STUDIES ON LEAF-SPOT DISEASE OF LETTUCE (*LACTUCA SATIVA* L.) CAUSED BY *CERCOSPORA LONGISSIMA* (CUG.) SACC.

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CHAPTER 1

INTRODUCTION

Lettuce (Lactuca sativa L.), a native of Southern Europe and Western Asia, is descended from the prickly lettuce (L. scariola L.) which is a wild lettuce and common to the roadsides and waste land in the world. It is an old world species cultivated for over 2,000 years and perhaps longer (Hill, 1937).

As a member of the Compositae, the plant produces a basal rosette of leaves and, later in the season, a stalk with flowers and fruits. It has a milky juice called Lactucarium containing Lactucarin ($C_{23}H_{36}O_2$) and Lactucin ($C_{40}H_{58}O_{13}$), etc., which have the function as anodyne and narcotic (Wu, 1957).

It is a popular plant of home gardens, for used in salads and for "prettying up" food at the table. The plant is rich in vitamins A, B, C, D and contains iron salts. It can be digested easily and is a good vegetable for anaemia and fiery temperament. In the recent decades, it has become a valuable commercial crop.

It is a common vegetable in Hong Kong and is cultivated by farmers in New Territories. In general, lettuce is sold in market through the whole year. Although it is inexpensive, yet, due to its quick-growing, good-production and suitability for interplanting with cruciferous vegetables, many farmers favor to cultivate it (Pao, 1973).

Lettuce cultivars have been developed into various forms.

Between 1966 and 1968, three hundred named seed samples of lettuce were recorded in the United Kingdom. Preferably, cultivars should be classified into groups on the basis of head type, absence or presence of anthocyanin, and seed colour. In general, lettuce cultivars are grouped into six types (Yan, 1935; Bowring, 1969).

(1) Butterhead lettuce

The head leaves overlap smoothly and regularly to produce a spherical or slightly flattened head. Leaves are thin, flexible, tender and usually with entire or crenated margins.

(2) Crisphead lettuce

The leaves form a spherical or slightly flattened dense head by overlapping each other smoothly and regularly. Leaves are brittle or crisp in texture and usually with dentate margins.

(3) Cos lettuce

Leaves touch each other at the apex to form an elongated head, but rarely overlap. Leaves are erect and long.

(4) Latin lettuce

Heads are oval. Leaves are less erect and shorter than Cos, and are leathery in texture.

(5) Loose leaf lettuce

Leaves do not form heads. The mature leaves are arranged loosely around the stem with no tendency

for the leaves to overlap or close.

(6) Stem lettuce

The edible part is the enlarged fleshy bolter stem. Rodenburg (1960) states that it is known by the names Romaine Asperge or Lactuca angustana.

In Hong Kong, farmers cultivate the local cultivar which is the loose leaf type, and butterhead, crisphead and cos lettuces which are introduced from the States.

Lettuce grows best where the seasons remain reasonably cool (5-25°C), and for this reason, it is grown extensively during the spring and in the fall. A good rich soil, retaining a fair amount of moisture, is essential, as lettuce develops its best qualities if it is grown quickly without any checks in growth. Temperature and radiation have important effects on morphogenesis of lettuce leaves and lettuce growing. Number of leaves, length and width of the largest leaf and total leaf area are affected primarily by the daily light energy, secondarily by the temperature range. Diurnal changes in temperature are preferable to constant temperature, especially at low light intensities. The optimum temperature is proportional to the daily light energy (Bensink, 1971; Verkerk & Spitters, 1973). The early Relative Growth Rates (RGR) of lettuce are sigmoidally related to temperature, RGR at 22°C is better than that at 10°C (Scaife, 1973). Head lettuce can produce firm heads only at 10-15°C and depends on a specific sum of heat, whereas time of harvest depends on the subsequent total radiation (Bierhuizen,

Ebbens & Koomen, 1973).

Lettuce is subjected to a variety of diseases during its growth and its distribution (Bohn, 1953; Chupp & Sherf, 1960; Ogilvie, 1961; Tibbitts & Rao, 1968; Leather & Hor, 1969; Thiobodeau & Minotti, 1969; Strobel & Mathre, 1970; Tanne, Nitzany & Avizohar-Hershenson, 1970; Ashkar & Ries, 1971; Hartnett & Lorbeer, 1971; Purcifull, Christie, Zitter & Bassett, 1971; Troutman, Gardner & Pew, 1971; Calvin & Sequeira, 1972; Coakley & Campbell, 1972; Colt & Endo, 1972; Duffus, 1972; Jarvis & Hawthorne, 1972; Tomlinson, 1972; Zink & Duffus, 1972; Fry, Close, Procter & Sunde, 1973; Sargent, Tommerup & Ingram, 1973; Pieczarka & Lorbeer, 1974).

A list of lettuce diseases and their causal agents is presented.

Physiological disturbances:

Tipburn—maturity during warm weather, or related to less calcium and more organic nitrogen in the nutrient solution.

Red heart—lack of sufficient oxygen.

Head blanching—effect of light deficiency.

Internal rib necrosis—sometimes induced on nearly mature plant by application of ammonium ion, or caused primarily by a combination of Beet western yellows virus and Lettuce mosaic virus.

Premature yellowing—associated with poor aeration, excessive soil moisture and salt accumulation in the root zone.

Rib blight—associated with rapid and succulent growth.

Root rot—soil conditions.

Viral diseases:

Mosaic—Lettuce mosaic virus

Vector: Aphid—*Myzus persicae* Sulzer

Yellowing

Aster yellows virus

Vector: Leafhopper—*Macrosteles fascifrons* Stal.

Beet yellows stunt virus and Beet western yellows virus

Vector: Aphids—*Nasonovia lactucae* L.
Myzus persicae Sulzer
Macrosiphum euphorbiae Thos.

Lettuce necrotic yellows virus

Vector: Aphid—*Hyperomyzus lactucae* L.

Big vein—Big vein virus

Vector: Fungus—*Olpidium brassicae* (Woron.) Dang.

Miscellaneous:

Bidens mottle virus

Dandelion yellow mosaic virus

Tobacco streak virus

Turnip mosaic virus

Bacterial diseases:

Slime rot—Erwinia carotovora Jones

Pseudomonas viridilivida (Brown) Holland

Pseudomonas cichorii Stapp.

Marginal spot—Pseudomonas marginalis (Brown) Stevens

Fungal diseases:

Black root rot—Thielaviopsis basicola (Berk. & Br.) Ferraris

Damping off (bottom rot)—Rhizoctonia solani Kuehn.

Pythium spp.

Botrytis cinerea Pers. ex Fr.

Gray mould (collar rot)—Botrytis cinerea Pers. ex Fr.

Sclerotinia drop (watery stem rot)—

Sclerotinia sclerotiorum (Lib.) de Bary

Sclerotinia minor Jagger

Downy mildew—Bremia lactucae Regel.

Anthracnose (ring spot or shot hole)—

Marssonina panattoniana (Berl.) Magn.

Leaf-spot—Cercospora longissima (Cug.) Sacc.

Septoria lactucae Pass.

Cladosporium spp.

Alternaria tenuis Nees ex Pers.

Powdery mildew—Erysiphe cichoracearum D. C.

Rust—Puccinia spp.

Leaf-spot disease of most plants can be induced by unfavourable water relationship, temperature and mineral

deficiencies or excess. They can also be incited by viruses, bacteria, fungi and insects. Lettuce diseases of which leaf-spot are the principal symptoms are most commonly caused by fungi.

The leaf-spot disease of lettuce, caused by Cercospora longissima (Cug.) Sacc. was studied.

The studies of environmental factors on growth, sporulation and conidial germination of C. longissima are presented, as well as studies on lettuce seed germination, growth, stomatal distribution and movement, and collection of lettuce cultivars. Infection studies were carried on the host-pathogen relationship, susceptibility of different host cultivars to the disease, pathogenicity of the pathogen, and the environmental factors on development of Cercospora leaf-spot disease.

CHAPTER 2

MORPHOLOGY AND CULTURAL CHARACTERISTICS OF *CERCOSPORA LONGISSIMA* (CUG.) SACC.

Introduction

Cercospora leaf-spot of lettuce (*Lactuca sativa* L.), caused by *C. longissima* (Cug.) Sacc. has been first recorded by Toro in 1929 in Colombia of South America. The organism found associated with it agrees with *C. lactucae* Stevenson described from similar material in Puerto Rico. Welles also described another *Cercospora* on lettuce and named it *C. lactucae* Welles. Both descriptions agree with that of *C. longispora* Cug. as given by Traverso. *C. longispora* was previously named and Saccardo changed the name to *C. longissima* (Cug.) Sacc.. Therefore this scientific name is adopted throughout the studies. However, Hennings described another *Cercospora* on lettuce, which he named *C. lactucae* P. Henn. before Stevenson and Welles described their species. But *C. lactucae* P. Henn. is very distinct from *C. longissima* (Toro, 1929).

Taxonomic notes

A very large number of fungi exist which are known only by their conidial stages. Since most of these imperfect stages (i.e. the asexual stages) are similar to those of known Ascomycetes, it is almost certain that many of the so-called imperfect fungi (Fungi Imperfecti) are actually Ascomycetes which have either lost their ascigerous stages as a result of

their evolutionary developments, or which possess ascigerous stages as yet undiscovered (Alexopoulos, 1962).

Since the perfect stage (i.e. the sexual stage) has not been discovered, Cercospora belongs to the Fungi Imperfecti (i.e. the Form-class of Deuteromycetes) with the reproductive stage similar to the asexual stage of some Mycosphaerella of the Ascomycetes.

