorial membranes.

Further investigations of the extra-haustorial membrane

in the resistant cultivars were carried out using SITS. This reagent binds to amino groups on the membrane surface (Maddy, 1964) and inhibits anion transport but has no effect on cation permeability (Knauf and Rothstein, 1971). Further, it stains only the plasma membranes of living cells (Benjamin and Katz, 1970). All the extra-haustorial membranes produced in susceptible cultivar, BS10, fluoresced brightly with SITS. Only 45-60% of extra-haustorial membranes in the resistant cultivars fluoresced, the majority of these exhibiting dull fluorescence (BS12-7 %, JI1047, JI1048 and JI1049 - approximately 80%, JI1050-57%). The modifications of fluorescence suggests that some of the amino groups on these extra-haustorial membranes were masked, had been structurally altered or were present in reduced numbers. Absence of SITS staining was also observed in haustorial complexes considered to be pre-necrotic and with low transport efficiency, which were isolated from a susceptible pea cultivar (Onward) (Spencer-Phillips and Gay, 1980). Ultrastructural studies of pre-necrotic complexes showed a reduction in the convulutions of the extra-haustorial membranes, which were associated with bead-like granules. The arrangement of these granules was occassionally symmetrical about the extra-haustorial membrane but they were often close or attached to the matrix, which was denser than in healthy complexes. In the present investigation, the non- or weakly fluorescent complexes in the resistant cultivars also did not appear to be necrotic and were not in necrotic host cells but structural modifications, similar to those described above, may have been responsible for the absence of or poor fluorescence.

Another difference observed between haustoria in susceptible and resistant pea cultivars was the time at which lobes on the haustorial body were initiated. Lobes were first observed on haustoria in cultivars BS10 JI1047 and JI1049 by 16 h after inoculation but delays of up to 2 h occurred in the other cultivars. Similar restriction of haustorial development has also been observed in the infection of oats by Erysiphe graminis f. sp. avenae, where the numbers, total length and surface area of the digitate processes was reduced in resistant cultivars (Carver and Carr, 1978a) These workers suggested that this may have been due to the effect of the host exerted by the extra-haustorial membrane However, it is likely that the growth of the haustorial body and development of lobes is facilitated by nutrients assimilated from the host cell This is supported by the observation that low rates of $^{35}S$ and $^{32}P$ transport to the surface fungal structures occur between 10 and 16 h after inoculation of wheat leaves with Erysiphe graminis (Martin and Ellingboe, 1978, Mount and Ellingboe, 1969, Slesinski and Ellingboe, 1971). This was during the period before production of secondary hyphae and when the haustorial body was developing but appendages were not evident. Thus the delay in lobe development on haustoria in cultivars BS12 JI1048 and JI1050 may be due to reduced surface area or efficiency of the extra-haustorial membrane resulting from modification in its components as discussed above. These, in time, may limit the quantities of nutrients transported from the host cell.

2 Effect of time of inoculation

The production of primary haustoria by Erysiphe graminis f sp. hordei (Hirata, 1967) and f. sp.avenae (Carver and Carr, 1978b) is unaffected by the photocycle However, the results obtained on susceptible pea cultivar, BS10 and resistant cultivars, JI1047 and JI1049, in the investigation of the effect of inoculation time on the production of haustoria by Erysiphe pisi show that the development of haustoria is delayed after "evening"- inoculation. This result cannot be attributed to a delay in appressorium formation, since results for haustoria are expressed as a percentage of mature appressoria. As the frequency of haustorium formation in cultivars BS10, JI1047 and JI1049 was higher 16 h after "morning"-inoculation than at 24 h after "evening"-inoculation, it is likely that the photocycle operating in the first 1 h of the infection process is the major factor influencing the numbers of haustoria produced. It seems, therefore that primary haustorium production is accelerated by light. However the frequencies of haustoria eventually become similar after both inoculation times as is shown by the similar frequencies of germinated conidia producing elongating secondary hyphae after 24 h (Tables 35 and 36) which are indicative of the presence of a functional haustorium (Masri and Ellingboe, 1966b). Also the frequencies of haustorium formation recorded at 48 h after "evening"-inoculation (Table 23) were slightly higher than those in "morning"- inoculated plants at 41 h (Table 24) which implies that the numbers become similar between 24 and 48 h after inoculation

V Production of secondary hyphae and colony development

In Erysiphe pisi secondary hyphae arise from conidia and from appressoria and the quantitative criterion defining elongating secondary hyphae is adopted from Slesinski and Ellingboe (1969) in their investigations of Erysiphe graminis development on wheat Such hyphae have individually attained a length greater than 12 μm continue to elongate and have a functional relationship with the host This definition is important in deciding if and when a compatible relationship has been established between the haustorium and the host, since secondary hyphal initials, which are rarely longer than 5 μm, have been recorded in the absence of a haustorium (Masri and Ellingboe, 1966b, McCoy and Ellingboe, 1966, Slesinski and Ellingboe, 1970). Hirata (1967) suggested that a secondary hyphal initial can be produced by Erysiphe graminis as a result of metabolic activity of the conidium before the haustorium begins to transport photosynthates to the surface fungal structures. Sporulation is also indicative of the efficiency of the haustoria in transporting photosynthates. Carver and Carr (1978a) found that fewer conidiophores were found by Erysiphe graminis f. sp. avenae on resistant oat cultivars, where haustoria were produced in reduced numbers, were smaller and developed fewer digitate processes than in a susceptible cultivar.

1 Effect of cultivar

Results of investigation of secondary hypha production by Erysiphe pisi showed that very few germinated conidia produced secondary hyphae on the resistant pea cultivars.

When elongating secondary hyphae were produced on these cultivars their appearance was delayed. Furthermore, the total number of secondary hyphae produced per conidium in the first 48 h of the infection process was reduced and, thereafter, the rate of elongation and therefore, the total mycelial length produced from each infection was smaller than on cultivar BS10. It has been shown above that fewer haustoria are produced in the resistant cultivars and this no doubt accounts partly for the failure of some conidia to produce secondary hyphae. However only a small proportion of conidia which had formed haustoria also produced secondary hyphae. A reduction in the proportion of parasitic units, associated with haustoria, which were capable of producing secondary hyphae has also been observed in incompatible combinations of wheat with Erysiphe graminis (Slesinski, 1969, Slesinski and Ellingboe, 1971, Stuckey and Ellingboe, 1974). This indicates a deficiency in solute transport by some haustoria produced in the resistant pea cultivars. Furthermore, the low extension rates and reduced total mycelial lengths attained when elongating secondary hyphae were produced imply that the associated haustoria are also partially physiologically deficient.

The quantities of photosynthates transported by haustoria in the resistant cultivars may have been restricted by the reduced surface area of the extra-haustorial membranes produced in the latter or by restricted lobe development, which was delayed in some cultivars (BS12 JI1048 and JI1050) (discussed above). The lobes of Erysiphe pisi are thought

to be especially concerned in solute transfer, as electron microscope autoradiography showed that they exhibit higher levels of $^{14}C$ assimilates than the haustorial body (Spencer-Phillips and Gay 1980). Furthermore, ATP-ase, which is thought to be a key factor in the transport of photosynthates, has been detected in the plasma membrane of the haustorial body and lobes (Spencer-Phillips and Gay, 1981). This implies that the degree of lobe development may influence the quantity of photosynthates reaching the surface fungal structures. Elongation of secondary hyphal initials produced by Erysiphe graminis, is augmented when the length and number of haustorial appendages are increased (Carver, 1975, Hirata, 1967). Carver and Carr (1978a) recorded reduced colony growth on resistant oat cultivars, where Erysiphe graminis haustoria showed a reduction in the number and length of appendages. On the other hand, Manners and Gay (1980) did not find any significant difference in $^{14}C$ distribution in haustorial complexes of Erysiphe pisi with differing degrees of lobe and matrix development, and concluded that haustoria of all ages (1-6 days) assimilated photosynthates at similar rates. However, the standard deviations in all the classes analysed were high and thus other factors influenced incorporation.