Taxonomy of this fungus:

Form-class Deuteromycetes

Form-order Moniliales

Form-family Dematiaceae

Form-genus Cercospora

In the Dematiaceae, both the hyphae and the conidia are typically dark, but sometimes the hyphae alone or the conidia only are dark. The form-genus Cercospora belongs to the section of Scolecosporae—conidia thread-like to worm-like, continuous or septate, hyaline or pale. It contains about 3,800 described form-species, many of which are destructive plant parasites causing leaf-spot diseases (Berger & Hanson, 1962a & b and 1963a & b; Sobers, 1968; Golatc & Meossi, 1971; Harvey & Wenham, 1972; Laviolette & Athow, 1972; Rathaiah & Pavgi, 1972; Marlatt, 1973; Spencer, 1973; Blazquez & Alfieri, 1974; Judd & Peterson, 1974). Studies on pathogenicity and cultural characteristic by Johnson and Valleau indicate, however, that many of these form-species are actually the same fungus attacking different hosts and described many times under different names (Johnson & Valleau, 1949).

Morphology

On agar medium, young hyphae of C. longissima are slender, 1.5-3.5 μm in width, with granular contents, septate, sometimes noduled, hyaline at first, but quickly acquiring pigmentation. The older hyphae are generally thicker, 3.0-6.0 μm wide, more closely septate, pale to moderate dark-brown or dark-green and often filled with oil globules. In old culture or in Rose Bengal and Streptomycin Plain Agar (Tsao, 1970), there are certain cells with special brown crystals. Some young and older vegetative hyphae with special brown crystals, prepared from the slide culture, are shown in Plate 2-1.

When sporulation occurs, conidiophores arise from hyphae deep within the substratum, from hyphae just beneath or on the surface of substratum, or from aerial hyphae. Sporulation usually develops on the younger growth at the peripheries of the colonies, but scattered conidiophores with conidia also form on the surface of the older portion of the colonies. Conidiophores are mostly amphigenous, fasciculate, moderate olivaceous brown, unbranched, septate (1-6 septa), 53.0-125.0 μm in length and 3.8-4.5 μm in width, bearing conidia singly on newly growing tips, and with obvious spore scars on the tips by deciduous conidia.

Conidia are hyaline, with 1-18 septa, sometimes up to 28, cylindrical to obclavate, with obconically truncate base and tapering obtuse apex, straight to curve, smooth, deciduous. The length varies from 11.3 μm to 270.0 μm with the base of

7.5 μm and the tip of 3.9 μm in width. Conidia with various length and septum number are shown in Plate 2-2.

Cultural characteristics

Different kinds of medium affect the colour, topography and general appearance of the colonies; but, in general, they are flat to slightly raised and felty. Colour of the colonies when viewed from the top is usually some shade of olive-gray; when viewed from the bottom through a glass plate and the substrate, the colour on most media is olivaceous-black.

Cultural characteristics of C. longissima on Plain Water Agar, Rose Bengal and Streptomycin Plain Agar, Nutrient Dextrose Agar, Potato Dextrose Agar, V-8 Juice Agar and Czapek-Dox V-8 Juice Agar are studied.

Composition of these agar media are:

Plain Water Agar (PWA)

Distilled water	1,000 ml
Agar	15 g

Rose Bengal and Streptomycin Plain Agar (RSA)

Distilled water	1,000 ml
Rose Bengal	30 mg
Streptomycin	30 mg
Agar	15 g

Nutrient Dextrose Agar (NDA)

Distilled water	1,000 ml
Beef extract	3 g

Peptone	10 g
Dextrose	10 g
Agar	17 g

Potato Dextrose Agar (PDA)

Distilled water	1,000 ml
Potato	200 g
Dextrose	20 g
Agar	17 g

V-8 Juice Agar (VJA)

Distilled water	900 ml
V-8 Juice	100 ml
Agar	17 g

Czapek-Dox V-8 Juice Agar (CVA)

Distilled water	900 ml
V-8 Juice	100 ml
Sodium nitrate	3 g
Potassium dibasic phosphate	1 g
Potassium chloride	0.5 g
Magnesium sulphate	0.5 g
Ferrous sulphate	0.01 g
Sucrose	30 g
Agar	17 g

Cultural characteristics on these six agar media are described as follows and shown in Plate 2-3.

(1) Plain Water Agar (PWA)

C. longissima can grow even on plain water agar. Hyphae

are hyaline, slender, loosely packed and spread out. The colony is pale. No conidia are produced.

(2) Rose Bengal and Streptomycin Plain Agar (RSA)

Growth is restricted. Colony is gray and irregular in shape. Hyphae are hyaline to pale brown. Many vegetative cells contain special brown crystals. Abundant conidia are produced.

(3) Nutrient Dextrose Agar (NDA)

The colony has a black periphery and a black core. The middle white to light gray portion has obvious radial folds.

(4) Potato Dextrose Agar (PDA)

The colony is dark green and felty. Hyphae are loosely packed.

(5) V-8 Juice Agar (VJA)

The colony has a black periphery and an uneven brownish gray core.

(6) Czapek-Dox V-8 Juice Agar (CVA)

The fungus grows and sporulates best on this agar medium. The colony possesses concentric rings of different shades of olive-gray and radial folds. The colonies seemed to inhibit each other and leave sterile bands in between.

The radial growth, sporulation and conidial size of C. longissima on NDA, PDA, VJA and CVA are studied later.

Variation in culture

Variants from the isolate often appeared in single-spore cultures of C. longissima. A similar range in color was observed when plates were reversed and the lower surfaces of colonies were examined. Through a series of subculturing, two distinct variants had been isolated. One variant possessed concentric rings of various shades of gray, and the other one was white in color (Plate 2-4). The white variant grew considerably faster than the gray one. Sporulation of the two variants was also different. Generally, the white variant produced less conidia, more slender and hyaline mycelium than the gray variant.

Radial growth and sporulation of the 10 days cultures of the white and gray variants on CVA at 25°C are shown in Table 2-1.

Table 2-1. Radial growth (mm in diameter) and sporulation ($\times 10^4$ conidia/cm^2) of different Cercospora longissima variants on CVA at 25°C.

Variants	Growth Diameter	Conidia No.
White	23.6±1.3	0.788±0.176
Gray	19.0±1.0	3.914±0.743

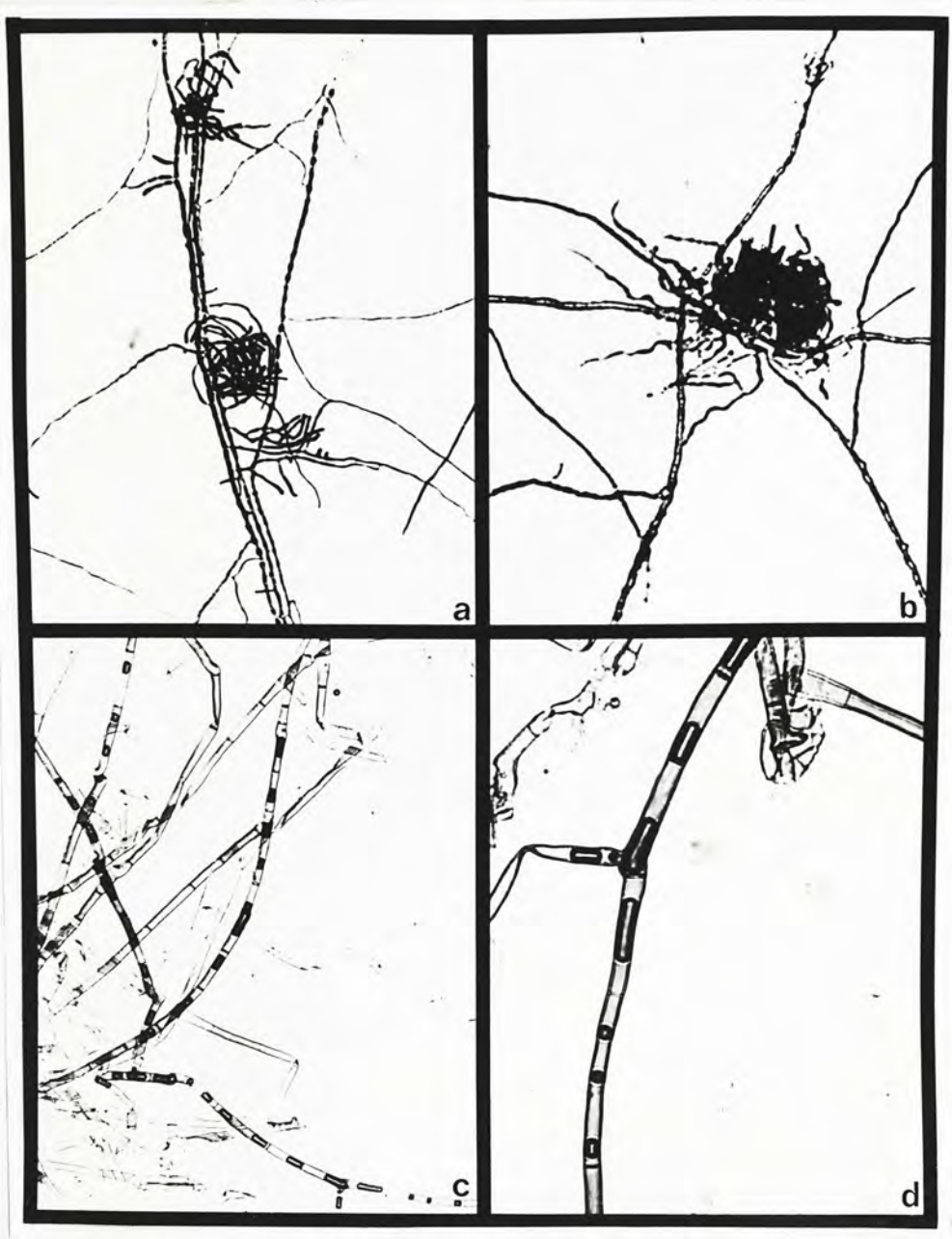


Plate 2-1. Vegetative hyphae of Cercospora longissima. (a) Young and slender hyphae with cluster of initial conidiophores. (x100) (b) Same as a. (x250) (c) Old and thicker hyphae with special brown crystals. (x100) (d) Same as c. (x250)

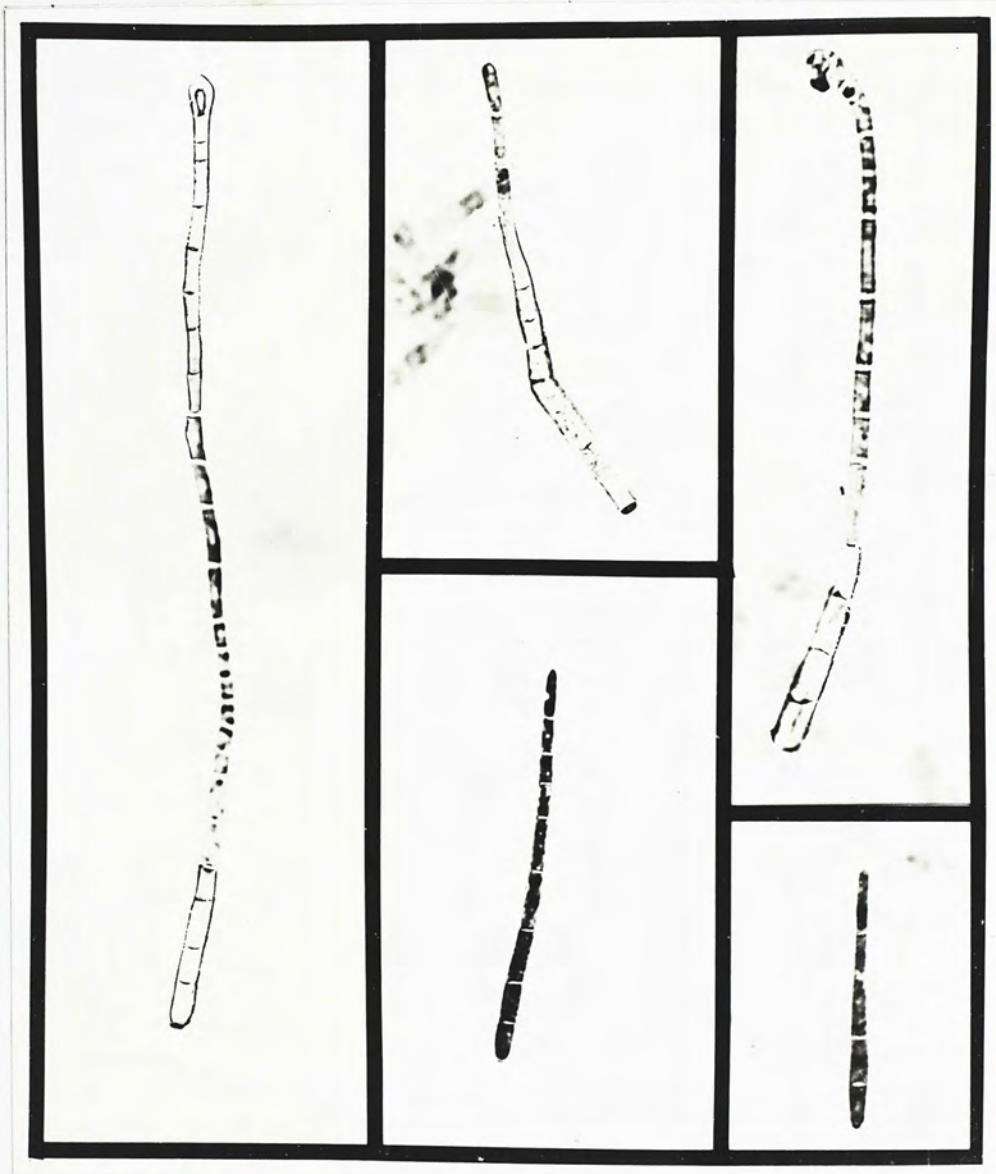


Plate 2-2. Deciduous conidia of Cercospora longissima. Number of septum varied from 1 to 18. (x500)

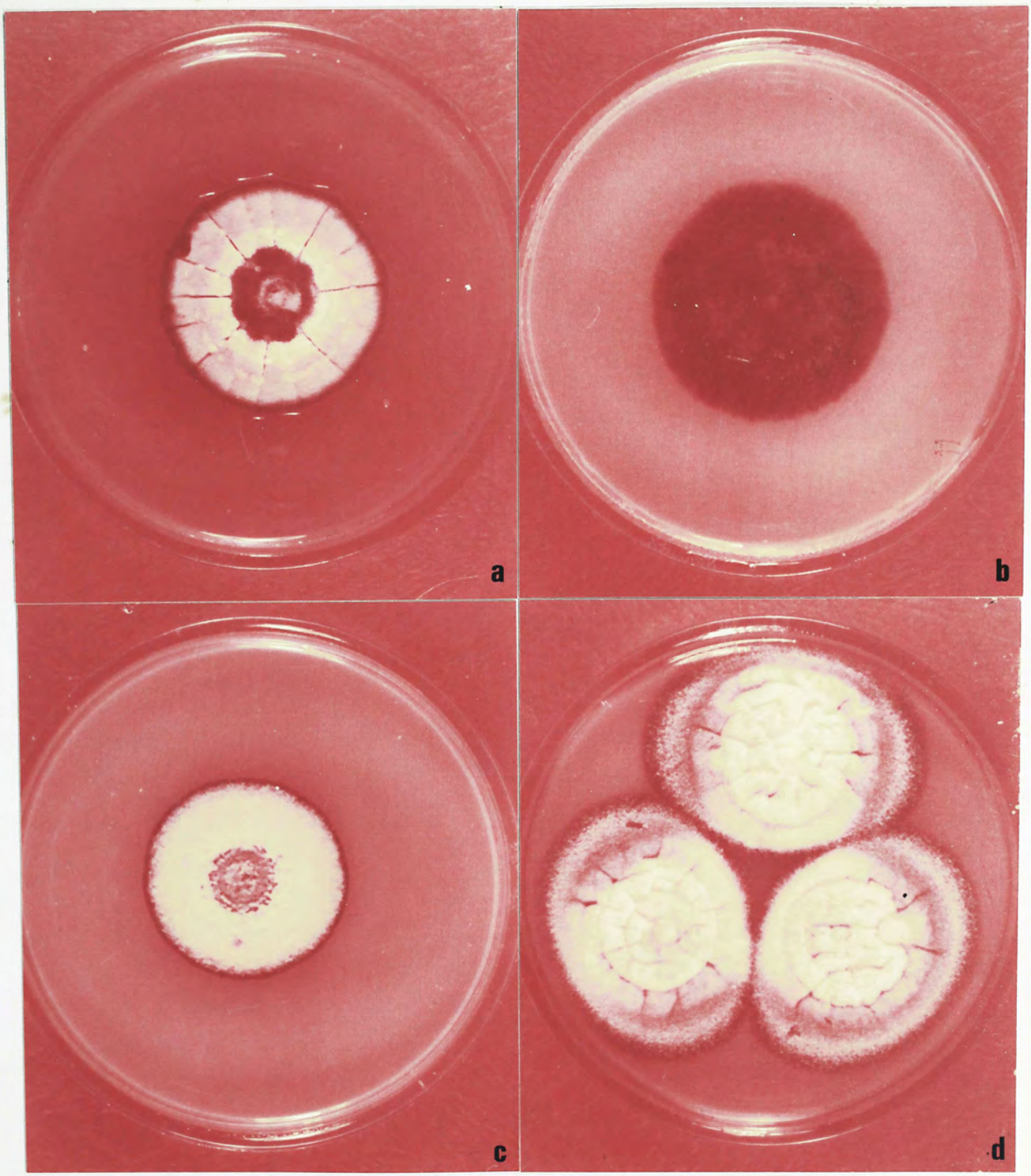


Plate 2-3. Cultural characteristics of Cercospora longissima on different agar media. (a) NDA (b) PDA (c) VJA (d) CVA