This suggests that transfer of photosynthates by haustoria in the resistant pea cultivars may have been inhibited by the modified β-polysaccharide and amino group content of the extra-haustorial membranes (discussed above, DIV1). Spencer-Phillips and Gay (1980) suggested that an exact molecular arrangement in the extra-haustorial membrane is necessary for efficient transport of photosynthates. They

recorded a reduction in $^{14}C$ assimilation by pre-necrotic haustorial complexes, isolated from susceptible pea cultivar, Onward, and provided evidence that this was correlated with failure by these complexes to fluoresce with SITS and with certain structural modifications in the extra-haustorial membranes (discussed above, DIV1). The failure by some extra-haustorial membranes in cultivars JI1049 and JI1050 to fluoresce with Calcofluor, the dull fluorescence exhibited by most extra-haustorial membranes in JI1047 and JI1048 implying the absence or reduced quantities of β-polysaccharides therein, and the fluorescence of the matrix of 50% of haustorial complexes in BS12 implying damage or increased permeability of the extra-haustorial membranes, can be correlated with reduced colony growth. The inhibition of colony development can similarly be correlated with the failure by approximately 40% of extra-haustorial membranes in all the resistant cultivars to fluoresce with SITS and the dull fluorescence exhibited by most of the other extra-haustorial membranes, implying absence, reduced numbers or masking of amino groups therein. The observation that high proportions of germinated conidia produced colonies on cultivars BS12 (Tables 37 and 38) and JI1049 (Table 38) implies that failed modification of the invaginated host plasma membrane is not the only factor influencing transport.

Colony development may also have been restricted by failure or delay in the production of secondary haustoria in the resistant cultivars. Although secondary appressoria were recorded on all the resistant cultivars by 48 h after inoculation secondary haustoria were seen only in BS12

at this time. Secondary haustoria may have been produced after 48 h but these were probably produced in reduced numbers and may have had altered extra-haustorial membranes, as discussed above (DIV1). Variations in the hyphal extension rates occurred on all the cultivars between 24 and 96 h but, as extension was not monitored at hourly intervals, it is not possible to establish the time of secondary haustorium initiation. Carver and Carr (1978b). in their investigations of Erysiphe graminis haustorium development in a susceptible oat cultivar, found that the extension rate always decreased approximately 4 h before secondary haustoria were produced and increased again when the haustoria produced digitate processes.

In the present investigation the delay in sporulation and subsequent low frequencies thereof on cultivars BS12 JI1048 and JI1049, and its absence even after 10 days. on the other resistant cultivars are further instances of inhibited colony development. Reduction in the number of conidiophores of Erysiphe graminis on resistant oat cultivars (Carver and Carr, 1978a) and reduced numbers of conidial chains on resistant wheat cultivars (Shaner, 1973) have also been observed.

2 Effect of host plant age

The numbers of germinated conidia on cultivar BS10 producing secondary hyphae were higher on 22- day than on 15- day plants until 24 h after inoculation after "evening"- inoculation and until 30 h on "morning"- inoculated plants. Similar trends were observed on all the resistant cultivars after "morning"- inoculation and on all except JI1048 after "evening"- inoculation The delay in production of

secondary hyphae by a proportion of conidia on BS10 and by all of them on the resistant cultivars is probably a result of the delay in appressorium formation, which occurs on 15- day plants in the early stages (0-6 h) of the primary infection process.

The leaves of some shrubs and trees have demonstrated increasing resistance to powdery mildews as they unfold and expand (Butt, 1971; Mence and Hildebrandt, 1966, Populer, 1972, Rogers 1959). The manifestation of increasing resistance on older leaves and plants is known as adult-plant resistance. Resistance of cereals to Erysiphe graminis has been found to increase with leaf age (Carver and Carr 1977, Graf-Marin 1934, Shaner, 1973), manifested in a reduction in colony size and number with decreased conidial chain density (Shaner, 1973). It seems unlikely that adult plant resistance operates in the five resistant pea cultivars as frequencies of secondary hypha formation eventually became similar in 15- and 22- day groups on all these cultivars.

3 Effect of time of inoculation

Time of inoculation was found to affect the production of secondary hyphae on all six test cultivars. This effect was manifested in lower proportions of germinated conidia producing secondary hyphae after "evening"- than "morning"- inoculation on cultivar BS10, the numbers becoming similar by 48 h on 15- day plants and by 72 h on 22- day plants. Greater variation occurred on the other cultivars but the general trend on 15- day plants was to a delay in secondary hypha production after "evening"- inoculation. In the 22-

day group, "morning"- inoculation resulted in higher frequencies of secondary hypha production on three cultivars (BS12, JI1048 and JI1050).

These results imply that a period of darkness beginning 1 h after inoculation was inhibitory to the production of secondary hyphae. This was not due to reduced frequencies of appressorium formation since the latter is enhanced by "evening"- inoculation (0-6 h) (Table 2) and frequencies become similar after both inoculation times by 24 h or later (Table 4). However the delay in the production of primary haustoria after "evening"- inoculation (Tables 23 and 24) may also be responsible for a consequent delay in secondary hypha production.

The production of secondary hyphae by Erysiphe graminis f. sp. tritici is also affected by the photocycle (Masri and Ellingboe, 1966a, Mount and Ellingboe, 1966) Maximal frequencies of secondary hypha initiation were obtained with a dark period lasting from 6 to 16 h after inoculation, with the greatest inhibition occurring with high light between 7 and 8 h after inoculation (Masri and Ellingboe, 1966a) The length of the dark period appeared to be the critical factor in determining the rate of secondary hypha elongation, synchrony being obtained when the dark period lasted from 6 to 20 h after inoculation. Inhibition of secondary hypha elongation occurred when a high light period extended from 9 to 10 h after inoculation (Mount and Ellingboe 1966) Erysiphe pisi and Erysiphe graminis, therefore appear to have similar physiological requirements for the development of secondary hyphae, ie an uninterrupted period of

darkness of at least 6 h for Erysiphe pisi and 12 h for Erysiphe graminis, before the initiation of haustorial appendages (14 to 16 h and 18 to 20 h, respectively).

The hyphal extension rate has been used to assess the contribution of host assimilates by secondary haustoria to colony growth (Carver and Carr, 1978b) In the present investigation it was not possible to assess whether the production of secondary haustoria was affected by the photocycle, as the extension rate was not monitored at hourly intervals No clear trends emerged in the variations of hyphal extension rates after the two inoculation times. Production of secondary and tertiary haustoria of Erysiphe graminis in barley (Nishiyama et al, 1966) and oats (Carver and Carr, 1978b). Carver and Williams 1980) in response to darkness has been reported. However, production of secondary haustoria but not tertiary, by Sphaerotheca fuliginea in cucumber is not affected by the photocycle (Hirata 1967).

## E CONCLUSION

The general conclusion from this investigation is that resistance to Erysiphe pisi in the five test cultivars was expressed at several stages of the developmental sequence; the effects were manifested in an inhibition of germination a delay in appressorium formation, inhibition of penetration and a reduction in the size and numbers of haustoria, whose extra-haustorial membrane was modified; subsequent to haustorium development being affected, colony development was curtailed. However, at none of the stages was development totally inhibited. Either the development of part of the conidial population was inhibited or a delay occurred and these effects resulted in a reduction in the numbers of parasitic units producing sporulating colonies.

The greatest effect was exerted on the following stages: (1) germination, where only 65% of the maximum germination frequencies recorded on cultivar BS10 (90%) were obtained on the resistant cultivars; (2) penetration, where only 8 to 15% of the penetration attempts recorded on cultivar BS10 by germinated conidia were observed on JI1048, JI1049 and JI1050, and only 46% of the BS10 value on BS12 and JI1047 As primary germ tubes, as well as penetration pegs, can cause papilla deposition, the actual number of successful penetrations was probably lower than indicated by the above figures; (3) haustorium formatien, where the low numbers of successfully produced haustoria, ranging from 30 to 60% of the numbers recorded on cultivar BS10 (90-100%) on the five resistant cultivars, were almost always surrounded by a modified extra-haustorial membrane. The most distinctive feature of the type of resistance observed in this investi-

gation is the lack of necrosis of either the host cell or the fungus.