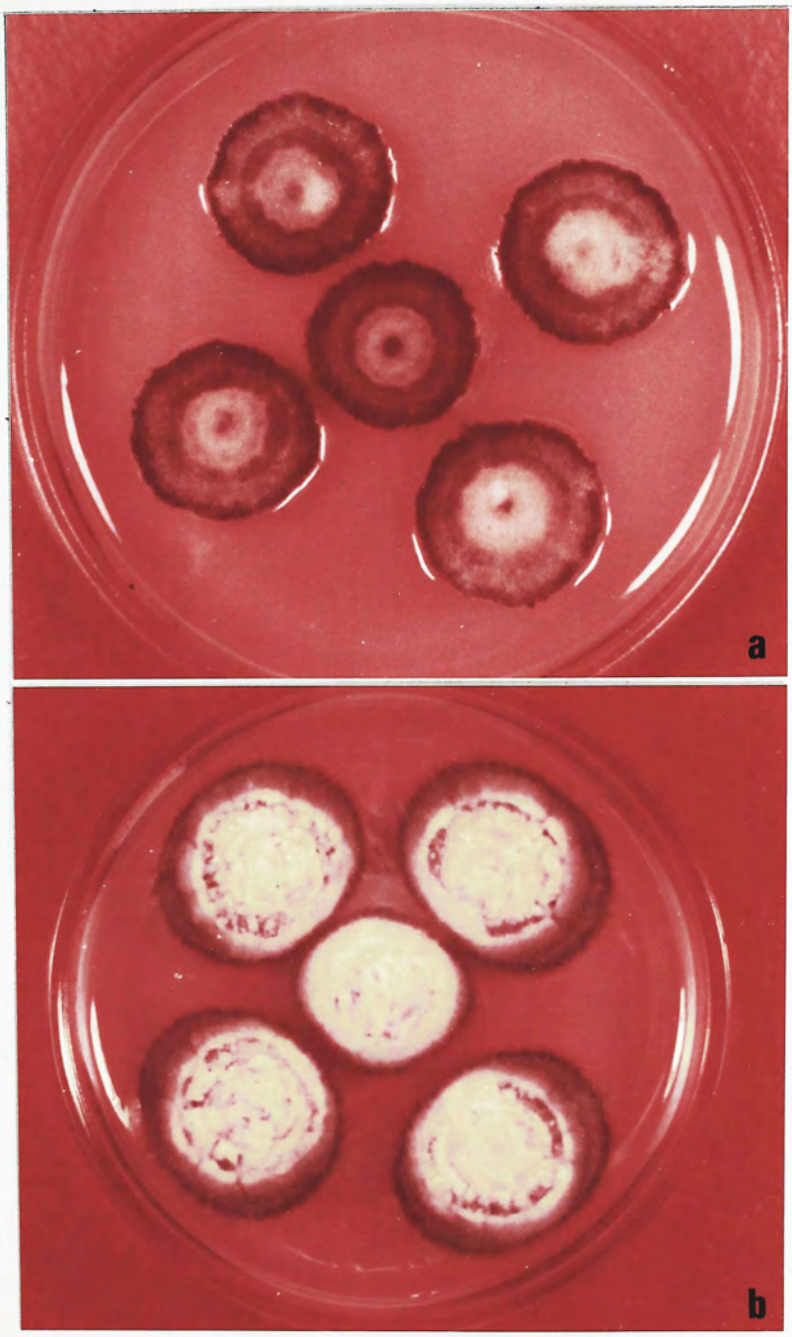


Plate 2-4. Variants of Cercospora longissima on CVA. (a) Gray variant. (b) White variant.

CHAPTER 3

NUTRITIONAL REQUIREMENTS, TEMPERATURE AND LIGHT EFFECTS ON GROWTH, SPORULATION AND CONIDIAL SIZE OF *CERCOSPORA LONGISSIMA* (CUG.) SACC.

Introduction

There are a number of factors influencing growth and sporulation of fungus (Hawker, 1950; Lilly & Barnett, 1951; Cochrane, 1958). In general, they are:-

External factors:

Environmental factors:

Temperature

Light

Humidity

Oxygen, carbon dioxide and volatile substances

Hydrogen-ion concentration

Type of medium (solid, semi-solid or liquid)

Gravity

Mechanical injury

Mechanical barrier to growth

Nutritional factors:

Concentration of nutrients

Nitrogen source

Carbon source

Micro-essential elements

Vitamins

Specific reproductive factors

Carbon-nitrogen ratio

Association with other organisms

Other physical factors:

Method of inoculation

Method of sterilization

Influence of the host

Internal factors

Age of the inoculum

Kind of the inoculum

Only three factors are under consideration for influencing the growth, sporulation and conidial size of Cercospora longissima (Cug.) Sacc. in these studies, i.e. the nutritional requirements, temperature and light effects.

1. Nutritional requirements

Species of Cercospora, in general, grow slowly and sporulate sparsely or not at all on most of the artificial media. Attempts to obtain sporulation of C. kikuchii (T. Matsu. & Tomoyasu) Gardner (1927) in artificial culture have been unsuccessful. Murakishi (1951) tried 15 different types of media but failed to obtain sporulation. Nagel & Dietz (1932) and Nagel (1934), working with C. beticola Sacc., found mycelial transfers resulted in sterile hyphae, whereas spore transfers yielded colonies that produced abundant sporulation. Diachun & Valleau (1941) obtained sporulation of C. nicotianae Ell. & Ev. by using a steamed tobacco leaf decoction mixed with 1.2% agar. Kilpatrick & Johnson (1956) dealt with 22 isolates of

Cercospora obtained from 14 different hosts. These fungi were grown on different kinds of plant decoction agar. All of the isolates tested produced spores on the agar medium prepared from carrot leaves steamed without pressure for one hour. Sporulation did not occur on the medium prepared from carrot roots, steamed with or without pressure, nor on the medium prepared from carrot leaves sterilized under pressure. Apparently the substance in carrot leaves that induces sporulation was changed chemically by pressure sterilization. Depth of agar also affected sporulation. When isolates were transferred to petri dishes containing only 10-15 ml of agar, sparse sporulation was observed, whereas in those dishes containing 25-40 ml of agar, abundant sporulation was obtained (Kilpatrick & Johnson, 1956). In C. zebrina Pass., optimum pH for germination was 5.2. Most mycelial growth occurred in media with an initial pH of 5.5. Maximum sporulation occurred at 100% R.H.; no spores developed at 95% R.H. or below. This species grew on all the synthetic and plant decoction media tested. Of the solid media, modified Richard's agar and potato-dextrose agar gave most radial growth. In liquid culture, most growth was obtained in cornmeal broth, modified Richard's broth and oatmeal broth. Most spores formed on V-8 juice agar (Berger & Hanson, 1962a & b and 1963a & b). In C. nicotianae, the optimum initial pH for mycelial growth on all solid and liquid media was 4.5. The optimum pH values for sporulation were 4.5 to 5 on V-8 juice agar and 6.5 on a medium containing DL-leucine, sucrose, yeast

extract and mineral nutrients. Maximum radial growth occurred on an agar medium containing 1.6 g of DL-leucine, 50 g of sucrose and 2.4 g of yeast extract per liter plus mineral nutrients. Sporulation was best on an agar medium containing 1.6 g of DL-leucine, 5 g of sucrose and 3.6 g of yeast extract per liter plus mineral nutrients. Sporulation was good on V-8 juice agar and supplemented tobacco decoction agar. Inclusion of peptone, protone, malt extract, or cholesterol in the medium and substitution of vitamins for yeast extract decreased sporulation. Longer conidia were produced on V-8 juice agar than on the best sporulation medium (Stavely & Nimmo, 1968a & b). In C. cruenta Sacc. and C. beticola Sacc., Czapek-Dox medium supported maximum growth of both species. Sporulations of these two species were good and fair respectively on carrot leaf decoction. Glucose supported maximum growth and sucrose supported good sporulation as compared with other carbon sources. Calcium nitrate, potassium nitrate or sodium nitrate supported good growth of both species. For sporulation, sodium nitrate was better than other nitrogen sources. Maximum growth and sporulation were found with pH 6.8 and 5.9 respectively in C. cruenta and with pH 6.4 and 7.1 in C. beticola (Verma & Agnihotri, 1972). C. beticola was partially deficient in three vitamins: thiamine, pyridoxine and choline. The deficiency in thiamine was more pronounced than the deficiencies in pyridoxine and choline. Thiamine only was essential for sporulation of the fungus (Mandahar, 1973). The studies of carbon and

nitrogen nutrition in liquid culture of C. omphakodes Ell. & Holw. with shaking, yielded best vegetative growth on fructose and tryptophan but no sporulation was observed (Judd & Peterson, 1974).

2. Temperature

Temperature was recognized by Bisby (1943) as an important natural factor governing the geographical distribution of the fungi. It has been found by various investigators that there is an optimum temperature for sporulation as well as for growth. The two optima may or may not be different. Higher and lower temperatures of incubation decreased the rate of growth and the number of spores produced.

Optimum temperature for growth and sporulation of C. zebrina on solid and liquid media was 24°C. Conidia and conidiophores attained maximum length at 24°C and 100% R.H. Temperature prior to formation of these structures had no effect on their length (Berger & Hanson, 1962b). The number of conidia produced by C. nicotianae in culture increased with time until a peak was reached and then declined. Incubation temperature determined the number of days required for peak sporulation to be reached and the abundance of conidia. Peak sporulation occurred 3, 4, 5, 5, 7, 8 and 12 days after flooding media with conidia and mycelia when media were incubated at 29°, 26°, 23°, 20°, 18°, 16° and 12°C respectively. The optimum temperature for sporulation was 18°C, but the optimum temperature for radial growth of the organism was 26°C. On V-8 juice agar, conidia

produced at 12°C were longer than those produced at 18° and 26°C, but there was no difference in conidial length at these three temperatures on the best sporulation medium of C. nicotianae (Stavely & Nimmo, 1969b). Optimum temperature for growth of both C. cruenta and C. beticola was observed to be 26°C (Verma & Agnihotri, 1972). The optimum temperature for growth of C. omphakodes was between 24° and 28°C (Judd & Peterson, 1974).

3. Light

Light also plays an important part in fungal physiology, e.g. growth rate, phototropism, photomorphogenesis, rhythmic processes and pigmentation (Carlile, 1965). Effect may be varied with the intensity of illumination, the length of exposure, the wave-length of light and different genera or species of the fungi.

Response of the species of Cercospora to light is very diverse. Klotz (1923) found that light effects were not very marked on growth and sporulation of C. apii Fres. Chowdhury (1944) considered that the rate of linear growth of C. sesami Zimm. was found to be greater and sporulation to be earlier and more copious in alternate light and darkness, less in continuous darkness and least in continuous light (Marsh, Taylor & Bassler, 1959). However, Johnson & Halpin used 5 weeks cultures of Cercospora on various media subjected to continuous illumination by incandescent or fluorescent light, alternate light (8 hr) and dark (16 hr) periods and complete darkness, they did not find any influence on conidial production by these treatments

(Johnson & Halpin, 1954). Yet, cultures of 22 isolates of Cercospora obtained from 14 different hosts exposed to daylight sporulated more abundantly than did those incubated in darkness. However, darkness did not suppress sporulation completely (Kilpatrick & Johnson, 1956). C. zebrina also had better sporulation in light than in dark, but light had no apparent effect on conidial length (Berger & Hanson, 1963a).

Little is known about the nutritional requirements, temperature and light effects on the growth, sporulation and conidial size of C. longissima (Cug.) Sacc. It is the purpose of this project to obtain adequate quantities of conidia for inoculation.

Materials and methods

The isolate used was obtained from the naturally infected leaves of lettuce (Lactuca sativa L.) from the Agriculture & Fisheries Department of Hong Kong and the local market.