Fungal development was also found to be affected by host plant age, with higher frequencies of germination, appressorium and secondary hypha formation occurring on 22- day than on 15- day plants in the first 24 h of the primary infection process. The time of inoculation with respect to the photocycle also affected fungal development. Appressorium formation was enhanced by "evening"- inoculation but haustorium formation and production of secondary hyphae occurred at higher frequencies after "morning"- inoculation.

Resistance to plant pathogens has been classified as vertical or differential, where a host cultivar is more resistant to some pathogen races than others (race-specific) and as horizontal or uniform, where the host is resistant to all races of a particular pathogen (race non-specific) (Van der Plank, 1963, 1969). He suggested that vertical resistance is manifested in the post-penetration phase of the infection process by mechanisms such as necrosis of the host cell and fungus or by phytoalexin production. Horizontal resistance, on the other hand, may operate before penetration by reducing germination and inhibiting development of appressoria or other infection structures. Race non-specific or horizontal resistance is usually polygenically inherited (Robinson, 1973), i.e. it is determined by many genes of individually small effect, and the characters governed by these genes, such as wax morphology and hairiness of the leaf surface, are not of a type which can be overcome by change in the genotype of the pathogen. Vertical resistance is

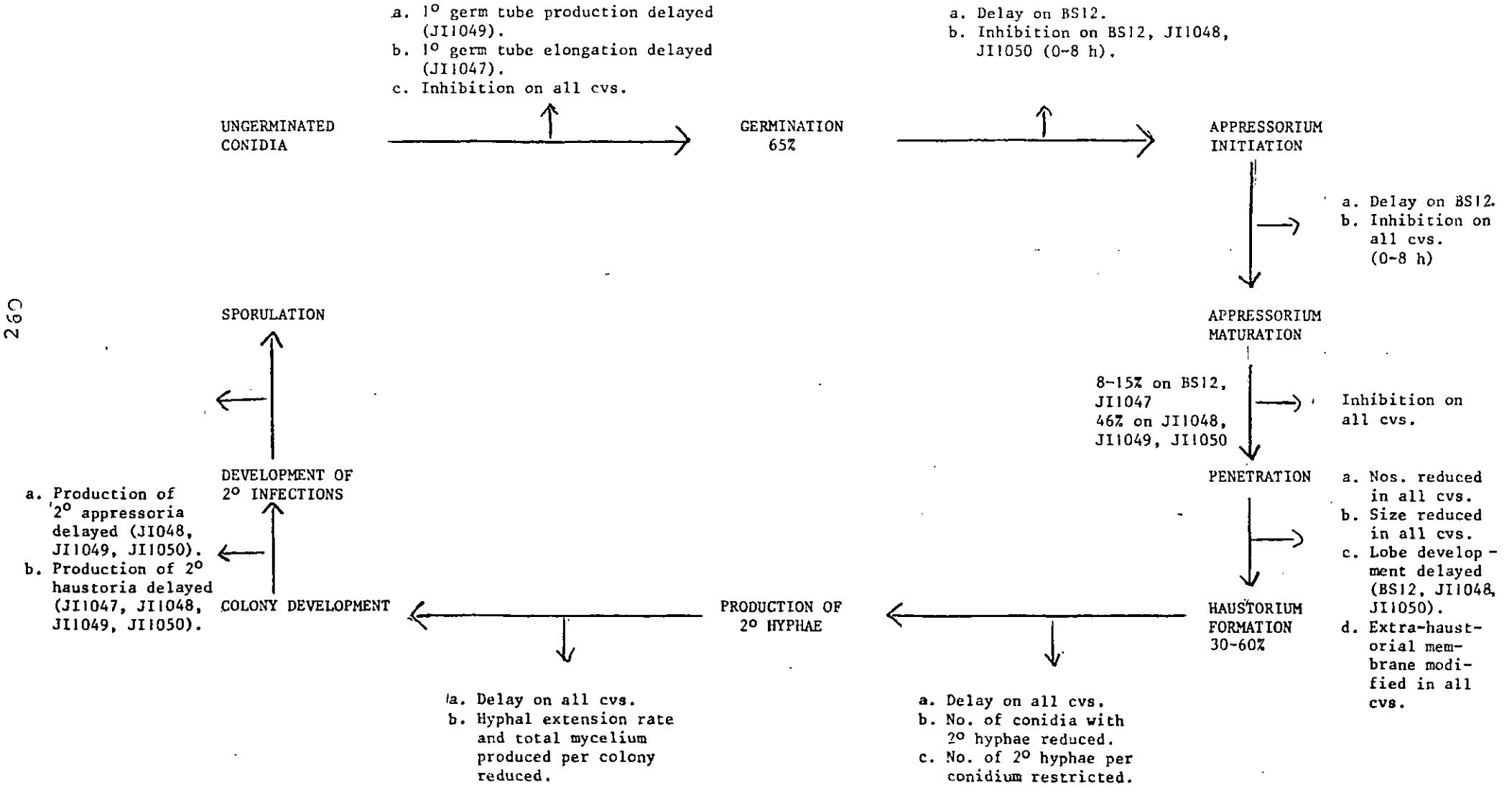
usually determined by monogenes or oligogenes (Robinson, 1973), where one or a few genes operate, their effects being easily distinguishable.

The results of the present investigation suggest that the resistance phenotype is under the control of several genes, each affecting a specific developmental stage. This form of resistance is possibly of the horizontal polygenically inherited type and may be race non-specific. The lack of necrosis of the host cell and fungus observed in the present investigation supports the possibility that race non-specific resistance operated in the five resistant pea cultivars, as one of the predominant expressions of race-specific resistance which is typically oligogenically inherited, in hypersensitivity (Robinson, 1971). However, there is always the danger, when using the term "race non-specific resistance", that a race will be found which is virulent, so the term "general resistance" is preferable.

However, it seems possible that only one resistance gene operates in the resistant pea cultivars. A single gene product may affect both the plant cuticle, causing inhibition of germination and appressorium formation, and the host plasma membrane, thereby inhibiting haustorium production.

Fig. 19 Effect of resistance on the developmental sequence of Erysiphe pisi in five resistant cultivars of pea.

The values recorded on the resistant cultivars are expressed as a % of BS10 results.



SECTION 2

# Induction of endophytic mycelium of powdery mildews in barley and cucumber

A INTRODUCTION

Infection by plant pathogens and the course it takes is very subject to environmental conditions. Predisposition has been defined as the effect of non-genetic conditions, acting before infection on the susceptibility of plants to disease (Yarwood, 1959, 1976). Infection may be influenced by environmental effects such as temperature, humidity, light, atmospheric pressure, nutrition and pH, by wounding, chemical treatment and prior inoculation of the plants. Investigation of the effect by predisposition to infection by plant pathogens may provide information about mechanisms involved in recognition of host and pathogen and, possibly, about the functions of the genes determining host response to infection.

Since Salmon (1904, 1905a) found that, following heat treatment, specificity of various grasses and cereals to powdery mildew infection was lost and leaves of Euonymus japonicus, resistant because of age, became susceptible to Oidium species (1905b). Much work has been done on the effect of heat predisposition on fungal and viral infections but little is, as yet, known about the mechanism of host-parasite interaction. Development of Erysiphe graminis on barley (Ouchi et al, 1975), Sphaerotheca fuliginea on cucumber (Yarwood, 1963), several viruses, Uromyces fabae, E. polygoni and Colletotrichum lindemuthianum on Phaseolus vulgaris (Yarwood 1956) was enhanced by heating the host before inoculation. Heat treatment rendered barley leaves susceptible to avirulent races of E. graminis f. sp. hordei

and to non-pathogens at appropriate doses (Ouchi et al, 1975, 1976); also, heat induced susceptibility in Phaseolus vulgaris to non-pathogen Sphaerotheca fuliginea (Yarwood, 1963).