1. Nutritional requirements

Growth, sporulation and conidial size of C. longissima on four different agar media were studied. The pH of these media was adjusted to 5 and the media were sterilized at 15 p.s.i. for 20 min. Nutrient dextrose agar (NDA), potato dextrose agar (PDA), V-8 juice agar (VJA) and Czapek-Dox V-8 juice agar (CVA) were used. A small piece of mycelial mat, about 1 mm in diameter, was transferred to the centre of each petri dish and incubated under room conditions with a temperature range of 20-25°C. The radial growth and the sporulation of the fungus on

different agar media were investigated on 3rd, 5th, 7th, 9th, 11th and 13th days after transferring. The effect of various media on radial growth of C. longissima was determined by measuring the diameter of growth on the media. For testing sporulation, the fungal colonies were cut with cork borers, according to the diameter of the colony, placed in 10 ml of sterile water and blended for 2 min with an electrical blender. Twelve counts of spore number on each test medium were made with a haemocytometer. Spore concentration was expressed as number of conidia per cm^2. Spore measurement was made from at least 100 conidia per trial with a calibrated eye-piece micrometer. Treatments were repeated three times further.

2. Temperature

Growth, sporulation and conidial size of C. longissima on CVA (pH=5) at different temperatures were studied. Colonies of 4 mm diameter were cut out with cork borer and transferred to plates of CVA. These plates were incubated at 5°, 10°, 15°, 20°, 25°, 30° and 35°C. Fluctuation of temperature was around ±1°. Radial growth and sporulation of the fungus at these seven temperatures were investigated on 3rd, 5th, 7th, 9th, 11th and 13th days after incubation. Spore measurement was also made. Observations were repeated three times.

3. Light

Pieces of mycelial mat of approximately 1 mm in diameter were transferred to the plates of CVA and incubated at 25°C for one week. Then the cultures were subjected to irradiation.

The cultures had 2 hr of darkness before and after irradiation with 30 min of red light (R_{30}) or with 25 min of far-red light (FR_{25}). Radial growth, sporulation and conidial size of the fungus, in comparison with the dark and light controls, were investigated on the 3rd day after irradiation. Treatments were repeated three times further.

Results and discussion

1. Nutritional requirements

(a) Mycelial growth

Effect of synthetic media on radial growth of C. longissima under room conditions with temperature range of 20-25°C is shown in Fig. 3-1. It is found that CVA supported maximum radial growth of the fungus. Growth was moderate in VJA and PDA, and poor in NDA. Radial growth was linear in two week cultures on all agar media tested.

(b) Sporulation

Sporulation of C. longissima on four different agar media at room conditions on the 3rd, 5th, 7th, 9th, 11th and 13th days after incubation is shown in Fig. 3-2. Sporulation was poor on PDA. Although NDA gave a quick sporulation on the 5th day after incubation, yet, the spore concentration on unit area dropped down rapidly. Sporulation on VJA fluctuated with the time of incubation. Therefore, VJA and NDA were not

suitable in production of adequate quantities of conidia for inoculation. Only CVA could give a steady conidial production. The number of conidia produced in this medium increased steadily with time of incubation. Spore concentration reached a maximum of 1.226×10^5 per cm^2 at the day 11 culture.

(c) Conidial size

Longer conidia were produced on CVA than on other agar media tested. The conidia had the average of 109.0 μm in length and 8 septa. The spore length varied from 18.8 μm to 266.3 μm and number of septum varied from 1 to 18. Conidia produced on NDA were the shortest, with the average length of 95.2 μm, varying from 22.5 μm to 176.3 μm and a range of 2 to 13 septa. Spore length and number of septum, with their ranges in parentheses, on different agar media are presented in Table 3-1.

Table 3-1. Spore length (μm) and number of septum on different agar media.

Spore \ Media	VJC	CVA	PDA	NDA
Spore Length	100.5 (22.5-225.0)	109.0 (18.8-266.3)	104.9 (30.0-176.3)	95.2 (22.5-176.3)
Septum Number	7 (1-17)	8 (1-18)	6 (2-11)	8 (2-13)

2. Temperature

(a) Mycelial growth

The effects of seven different temperatures on radial growth of C. longissima on CVA are shown in Fig. 3-3. The fungus could grow at 15-30°C, with 25°C being the optimum temperature. Little growth occurred below 10°C or above 35°C.

(b) Sporulation

The temperature effect on sporulation of the fungus on CVA is shown in Fig. 3-4. The number of conidia produced by C. longissima in culture increased with time of incubation until a maximum of sporulation was reached and then declined. All seven temperatures tested, reached the maximum of sporulation on the 5th day after incubation. Different incubation temperatures determined the abundance of conidia but not the peak of sporulation. The optimum temperature for sporulation of C. longissima was 25°C. Less conidia was produced in plates incubated at 10°C and 35°C.

(c) Conidial size

Temperature also had an effect on the size of conidia. Conidia produced at 25°C were, on the average, longer than those formed at higher or lower temperatures. Conidia produced on CVA at 25°C were found to have an average length of 103.8 μm and a range of 15.0-270.0 μm. Number of septum varied from 1 to 18 with the

mean of 8. Temperature effects on spore length and number of septum, with their ranges in parentheses, are given in Table 3-2.

Table 3-2. Spore length (μm) and number of septum on CVA at different temperatures.

Temp.	5°C	10°C	15°C	20°C	25°C	30°C	35°C
Spore Length	92.8 (22.5-176.3)	98.7 (26.3-146.3)	70.0 (15.0-146.2)	86.4 (22.5-183.8)	103.8 (15.0-270.0)	75.4 (22.5-243.8)	91.0 (30.0-187.5)
Septum Number	6 (1-13)	6 (1-16)	6 (1-16)	7 (2-12)	8 (1-18)	7 (1-15)	7 (1-15)

3. Light

(a) Mycelial growth

Light effect on radial growth of C. longissima on CVA at 25°C is presented in Table 3-3. From the results, all different light treatments did not show any significant effect on radial growth of this fungus. It had approximately equal growth rate under different light treatments.

Table 3-3. Radial growth (mm in diameter) of Cercospora longissima on CVA with different light treatments.

Treatments	Light	Dark	R_{30}	FR_{25}
Growth Diameter	21.0±2.4	20.6±2.5	21.0±1.6	21.4±2.4

(b) Sporulation

Sporulation of C. longissima was found not to be light-dependent. There was no significant difference between production of conidia at different light treatments, i.e. continuous light, continuous dark, R_{30} and FR_{25}. The results are shown in Table 3-4.

Table 3-4. Sporulation (x 10^4 conidia/cm^2) of Cercospora longissima on CVA with different light treatments.

Treatments	Light	Dark	R_{30}	FR_{25}
Conidia Number	3.535±0.910	2.992±0.617	3.716±0.754	3.071±0.996

(c) Conidial size

Conidia produced for different light treatments, had some sort of difference in both the length and number of septum. Spore length and number of septum of C. longissima, with their ranges in parentheses, with different light treatments are shown in Table 3-5. Continuous irradiation produced the longest conidia, whereas, conidia formed in complete darkness were the shortest. Treatment of R_{30} also had the effect on production of longer conidia and FR_{25} had the inhibitory effect. However, light effect on number of septum was not very significant.

Table 3-5. Spore length (μm) and number of septrum on CVA with different light treatments.

Treatments	Light	Dark	R_{30}	FR_{25}
Spore Length	119.9 (15.0-251.2)	79.7 (15.0-247.5)	103.0 (22.5-217.5)	97.8 (26.2-183.8)
Septum Number	9 (1-24)	7 (1-21)	7 (1-18)	8 (1-19)

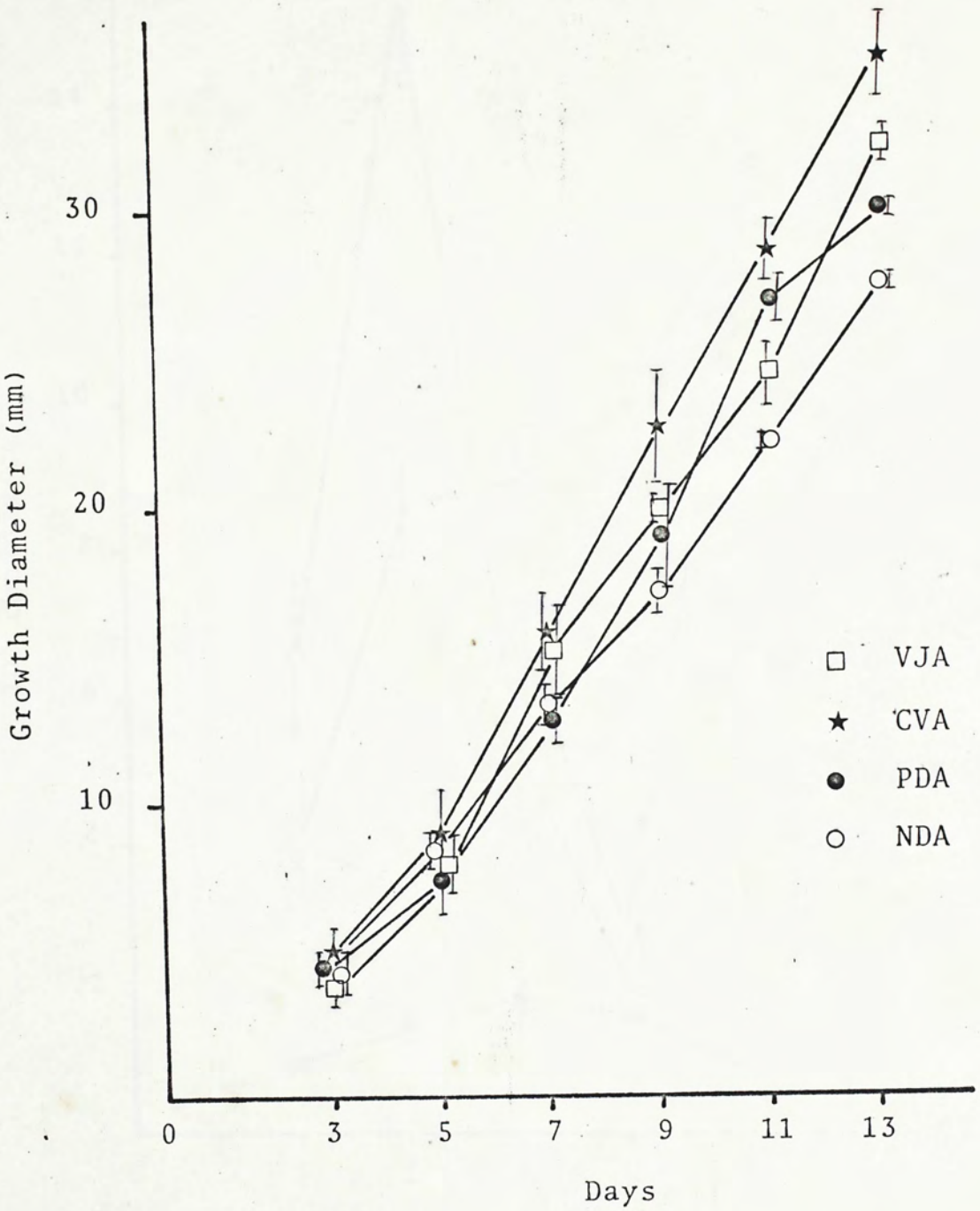


Fig. 3-1. Radial growth of Cercospora longissima on different agar media.

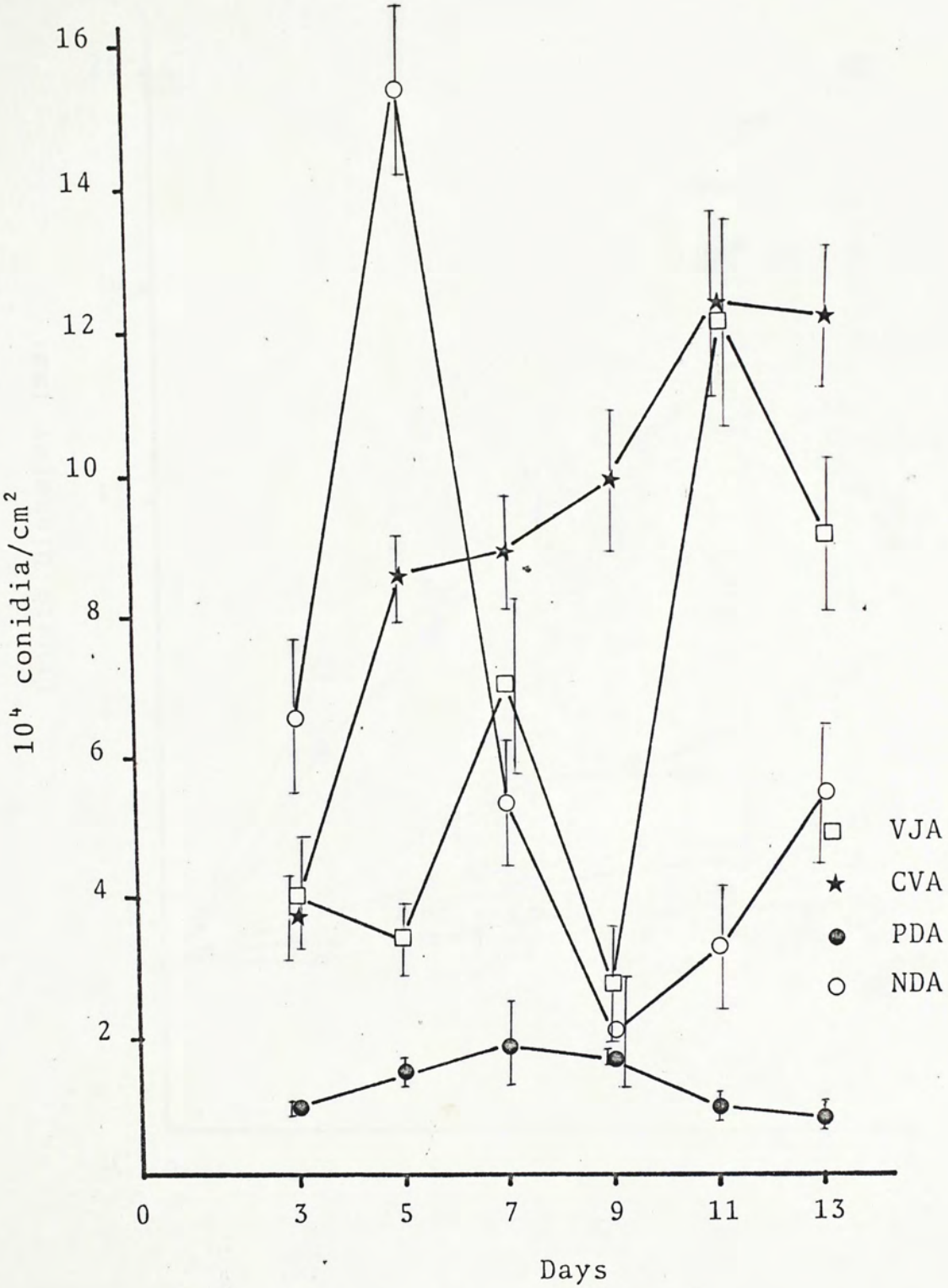


Fig. 3-2. Sporulation of Cercospora longissima on different agar media.

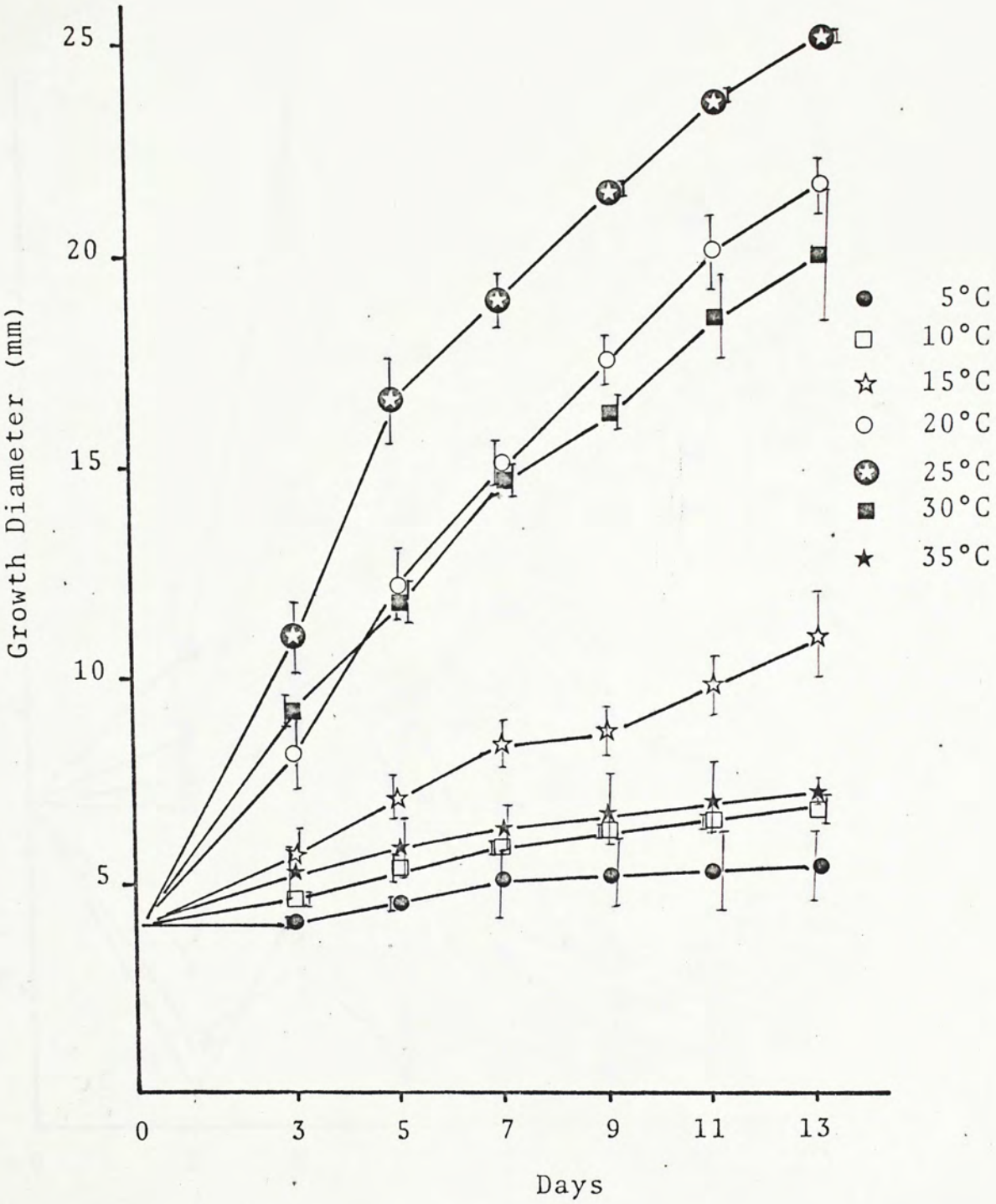


Fig. 3-3. Radial growth of Cercospora longissima on CVA at different temperatures.