Salmon (1906) named the alteration of the host response of cereals and grasses to powdery mildews, caused by heat and chemical treatment and by wounding of the leaves, "xenoparasitism" and found that it was usually accompanied by the development of endophytic mycelium. This was manifested by penetration through the adaxial epidermal layer and inward growth of the hyphae through the intercellular spaces of the mesophyll. Haustoria were formed in the mesophyll bundle sheath and abaxial epidermal cells and sporulation occurred in the intercellular spaces. Endophytic mycelium was first recorded by Neger (1901), in E. cichoracearum infecting Sonchus oleraceus in the summer months, and has since been recorded in leaves by many workers in various plant-parasite combinations. It developed in E. polygoni in damaged tissue of Brassica species (Searle, 1920); in E. graminis in exposed mesophyll of barley (Mackie, 1928); in E. cichoracearum in exposed mesophyll of lettuce, particularly if the latter was subjected to frost damage (Schnathorst, 1959); in S. fuliginea in the non-host Phaseolus vulgaris following abrasion, heat treatment and rust infection, with greater mildew development in the latter case if the rust was heat-killed (Yarwood, 1963); in E. graminis, S. fuliginea and E. cichoracearum in heat-treated leaves of barley, cucumber and sunflower, respectively; in

S. fuliginea in non-host sunflower, following heat treatment or chilling and after prior infection of bean leaves by rust; and in E. cichoracearum and S. fuliginea in sunflower and cucumber following heat-treatment of the roots (Jarvis, 1964).

The purpose of this investigation, which was based on unpublished work by Jarvis (personal communication), was to (1) observe the effect of heat treatment and chilling of barley leaves and cucumber cotyledons and of heat treatment of the roots of these plants, on the ectophytic development of E. graminis and S. fuliginea, respectively, and (2) attempt induction of endophytic mycelium of these fungi in their respective hosts using the predisposing treatments outlined above.

In addition, translocation of the effect of heat was investigated. Translocation heat injury, which is defined as the injury of unheated leaves which results when other leaves on the same plant are heated, was demonstrated in Phaseolus vulgaris and cowpea (Yarwood, 1961). Heat treatment of one of a pair of cucumber, cowpea and bean leaves, was believed to enhance infection by various viruses in the untreated opposite leaf (Nienhaus and Yarwood, 1963, Yarwood et al, 1962). Two substances formed in bean after heat treatment and translocated have been demonstrated in extracts of heated plants (English and Bonner, 1937, English et al, 1939). Jarvis (1964) also concluded that a stimulus inducing endophytism by S. fuliginea, produced in response to heat treatment and chilling of a cucumber cotyledon, was translocated to the opposite unheated

cotyledon. Sap extracts from heat treated cucumber cotyledons, applied to the unheated twin opposite, induced endophytism. The translocation of induced stimuli were investigated in this study using S. fuliginea and cucumber in an attempt to reproduce Jarvis's (1964) demonstration of this phenomenon.

Jarvis (1964) found that the determining factors in the induction of endophytic mycelium were the temperature used and the duration of the treatment, the appropriate dose varying with different host-pathogen combinations. Furthermore, induction of endophytism in cucumber and sunflower was affected by the time of day and year when the plants were treated and by host plant age. The conditions which resultedin the greatest development of endophytic mycelium in barley and cucumber (Jarvis, personal communication) were used in this investigation.

In summary, this study to investigate the effect of heat treatment of leaves and roots and ice treatment of leaves of E. graminis f. sp. hordei and S. fuliginea in barley and cucumber, respectively, was carried out in order to elucidate the development of the pathogen and the predisposition of the host to infection.

## B MATERIALS AND METHODS

### I HOST PLANTS AND INOCULUM

#### 1 Organisms and sources

The experiments were carried out using Sphaerotheca fuliginea on Cucumis sativus L. cv. Wisconsin SLR18, seeds of which were kindly provided by I.C.I. (Plant Protection Ltd), Jealotts Hill, Bracknell and Erysiphe graminis f. sp. hordei on Hordeum vulgare L.,cv Golden Promise.

#### 2 Cultivation of host plants

Cucumber seeds were germinated in the dark for 24 h at 27°C on damp filter paper in a petridish. Germination of barley seeds before sowing was unnecessary. Seeds were sown in vermiculite in 5 inch pots, four seeds per pot, and maintained in a growth room with 16 h daylength at a mean temperature of 22°C and a relative humidity of 65%. Illumination was provided by a combination of fluorescent and tungsten lights providing to 22 watts $m^{-2}$ at the base of the plant and 37 watts in $m^{-2}$ immediately above the plant.

#### 3 Maintenance of inoculum

Cultures of Erysiphe graminis and Sphaerotheca fuliginea were maintained on barley (cv. Golden Promise) and cucumber (cv. Wisconsin) plants in a greenhouse with a16 h daylength at an appropriate temperature of 22°C and a relative humidity of 65-70%. Illumination was provided by a combination of natural, fluorescent and tungsten lights resulting in a light intensity of at least 25 watts $m^{-2}$.

4 Inoculation of host plants

4.1 Production of young conidia

An inoculum, consisting of newly matured conidia, was obtained by blowing old conidia from infected leaves 24 h before inoculation.

4.2 Inoculation procedure (Nair and Ellingboe, 1962)

Conidia were collected on glass slides from young pustules or colonies by gently tapping the leaves with a glass rod, then blowing gently on the slide to remove clumps of conidia. The conidia were picked up by rolling a sterilised absorbent cotton swab over the slide and transferred to the leaf by rolling the swab over the surface in one forward and backward movement. This inoculation procedure resulted in the deposition of approximately 50 conidia per 50 $mm^{-2}$ of leaf.

5 Time of inoculation

Inoculation was carried out at 0800 h at the end of the 16 h light period.

6 Hot water treatment

Leaves on the intact plant were dipped into a water bath, maintained at a constant temperature, for various periods of time.

7 Chilling

Leaves on the intact plant were dipped into a beaker of water containing ice for various periods of time.

C EXPERIMENTAL PROCEDURES AND RESULTS

I Induction of endophytic mycelium by sub-lethal hot water treatment.

a Barley : Erysiphe graminis f. sp. hordei

1 Heat-treatment (50°C) before inoculation : 12 and 24 h intervals.

1.1 Introduction

The purpose of this experiment, based on work done by Jarvis (1964, personal communication), was to investigate the effect of hot water treatment of barley leaves before inoculation on the induction of endophytic mycelium. He immersed barley leaves in water at 50°C for periods of 0.5 to 30 sec at 0800 h, inoculating immediately, 12 and 24 h after treatment. Endophytic mycelium, indicated by colony development on the abaxial uninoculated epidermis, was found 7 days after inoculation of leaves inoculated 12 h after 5 sec of treatment and 24 h after 1, 2, 5 and 10 sec of treatment. The endophytic mycelium was similar to that described by Salmon (1906). The present investigation was done using the effective experimental conditions used by Jarvis (personal communication).

1.2 Experimental

Nineteen days after sowing the susceptible barley cultivar, Golden Promise, when the third leaf had emerged, the leaves of six plants were heat-treated (Section 2 B I.6) at 50°C for 1, 2, 5 and 10 sec at 0800 h. The adaxial epidermis was inoculated 24 h after each treatment and a further six plants were inoculated 12 h after 5 sec of treatment at 50°C. These and six inoculated untreated

plants were incubated in a growth room. Samples were taken 48 h and 7 days after inoculation, using three plants at each sampling time. The surface fungal structures were removed from the adaxial epidermis of the 48 h samples on Sellotape strips (Section 1 BII 3.1) and, after staining with lactophenol cotton blue (Section 1 BII 1.2), counts were made of conidia producing elongating secondary hyphae on the inoculated leaf surface. The leaves were cut into pieces, approximately 10 mm x 10 mm, cleared in lactic acid : chloroform : methanol (Section 1 BII 2.2), stained with acid fuchsin (Section 1 BII 1.4) and mounted in 50% glycerine for observation. Counts were made of haustoria in the adaxial epidermis. Observations were made of mycelial development and sporulation on both epidermes of the 7-day samples. The density of mycelium was assessed using the following arbitrary scale, which represented the percentage of the leaf covered : 1) - no cover; (2) less than 25%; (3) 25 to 50%; (4) 50 to 75%; (5) 75 to 100%. The same scheme was used to assess the number of sporulating colonies. The surface mycelium was then brushed from the leaves, to facilitate observation of internal fungal development, and the leaves were cut into small pieces and cleared (Section 1 BII 2.2). The sub-epidermal tissue of both 48 h and 7-day samples was examined for the presence of endophytic mycelium. The adaxial epidermis was then stripped off and the mesophyll further examined. The results showing haustorium formation and the production of elongating hyphae (expressed as a percentage of appressoria) are recorded in Table 53a.

1.3 Results

No colony development was observed on the abaxial uninoculated epidermis after any of the heat treatments, irrespective of the time of treatment, and no endophytic mycelium was seen. Greater frequencies of haustoria and secondary hyphae were recorded for the adaxial epidermis of leaves inoculated 12 h after 5 sec of treatment (62.1% and 52%, respectively) and in those inoculated 24 h after treatment for 5 sec (63.8% and 50.0%, respectively) and 10 sec (69.2% and 56.7%, respectively) than in the untreated leaves (approximately 55% and 45%, respectively) (Table 53a). The numbers of haustoria and conidia with elongating secondary hyphae in leaves treated for 1 and 2 sec were similar to those recorded in untreated leaves. Colony development, 7 days after inoculation, was greater on plants inoculated 12 h after treatment for 5 sec and 24 h after 5 and 10 sec treatments, rating 5 on the arbitrary scale, than after the other treatments and on untreated plants, where a rating of 4 was recorded. Sporulation was unaffected by heat treatment, a rating of 4 being recorded on treated and untreated plants.

2 Heat-treatment (50°C) before inoculation : 3 and 6 h intervals

2.1 Introduction

As no endophytic mycelium was observed in barley leaves heated 12 and 24 h before inoculation (Section 2 C Ia 1.3), it seems possible that the stimulus inducing endophytism may have disappeared or decreased in the period between heat-treatment and inoculation. Heat-induced

susceptibility in barley to avirulent races of Erysiphe graminis f. sp. hordei and to non-pathogens diminishes exponentially as a function of time, disappearing by 24 h after heat-treatment (Ouchi et al, 1975). The following experiment was done to investigate the effect of shortening the period between heat-treatment andinoculation on the induction of endophytic mycelium.

2.2 Experimental

The abaxial leaf epidermes of 19-day barley plants were inoculated 3 and 6 h after heat-treatment for 1, 2, 5 and 10 sec at 50°C at 0800 h. These and six inoculated untreated plants were incubated in a growth room. Samples, taken 48 h and 7 days after inoculation, were prepared for observation and results recorded (Table 53a) as described above (Section 2 C Ia 1.2).

2.3 Results

No mycelial development was observed on the abaxial uninoculated epidermis of heat-treated leaves nor any endophytic mycelium recorded. The frequencies of haustorium and secondary hypha production were greater on leaves treated for 5 sec (3 h interval 59.4% and 40.2%, respectively, 6 h interval - 64.8% and 56.8%, respectively) and 10 sec (3 h interval - 68.5% and 55.1%, respectively, 6 h interval - 67.2% and 59.2%, respectively) than on untreated leaves (approximately 55% and 48%, respectively) (Table 53a). The values recorded on the untreated leaves were similar to those on leaves heated for 1 and 2 sec for both 3 and 6 h intervals before inoculation. Mycelial development was greater after 5 and 10 sec of treatment (rated 5 with both 3 and 6 h intervals between heat-treatment and inoculation)

than on untreated leaves (rated 4). Similar degrees of mycelial development were recorded on leaves treated for 1 and 2 sec, 3 and 6 h before inoculation, and on untreated leaves. Sporulation was unaffected by heat-treatment, a rating of 4 being recorded on treated and untreated leaves.

3 Heat treatment (45°C, 55°C) before inoculation : 12 and 24 h intervals

3.1 Introduction

No endophytic mycelium was induced by any of the heat-treatments above (Section 2 C Ia 1.3, 2.3), irrespective of the time of inoculation. It seems possible that the temperature (50°C) was inappropriate for inducing endophytism in the barley cultivars, Golden Promise, used in this investigation. It has been shown that the induction of susceptibility in barley to avirulent races of Erysiphe graminis f. sp. hordei and to non-pathogens occurs only after treatment at certain temperatures for specific periods of time (Ouchi et al, 1975, 1976) and that the appropriate dose varies with different host : pathogen combinations. The purpose of this experiment was to investigate the effect of temperatures above and below 50°C on the induction of endophytic mycelium.

3.2 Experimental

The adaxial leaf epidermis of six 19-day barley plants were inoculated 12 and 24 h after heat-treatmnet at 45°C for 5, 10 and 15 sec and at 55°C for 1, 2, 5 and 10 sec at 0800 h. These and six untreated plants were incubated in a growth room. Samples, taken 48 h and 7 days after inoculation were prepared for observation and results recorded (Table 53a) as described above (Section 2 C Ia 1.2).

3.3 Results

No mycelial development was observed on the abaxial uninoculated epidermis of heat-treated leaves nor any endophytic mycelium observed. Frequencies of haustorium formation and secondary hypha formation were higher after 15 sec of treatment at 45°C (12 interval - 59.8% and 47.2%, respectively; 24 h interval - 60.9% and 48.1%, respectively) than on untreated leaves (approximately 51% and 41%, respectively) (Table 53a). The results recorded after the other heat-treatments were similar to those on untreated leaves (approximately 50% and 40% for haustorium and secondary hypha production, respectively). Colony development was more extensive after 15 sec of treatment at 45°C with both 12 and 24 h intervals, rated 5, than on untreated leaves, rated 4. A rating of 4 was recorded for colony development after the other heat treatments used in this experiment. Sporulation was unaffected by heat-treatment, a rating of 4 being recorded on treated and untreated plants.

4 Heat-treatment after inoculation

4.1 Introduction

No endophytic mycelium or colony development on the uninoculated abaxial epidermis of barley leaves were observed when heat-treatment was carried out before inoculation (Section 2 C Ia 1.3, 2.3, 3.3). The purpose of this experiment was to investigate the effect of heat-treatment of barley leaves after inoculation on the induction of endophytic mycelium, using the heat-treatments (45°C for 15 sec, 50°C for 5 and 10 sec) which resulted in the greatest enhancement of ectophytic fungal development above (Section

2 C Ia 1.3, 2.3, 3.3). Jarvis (1964, personal communication) recorded the development of endophytic mycelium of Sphaerotheca fuliginea and Erysiphe cichloracearum in cucumber cotyledons and sunflower leaves, respectively, which were heat-treated after inoculation.

4.2 Experimental

The leavesof six 19-day barley plants were heat-treated at 45°C for 15 sec and at 50°C for 5 and 10 sec, 12 and 24 h after inoculation at 0800 h. These and six inoculated untreated plants were incubated in a growth room. Samples, taken 48 h and 7 days after inoculation, were prepared for observation and results recorded (Table 53b) as described above (Section 2 C Ia 1.2).

4.3 Results

No mycelial development was observed on the abaxial uninoculated epidermis of heat-treated leaves nor any endophytic mycelium recorded. Similar frequencies of haustorium formation (approximately 55%) and secondary hypha production (approximately 45%) were recorded on treated and untreated leaves (Table 53b). There was also no difference in colony formation (rated 5) and sporulation (rated 4) on treated and untreated leaves.

b Cucumber : Sphaerotheca fuliginea

1 Heat treatment before inoculation

1.1 Introduction

The purpose of this investigation was to discover the effect of wet-heat treatment, carried out before inoculation, on the induction of endophytic mycelium of Sphaerotheca fuliginea in cucumber. Jarvis (1964) recorded

Tables 53a and b. Production of haustoria (elongating secondary hyphae (ESH) both expressed as a % of total appressoria) by Erysiphe graminis on the adaxial epidermis of barley leaves 48 h after inoculation, at various periods before 53a) and after (53b) heat treatment. The figures in brackets represent the results recorded on untreated leaves.