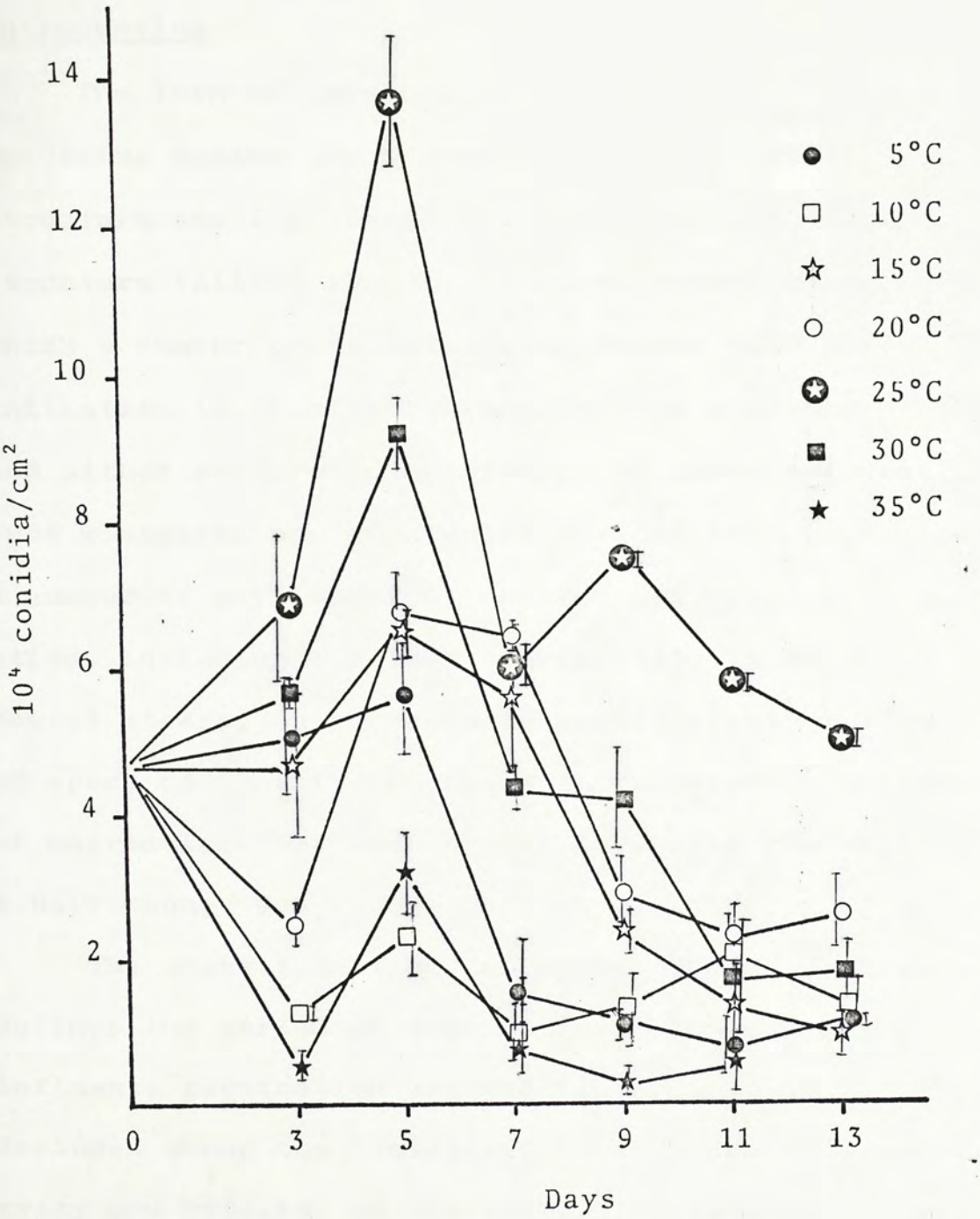


Fig. 3-4. Sporulation of Cercospora longissima on CVA at different temperatures.

CHAPTER 4

CONIDIAL GERMINATION OF CERCOSPORA LONGISSIMA (CUG.) SACC.

Introduction

The term of germination comprises the processes and changes occurring during the resumption of development of a resting structure and its transformation to a morphologically different structure (Allen, 1965). It is an irreversible process in which a number of simultaneous events take place. Overall, initiation is usually followed by the swelling of the spore, and either rupture or absorption of the spore coat. The germ-tube elongates and eventually divides into vegetative cells. A number of physiological changes are associated with germination, including the loss of viability in response to environmental stress, an increase in permeability, a loss or changes of spore components, increase in respiratory activity, synthesis of macromolecules, and changes in enzyme composition (Sussman & Halvorson, 1966).

The transition from the spore to the vegetative stage defines the period of germination. Two classes of phenomena influence germination are the 'internal' and 'external' factors. Included among the 'internal' factors are the maturity, longevity and vitality of the spore. 'External' factors include temperature, oxygen, carbon dioxide, hydrogen-ion concentration, water, light, conditions of spore formation, a presoaking treatment, nutrition and spore density, etc. (Lilly & Barnett,

1951; Cochrane, 1958).

Neglect of the 'internal' factors on conidial germination, most workers worked on the environmental factors affecting conidial germination. Optimum temperature for conidial germination of Cercospora zebrina Pass. was 24°C (Berger & Hanson, 1963a). Conidia of C. arachidicola Hori. required a saturated or near-saturated atmosphere to germinate at optimum temperature of 20-30°C (Oso, 1972). Conidial germination of C. omphakodes Ell. & Holw. occurred from 10-30°C and 70-100% R.H. (Judd & Peterson, 1972). However, little is known about the conidial germination of C. longissima (Cug.) Sacc.

The following studies were made to determine the mode of germination, the temperature requirement, and the light effects of red (R) and far-red (FR) on conidial germination of C. longissima.

It has been well known that R light stimulated the germination of lettuce seeds since 1934. Three years later, Flint and McAlister found that blue and FR wavelengths were inhibitory (Flint & McAlister, 1937). In 1952, the stimulating effect of R light on seed germination was found to be reversed by immediately following the R treatment with FR. By repeatedly alternating R and FR treatments, the Borthwick group found that the light applied last determined whether the seeds germinated or not, R promoting and FR inhibiting the promotion by R (Borthwick, Hendricks, Parker, Toole & Toole, 1952). It was concluded that a blue-green pigment capable of existing in two

interconvertible forms existed in plants. One form was thought to absorb R light and to be changed into a form of a slightly different colour, which was then capable of absorbing mostly FR. The FR-absorbing form (P_{fr}) was believed to be changed back to the R-absorbing form (P_r) as it absorbed FR light. In addition, it has been found that P_{fr} will slowly convert to P_r in the dark. This slow conversion to the P_r form is under thermal control. Initial success in purification of the pigment came in 1959 and the pigment was then named PHYTOCHROME. The absorption spectrum of a purified solution of oat phytochrome between 300-800 nm was worked out by Butler et al. in 1965.

The P_r of phytochrome proved to be blue-green and the P_{fr} of green-yellow coloured. In 1966, it was reported that the chromophore group giving the protein the two colours is an open-chain, metal-free, tetrapyrrole pigment similar to the phycobilin of allophycocyanin. In 1971, Rudiger proposed the structure for the reversible changes occurred in the chromophoric group in acid and base buffer solutions and its linkage to the apoprotein.

The form produced by R light was apparently the active form which has reversible influences upon a large number of physiological responses. The mechanisms of phytochrome action that received consideration are: (1) control over an enzyme action, (2) gene function, and (3) membrane permeability. With passage of time and accumulation of a broader array of

P_{fr}-mediated responses, the idea of a physical rather than chemical basis of control gained headway.

Phytochrome has been reported from every major taxonomic group of plants. However, no convincing evidence for the involvement of phytochrome in fungi has yet been shown (Salisbury & Ross, 1969; Clayton, 1971; Mitrakos & Shropshire, 1972).

Therefore, it is the aim in the following experiment to find out whether there is phytochrome-mediated response involved in the conidial germination of C. longissima.

Materials and methods

Abundant conidia were obtained from the CVA medium.

1. Mode of conidial germination

Conidia were scraped into a drop of sterile distilled water on a glass slide. The slides were placed in a sterilized petri dish moistened with wetted filter paper as a humidity chamber. The petri dish with slides was then incubated at room temperature (21-24°C). Germination of conidia was observed microscopically at two hours interwals. Conidium was considered germinated if the germ-tube was as long as or more than the diameter of the conidium.

2. Temperature

Colonies on CVA were scraped into 10 ml of sterile distilled water and blended for 2 min in a Mitsubishi electric blender. Every 1 ml of conidial suspension was kept in a universial bottle. The conidial suspensions were incubated

for 24 hr at temperatures of 5°, 10°, 15°, 20°, 25°, 30° and 35°C. Fluctuation of temperature was around ±1°C. Germination percentage were counted three times immediately after blending and incubation for 24 hr. Experiment was repeated thrice. Results were expressed as Germination Index (G.I.).

$$G.I.=\frac{\text{Germination percentage after 24 hr incubation}}{\text{Germination percentage before incubation}}$$

3. Effects of red and far-red lights

Conidial suspensions were kept in darkness for 2 hr before and after irradiation with R (660 nm) and FR (730 nm) lights. In the first trial, duration of irradiation were 5, 10, 15, 20, 25 and 30 min. In the second trial, conidial suspensions were irradiated at regular intervals of 2 min within the effective irradiation period. In the third trial, conidial suspensions were post-irradiated with the antagonistic spectral type, i.e. FR and R respectively. Germination percentages were counted thrice for each light-treatment after 24 hr incubation at 25°C and compared with the light and dark controls. Results were expressed as G.I.

Results and discussion

Conidia of C. longissima are hyaline, with 1-18 septa, cylindrical to obclavate, with obconically truncate base and tapering obtuse apex, smooth, deciduous. The length varies from 11.3 μm to 270.0 μm, with the base of 7.5 μm and the tip of 3.8 μm in width.

1. Mode of conidial germination

Germination of conidia were observed after 20 hr incubation in saturated atmosphere and at room temperature (21-24°C). Germ-tubes may arise from any cells, but the basal cell usually germinated first, followed by germination of the terminal cell. Occasionally the apical cell, or even the center cell, would germinate first. The germ-tubes, protruding from the conidia, usually extended to a certain length before branching, or occasionally they may branch at the germination point in bi-directions. Swelling of the conidium, one of the criteria of germination for other fungal spores, was not observed, even after germ-tube protrusion. No secondary conidial formation was observed in this species.