Table 53a — INTERVAL BETWEEN HEAT-TREATMENT AND INOCULATION (h)

| Heat-treatment | | 3 | | 6 | | 12 | | 24 | |
|---|---|---|---|---|---|---|---|---|---|
| Temp (°C) | Duration(s) | H | ESH | H | ESH | H | ESH | H | ESH |
| 45 | 5 | - | - | - | - | 51.7 (49.8) | 40.2 (40.1) | 52.8 (50.1) | 40.7 (42.9) |
| | 10 | - | - | - | - | 50.8 (51.7) | 41.3 (42.3) | 51.7 (51.2) | 38.2 (41.3) |
| | 15 | - | - | - | - | 59.8 (52.1) | 47.2 (40.2) | 60.9 (51.3) | 48.1 (41.8) |
| 50 | 1 | 50.2 (51.2) | 41.8 (40.2) | 53.8 (53.1) | 44.7 (45.0) | - | - | 52.4 (53.1) | 41.2 (45.1) |
| | 2 | 54.3 (53.8) | 40.7 (42.1) | 52.1 (53.1) | 40.1 (42.1) | - | - | 56.1 (55.9) | 42.1 (44.8) |
| | 5 | 59.4 (52.1) | 50.2 (47.9) | 64.8 (55.8) | 56.8 (48.2) | 62.1 (54.1) | 52.0 (46.1) | 63.8 (54.9) | 50.0 (43.2) |
| | 10 | 68.5 (51.2) | 55.1 (45.8) | 67.2 (53.2) | 59.2 (47.4) | - | - | 69.2 (55.3) | 56.7 (41.4) |
| | 1 | - | - | - | - | 51.2 (50.9) | 43.8 (42.7) | 51.7 (52.1) | 40.9 (38.7) |
| | 2 | - | - | - | - | 53.6 (54.7) | 41.2 (41.8) | 50.1 (50.3) | 42.8 (40.1) |
| | 5 | - | - | - | - | 52.7 (53.8) | 44.7 (42.1) | 51.8 (52.8) | 41.7 (39.1) |
| | 10 | - | - | - | - | 50.2 (51.7) | 40.1 (43.4) | 52.3 (53.7) | 41.2 (40.7) |

Table 53b — INTERVAL BETWEEN INOCULATION AND HEAT TREATMENT (h)

| Heat-Treatment | | 12 | | 24 | |
|---|---|---|---|---|---|
| Tamp (°C) | Duration(s) | H | ESH | H | ESH |
| 45 | 15 | 54.6 (55.8) | 43.9 (40.1) | 52.1 (53.9) | 44.9 (43.1) |
| 50 | 5 | 53.7 (56.8) | 45.2 (46.3) | 55.8 (56.1) | 43.7 (47.1) |
| | 10 | 55.2 (53.8) | 45.8 (44.9) | 54.7 (53.8) | 46.8 (45.9) |

The development of endophytic mycelium of S. fuliginea in cucumber cotyledons heated at 45, 50 and 55°C for periods of ½ to 120 sec at 0800 h, 12 h before inoculation.

1.2 Experimental

The adaxial epidermes of six 14-day cucumber plants were inoculated 12 and 24 h after heat-treatment of the cotyledons at 45°C for 15, 30, 60 and 90 sec, 50°C for 5, 10 and 30 sec and 55°C for 1, 2, 5 and 10 sec at 0800 h. These and six inoculated untreated plants were incubated in a growth room. Samples taken 48 h and 7 days after inoculation, were prepared for observation and results recorded (Table 54) as described above (Section 2 C Ia 1.2).

1.3 Results

No mycelial development was observed on the abaxial uninoculated epidermis of heat-treated cotyledons nor any endophytic mycelium recorded. The frequencies of haustorium and secondary hypha production were greater on cotyledons heat-treated at 45°C for 60 (6.3.3% and 58.1%, respectively) and 90s (65.1% and 56.3%, respectively), at 50°C for 10 (59% and 50%, respectively) and 30s (62.7% and 53.8%, respectively) and at 55°C for 5 (60.8% and 52.9%, respectively) and 10s (61.2% and 54.1%, respectively) than on untreated cotyledons (approximately 53% and 45%, respectively) (Table 54). The numbers of appressoria producing haustoria and elongating secondary hyphae after the other heat-treatments were similar to those produced in untreated cotyledons. Colony development was more extensive (rated 5) on cotyledons treated at 45°C for 60s and 90s, at 50°C for 10 and 30s and at 55°C for 5 and 10s than on untreated cotyledons

(rated 4). A rating of 4 was also recorded after the other heat-treatments used in this experiment. Sporulation was unaffected by heat-treatment, a rating of 4 being recorded on all treated and untreated cotyledons.

2 Heat-treatment after inoculation

2.1 Introduction

The purpose of this investigation was to discover the effect of heat-treatment, applied after inoculation, on the induction of endophytic mycelium in cucumebr, as treatment before inoculation did not induce endophytism. Jarvis (1964, personal communication) heat-treated 14-day cucumber cotyledons at 45°C for 15 to 120s, at 50°C for 1 to 60s and at 55°C for 0.5 to 10s, 0.5, 2, 3, 5 and 7 days after inoculation. The abaxial, uninoculated epidermis showed the most extensive colonisation and supported the highest degree of sporulation on the abaxial when cotyledons were inoculated 2 days after treatment at 55°C for 1, 2 and 5 sec.

2.2 Experimental

The adaxial epidermes of six 14-day cucumber cotyledons were inoculated at 0800 h and 24 and 48 h before heat treatment at 45°C for 15, 30, 60 and 90 sec, at 50°C for 5, 10 and 30 sec and at 55°C for 1, 2, 5 and 10 sec. These and six inoculated untreated plants were incubated in a growth room. Samples, taken 48 h and 7 days after inoculation, were prepared for observation and results recorded (Table 54) as described above (Section 2 C Ia 1.2).

2.3 Results

No mycelial development was observed on the abaxial

Table 54 Production of haustoria (H) and elongating secondary hyphae (ESH) (both expressed as a % to total appressoria) by Sphaerotheca fuliginea on the adaxial epidermis of cucumber cotyledons 48 h after inoculation at various periods before and after heat-treatment. The figures in brackets represent the results recorded on untreated cotyledons.

| Heat-treatment | | HEAT-TREATMENT BEFORE INOCULATION | | | | HEAT-TREATMENT AFTER INOCULATION | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | INTERVAL (h) | | | | INTERVAL (h) | | | |
| | | 12 | | 24 | | 24 | | 48 | |
| Temp(°C) | Duration(s) | H | ESH | H | ESH | H | ESH | H | ESH |
| 45 | 15 | 52.1 (50.8) | 47.6 (45.1) | 50.6 (51.7) | 47.2 (45.1) | 50.1 (52.7) | 47.1 (45.7) | 50.6 (52.9) | 46.7 (45.1) |
| | 30 | 50.8 (52.1) | 45.9 (38.6) | | 45.2 (47.2) | 53.4 (51.1) | 45.2 (45.6) | 53.1 (51.6) | 45.4 (45.6) |
| | 60 | 63.3 (53.1) | 58.1 (41.2) | 52.8 (53.9) | 45.1 (43.9) | 51.6 (52.3) | 46.8 (43.2) | 50.7 (51.9) | 47.0 (43.0) |
| | 90 | 65.1 (52.8) | 56.3 (37.4) | 52.9 (53.2) | 44.3 (45.3) | 52.6 (53.1) | 44.1 (47.1) | 50.4 (52.7) | 47.2 (43.9) |
| 50 | 5 | 50.8 (50.7) | 45.0 (44.7) | 52.1 (51.8) | 45.6 (44.9) | 51.3 (50.8) | 46.1 (46.9) | 52.9 (51.6) | 48.1 (44.7) |
| | 10 | 59.0 (50.3) | 50.0 (45.0) | 53.8 (50.6) | 47.2 (45.8) | 52.1 (53.1) | 45.8 (45.8) | 53.9 (50.9) | 46.3 (45.9) |
| | 30 | 62.7 (53.8) | 53.8 (43.9) | 51.7 (53.8) | 45.9 (46.1) | 52.6 (53.2) | 44.7 (43.1) | 51.3 (48.6) | 46.1 (46.1) |
| 55 | 1 | 51.6 (53.1) | 47.1 (45.1) | 50.6 (52.7) | 44.7 (45.0) | 50.1 (51.6) | 47.8 (43.9) | 53.1 (49.4) | 44.1 (43.7) |
| | 2 | 52.6 (52.7) | 46.3 (43.8) | 50.9 (53.1) | 45.1 (44.7) | 50.6 (51.7) | 45.2 (47.1) | 52.7 (50.6) | 45.0 (44.2) |
| | 5 | 60.8 (51.8) | 52.9 (45.2) | 52.1 (49.9) | 46.8 (42.9) | 53.2 (54.1) | 46.1 (46.8) | 52.3 (53.1) | 45.3 (45.8) |
| | 10 | 61.2 (50.8) | 54.1 (44.7) | 51.7 (53.8) | 44.9 (44.1) | 52.8 (52.8) | 45.1 (45.1) | 51.8 (52.8) | 46.7 (44.1) |

uninoculated epidermis of heat-treated cotyledons nor any endophytic mycelium recorded. Frequencies of haustorium and elongating secondary hypha production were similar on all the treated and untreated cotyledons (approximately 53% and 45%, respectively) (Table 54). Colony development and sporulation were unaffected by heat-treatment, ratings of 4 being recorded on both treated and untreated cotyledons.

3 Diurnal variation in the induction of endophytic mycelium

3.1 Introduction

As no endophytic mycelium was found in cucumber cotyledons heat-treated 12 and 24 h after inoculation at 0800 h (CI b 2.3). This experiment was done to investigate diurnal variation in, and the effect of host plant age on, the induction of endophytic mycelium. Jarvis (1964, personal communication) found that the induction of endophytic mycelium of Sphaerotheca fuliginea in cucumber cotyledons depended on the time of day when hot water treatment was carried out and that it was affected by host plant age. In his preliminary investigations (Jarvis, 1964) found that endophytic mycelium was induced in 11-day cucumber cotyledons heat-treated at 0700 h, following inoculation at 1700 h the previous days and in 14-day cotyledons after heating at 1300 h. In further investigations (Jarvis, personal communication), he inoculated 11-, 14- and 18-day cucumber plants at 0800 h and heat-treated the cotyledons at 50°C for 1, 3, 5, 10 and 15 sec the following day at 0800, 1300 and 1700 h. Colony development was observed on the abaxial uninoculated surface of 11-day cotyledons after treatment at 1300 h but only after exposure for 1 sec at 1700 h. On 14-day plants the greatest mycelial

development on the abaxial epidermis was observed after treatment at 1700 h.

3.2 Experimental

Three 11- and 14-day cucumber plants were heat-treated at 50°C for 1, 3, 5, 10 and 15 sec at 1300 and 1700 h following inoculation of the adaxial epidermis at 0800 h the previous day. The treated and three inoculated untreated 11- and 14-day plants were incubated in a growth room where the 16 h light period began at 1600 h. Samples taken 48 h and 7 days after inoculation, were prepared for observation and results recorded as described above (C Ia 1.2).

3.3 Results

No mycelial development was observed on the abaxial uninoculated epidermis of heat-treated plants nor any endophytic mycelium recorded. Frequencies of haustorium and secondary hypha production (52.0% and 45.0%, respectively) were similar on treated and untreated cotyledons in both age groups after heat-treatment at both 1300 and 1700 h. Colony development and sporulation were unaffected by host plant age and time of heat-treatment, a rating of 4 being recorded for both on treated and untreated plants.

4 Translocation of the stimulus predisposing cucumber to the endophytic mycelium of Sphaerotheca fuliginea

4.1 Introduction

The purpose of this experiment was to investigate the induction of endophytic mycelium in an untreated inoculated cucumber cotyledons, following heat-treatment of the opposite cotyledon. Yarwood (1961) showed that the effect of heat injury was translocated from one leaf to its twin opposite in Phaseolus vulgaris and cowpea. Jarvis (1964, personal

communication) found that when one of a pair of 14-day cucumber cotyledons was heat-treated at 45, 50 and 55°C for periods of ½ to 120 sec, 12 h before or up to 7 days after inoculation, the mycelium of Sphaerotheca fuliginea became endophytic in both cotyledons. The most extensive mycelial development on the abaxial uninoculated epidermis of the untreated cotyledon was observed after 90 sec of treatment at 45°C 2 days after inoculation (Jarvis, personal communication). In his investigation of diurnal variation in the induction of endophytic mycelium, Jarvis (personal communication) found the greatest translocation effect in 11- and 14-day plants heat-treated 24 h after inoculation at 1700 h. A bimodal response was observed in the curve relating to the degree of stimulus translocation in 14- and 18-day plants treated for different periods at 50°C at 1300 h with greatest trnaslocation occurring after treatments shorter than 5 sec and longer than 10 sec.

4.2 Experimental

The adaxial epidermis of 11- and 14-day cucumber cotyledons was inoculated at 1700 h. One of each pair of cotyledons was heat-treated at 45°C for 90 sec, 50°C for 10 sec and 55°C for 10 sec at 1300 and 1700 h the following day. These and six inoculated untreated plants were incubated in a growth room. Samples taken 48 h and 7 days after inoculation, were prepared for observation and results recorded as described above.

4.3 Results

Fungal development on the untreated cotyledon of each pair was unaffected by heat-treatment of its twin opposite, similar frequencies of haustorium and elongating secondary

hypha production being recorded on both (approximately 53% and 45%, respectively). The degree of colony development and sporulation (rated 4.4, respectively) was also similar on treated and untreated cotyledons.

II Development of endophytic mycelium in exposed mesophyll tissue

1 Introduction

The development of endophytic mycelium of Erysiphe graminis and Sphaerotheca fuliginea in mesophyll tissue inoculated after stripping the adaxial epidermis from barley leaves and cucumber cotyledons, respectively, was investigated. Endophytic mycelium of Erysiphe graminis in barley and oat leaves (Salmon, 1906) and of Erysiphe cichoracearum in lettuce (Schnathorst, 1959) was observed in the intercellular spaces of mesophyll inoculated after removal of the epidermis. Induction has also been attempted with Erysiphe pisi and Erysiphe graminis in pea and wheat, respectively (Webb, 1979) and although intercellular hyphae were not formed haustoria did develop in pea mesophyll.

2 Experimental

The adaxial epidermis was stripped off 19-day barley leaves and 14-day cucumber cotyledons and the exposed mesophyll was inoculated with conidia. The plants were incubated in a growth room. Samples, taken 7 days after inoculation, were prepared for observation and results recorded as described above (Section 2, C Ia 1.2).

3 Results

No colony development was observed on the abaxial uninoculated epidermis of the barley leaves or the cucumber cotyledons. Less than 5% of the conidia germinated on the exposed mesophyll and no endophytic mycelium developed.

III Induction of endophytic mycelium by exposure to high RH

1 Introduction

The effect of high RH on the development of endophytic mycelium of Erysiphe graminis and Sphaerotheca fuliginea in barley leaves and cucumber cotyledons, respectively, was investigated; the treatment has been found to predispose barley to endophytism by E. graminis (Mackie, 1928). He recorded the development of E. graminis in the leaves of some barley cultivars, which were incubated, after inoculation, under bell jars at a high RH. Ectophytic growth was poor under these conditions but improved when the plants were removed from the bell jars. Furthermore, the experiments done by Salmon (1906), in which endophytic mycelium of Erysiphe graminis was recorded in barley and oat leaves damaged by heat, were carried out using plants which were incubated in a damp chamber.

2 Experimental

Six 19-day barley and 14-day cucumber plants were inoculated and incubated under transparent plant pot covers, raised slightly on one side to provide ventilation in a growth room. Six inoculated barley and cucumber plants

were also incubated, uncovered. Samples, taken 48 h and 7 days after inoculation, were prepared for observation and results recorded as described above (Section 2, C Ia 1.2).

3 Results

Ectophytic fungal development was poor under conditions of high RH, with less than 10% germination on both barley and cucumber cotyledons. No endophytic mycelium was observed in these plants. Fungal development proceeded normally on the uncovered plants, with similar frequencies of haustorium and secondary hypha production and colony development to those recorded on untreated plants in previous experiments (Tables 53 and 54).

## IV Induction of endophytic mycelium by ice-treatment of leaves

1 Introduction

The pupose of this experiment was to investigate the effect of immersing barley leaves and cucumber cotyledons in ice water, after inoculation, on the induction of endophytic mycelium of the respective powdery mildews. Jarvis (personal communication) inoculated 14-day cucumber cotyledons at 0800 h and then immersed them in ice-water at 1700 h on the same day and at 0800 h and 1700 h on the following day for 5, 10, 15, 30 and 45 sec. He recorded mycelial development and sporulation on the abaxial uninoculated epidermes after all the treatments and the greatest enhancement of fungal development occurred after 5 sec of treatment 1½ days after inoculation and 30 and 45 sec 1 day after inoculation. Although colony development on the

uninoculated epidermis was indicative of the presence of endophytic mycelium, none was detected. However, its presence was not ruled out as its distribution was sparse after heat treatment.

2 Experimental

Six 19-day barley leaves and 14-day cucumber cotyledons were inoculated at 0800 h and were ice-treated at 0800 h for 30 and 45 sec and at 1700 h for 5 sec on the following day. These and six inoculated untreated barley and cucumber plants were incubated in a growth room. Samples, taken 48 h and 7 days after inoculation, were prepared for observation and results recorded as described above (Section 2, C Ia 1.2).

3 Results

There was no difference in the fungal development recorded on treated and untreated barley leaves and cucumber cotyledons. Frequencies of haustorium and elongating secondary hypha production (52% and 45%, respectively) and the degree of colony development and sporulation (4.4, respectively) were similar on treated or untreated leaves and cotyledons.

V Induction of endophytic mycelium by heat-treatment of the roots

1 Introduction

The purpose of this experiment was to investigate the effect of immersing the roots of barley and cucumber plants in water on the development of endophytic mycelium of the respective powdery mildews. Jarvis (personal communication) immersed the roots of 14-, 18-, 19- and 22-day

cucumber and sunflower plants in water at 50°C for 1, 3, 5, 10, 15, 30 and 60 sec at 1700 h after inoculating the plants at 0800 h on the same day. Colony development was observed on the uninoculated abaxial epidermis of 14-day cucumber cotyledons after 3, 5 and 15 sec of heat-treatment of the roots but no endophytic mycelium was found. Only a small amount of mycelial development was recorded on the inoculated surface of plants in this age group after all the treatments. On 19- and 22-day plants more fungal development was recorded on the inoculated epidermis than on younger plants after all the treatments, with greatest mycelial development occurring after treatment of the roots for 5, 10 and 15 sec. Colony development was observed on the uninoculated leaf epidermis of sunflower leaves after 1, 3 and 5sec of treatment of the roots but no endophytic mycelium was detected.

2 Experimental

The leaves of three 19-day barley plants and the cotyledons of 14- and 22-day cucumber plants were inoculated at 0800 h. At 1700 h on the same day the plants were removed from the pots, soil adhering to the roots was removed by washing, and the roots were immersed in a water bath at 50°C for 1, 3, 5, 10 and 15 sec. These and three inoculated untreated barley and cucumber plants were incubated in a growth room. Samples, taken 7 days after inoculation, were prepared for observation and results recorded as described above (Section 2, C Ia 1.2).

3 Results

Fungal development was unaffected by heat-treatment

of the roots of barley and cucumber plants of any of the ages tested. Colony development and sporulation (4.4) on the adaxial inoculated epidermis were similar on treated and untreated plants. No fungal development was observed on the abaxial uninoculated epidermis nor any endophytic mycelium recorded. Furthermore, the leaves and cotyledons on treated plants did not exhibit any signs of heat injury.

## D DISCUSSION

The results of this investigation showed that periods of hot water treatment before and after inoculation, exposure to high RH and chilling of barley leaves and cucumber cotyledons failed to induce endophytic mycelium of Erysiphe graminis and Sphaerotheca fuliginea, respectively. There are several possible reasons for the failure to induce endophytic mycelium by heat-treatment. The heat dose may have been inappropriate for modifying the mechanism which confines the fungi to the epidermal layer. Jarvis (1964, personal communication) found that only certain heat-treatments resulted in the development of endophytic mycelium in barley and cucumber, both temperature and duration of the treatment having an effect. Furthermore, the amount of endophytic mycelium produced was affected by different heat-treatments some resulting in only a sparse internal mycelium. Jarvis (1964, personal communication) showed that the stimulus inducing endophytism in cucumber was caused by a chemical, which, when extracted from a heat-treated cotyledon and applied to the opposite inoculated untreated cotyledon, induced endophytic mycelium of S. fuliginea in the latter. The heat-treatments used in the present investigation may have induced insufficient quantities of the chemical stimulus to promote endophytic fungal development or only internal development too sparse to be detected. S. fuliginea became increasingly difficult between June and January. The period when the present investigation was done, November to April, may similarly have been an unsuitable time of year for induction of internal fungal development, although only the November to January period

coincides with Jarvis's results.

Enhancement of fungal development was observed on both hosts after certain wet-heat treatments (barley - 15 sec at 45°C, 12 and 24 h before inoculation, 5 sec at 50°C, 3, 6, 12 and 24 h before inoculation and 10 sec at 50°C, 3, 6, and 24 h before inoculation; cucumber - 60 and 90 sec at 45°C, 10 and 30 sec at 50°C and 5 and 10 sec at 55°C, 12 and 24 h before inoculation). Fungal development was unaffected by heat-treatment carried out after inoculation, by chilling of leaves and cotyledons and by heat-treatment of the roots. No enhancement of fungal development was observed on an inoculated untreated cucumber cotyledon after heat treatment of its twin opposite, implying that the effect of heating the cotyledon was not translocated.

Enhancement of the development of Erysiphe graminis f. sp. hordei has also been observed on compatible barley cultivars, following dry heat treatment (Ouchi et al, 1975). This was manifested in an increase in the number of conidia producing elongating secondary hyphae and in the length of the latter. They suggested that susceptible plants possess a degree of inherent resistance to fungal pathogens, which is broken down by heat treatment, and called the phenomenon "heat-induced susceptibility". A similar break-down in such resistance may have been caused by the wet heat treatments used in the present investigation. It has been suggested that the induction of susceptibility to avirulent races of Erysiphe graminis by heat-treatment of barley leaves (Ouchi et al, 1975, 1976) is caused by

suppression of the first phase of phytoalexin production (Oku et al, 1975 a and b, 1979), which occurs during the period of hyphal penetration of the epidermis, with high levels between 12 and 20 h after inoculation. However, compatible fungi are believed to suppress the production of phytoalexins at this developmental stage, inducing phytoalexin production only after the establishment of infection. No phytoalexin activity was detected between 12 and 20 h after inoculation in a compatible barley cultivar (Oku et al, 1975a). However, Oku et al (1975b) recorded a small amount of phytoalexin production between 8 and 16 h after inoculation with E. graminis on a compatible barley cultivar. It, therefore, seems possible that very small quantities of phytoalexin are induced in compatible host-parasite combinations which restrict infection establishment. In the present investigation hot water treatment of barley leaves and cucumber cotyledons may similarly have suppressed the minimal phytoalexin production in these plants, resulting in enhancement of infection. The lack of enhancement of fungal development on barley leaves and cucumber cotyledons heat-treated after inoculation implies that phytoalexin production had already been induced and could not be reversed by heat-treatment. However, detailed investigations of phytoalexin production are necessary to test these suggestions.

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