The sequence of conidial germination of C. longissima after 20 hr incubation at 2 hr intervals is shown in Plate 4-1.

2. Temperature

Seven different temperatures, i.e. 5°, 10°, 15°, 20°, 25°, 30° and 35°C were tried for conidial germination of C. longissima. Conidial germination occurred from 15-30°C with 25°C being the optimum temperature. Germination Indexes at these seven temperatures are shown in Table 4-1.

Table 4-1. Germination Indexes of Cercospora longissima at different temperatures.

Temp.	5°C	10°C	15°C	20°C	25°C	30°C	35°C
G.I.	1.00 ±0.09	1.23 ±0.19	1.88 ±0.21	2.32 ±0.70	3.89 ±0.06	2.86 ±0.32	1.00 ±0.05

3. Effects of red and far-red lights

In comparing the light control with the dark one, we can find that conidial germination of C. longissima is light-dependent. In the first trial, irradiation with R light for 30 min (R_{30}) was found to be the most effective in promoting the conidial germination and irradiation with FR light for 25 min (FR_{25}) was the most effective in inhibition of the conidial germination. Effects of R and FR lights at regular intervals of 5 min are presented in Table 4-2 (a).

In the second and third trials, further evidences were gained to support that both R_{30} and FR_{25} were the most effective irradiation periods in controlling conidial germination of C. longissima. Effects of R and FR lights at regular intervals of 1 min between the effective range are presented in Table 4-2 (b). Also, a reversible photoreaction controlling of conidial germination was worked out and shown in Table 4-2 (c). The effects of promotion and inhibition of conidial germination can be cancelled by the post-irradiation with the antagonistic spectral type, i.e. FR and R lights respectively at the most effective irradiation period. Only the light applied last, determined whether the conidia germinated or not. Therefore, phytochrome may be predicted to be presented in the conidia of C. longissima owing to the photoreversible effects of R and FR lights on conidial germination. However, due to the longer irradiation period required in comparison with those in higher plants which usually need only a few minutes to initiate the

responses, phytochrome in fungi may be very dilute or in some sort of differences with that in higher plants.

Table 4-2. Germination Indexes of Cercospora longissima at different irradiation periods in minutes.

(a) Periods of 5 min interval

Treatments	Light	R_5	R_{10}	R_{15}	R_{20}	R_{25}	R_{30}
G. I.	7.17 ±0.92	3.13 ±0.18	1.38 ±0.18	2.56 ±0.09	2.50 ±0.53	2.94 ±0.62	7.88 ±1.38
Treatments	Dark	FR_5	FR_{10}	FR_{15}	FR_{20}	FR_{25}	FR_{30}
G. I.	1.44 ±0.27	2.83 ±0.53	3.50 ±0.35	2.75 ±0.50	2.06 ±0.09	1.94 ±0.09	2.69 ±0.27

(b) Periods of 2 min interval

Treatments	Light	R_{25}	R_{27}	R_{29}	R_{30}	R_{31}	R_{33}	R_{35}
G. I.	3.51 ±0.16	3.34 ±0.24	3.68 ±0.08	3.17 ±0.33	3.97 ±0.32	4.02 ±0.24	3.57 ±0.40	1.36 ±0.28
Treatments	Dark	FR_{21}	FR_{23}	FR_{25}				
G. I.	1.70 ±0.32	3.51 ±0.48	2.38 ±0.04	2.10 ±0.08				

(c) Periods of alternate light qualities

Treatments	Light	R_{30}	$FR_{15}R_{30}$	$FR_{25}R_{30}$	$FR_{15}R_{15}$	$FR_{25}R_{15}$
G. I.	2.29 ±0.27	3.17 ±0.15	3.00 ±0.06	3.20 ±0.10	1.55 ±0.06	2.45 ±0.03
Treatments	**Dark**	FR_{25}	$R_{30}FR_{25}$	$R_{15}FR_{25}$	$R_{30}FR_{15}$	$R_{15}FR_{15}$
G. I.	1.97 ±0.22	1.90 ±0.12	1.50 ±0.25	2.35 ±0.26	2.03 ±0.24	2.09 ±0.14

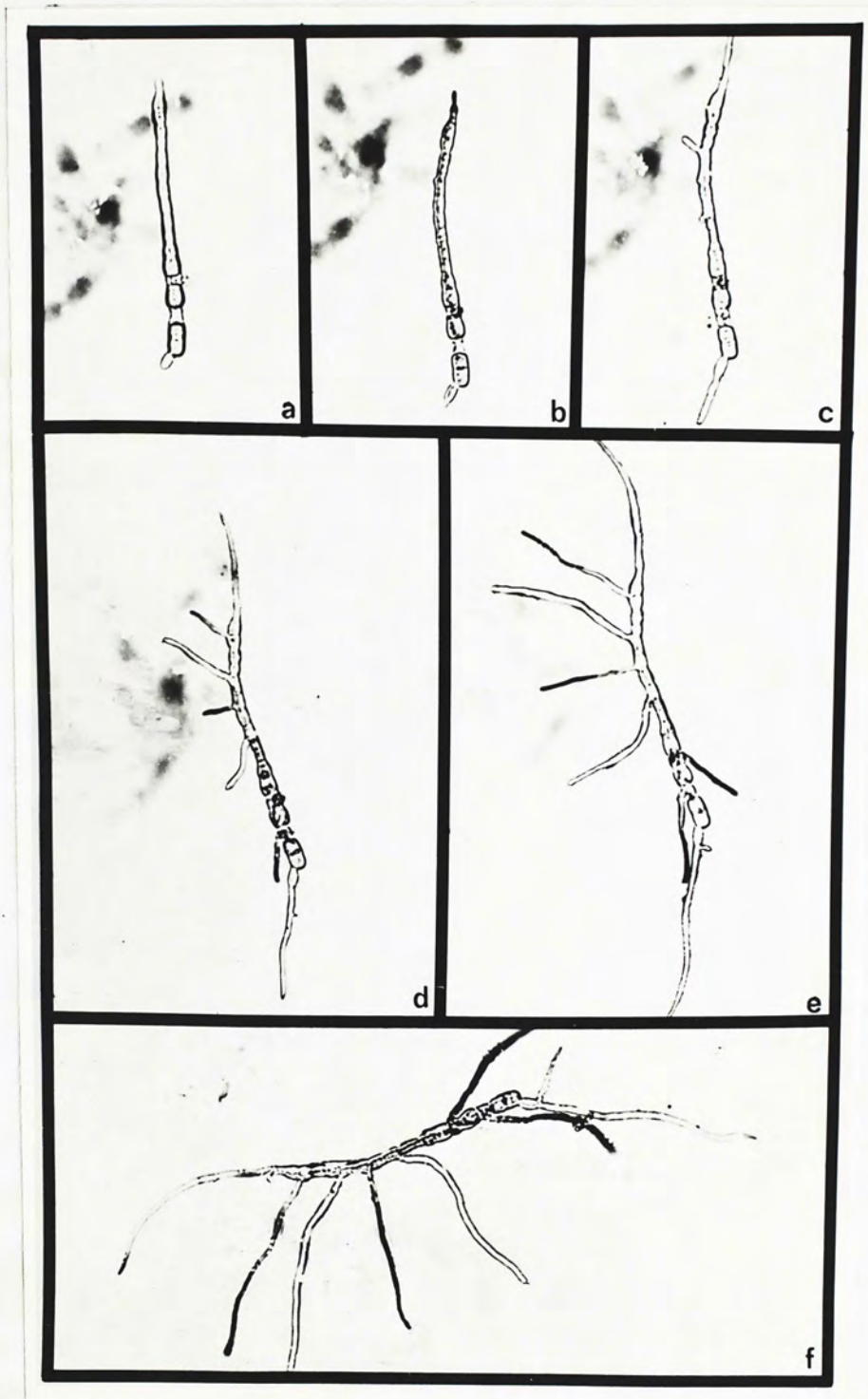


Plate 4-1. Sequence of conidial germination of Cercospora longissima from 20 hr to 30 hr, at 2 hr intervals, after incubation (a-f).

CHAPTER 5

SEED GERMINATION OF LETTUCE (*LACTUCA SATIVA* L.)

Introduction

The process of germination may be defined as that sequence of steps beginning with the uptake of water and leading to the rupture of the seed coat by the radicle or by the plumule. Cell divisions and enlargements in the embryo and an overall increase in metabolic activity accompany these steps. Although actual germination begins long before the rupture of the seed coat, germination is usually determined visibly by observing the protrusion of the radicle or plumule. The germination of seed may be blocked by absence of some external factors, i.e. water, proper temperature or proper mixture of gases. However, many seeds may be placed in an environment considered adequately for germination and still not germinate because of some internal factors. This may be caused by a hard seed coat impermeable to water or gases, or physically resistant to embryo expansion; an immature embryo; a need for after-ripening; presence of a substance inhibiting germination; specific light or temperature requirement (Crocker & Barton, 1957; Devlin, 1969).

Lettuce (*Lactuca sativa* L.) seeds are plumed achenes. Blockage of germination is usually due to the presence of germination inhibitors. In recent years, many workers have been working on the germination inhibitors and promoters of lettuce seed (Gawronski, 1970a-d; Hradilik, 1970; Stanislawski, Indeka

& Stanislawska, 1971; Bex, 1972; Chatterji, Harsh, Sankhla & Sankhla, 1972; Negm, Smith & Kumamoto, 1972 & 1973; Sankhla & Sankhla, 1972; Berrie & Robertson, 1973; Chowdhury & Chatterjee, 1973a & b; Khan, Tao & Roe, 1973; Sankhla, Harsh, Vyas, Bohra & Sankhla, 1973; Sharples, 1973; Speer, 1973; White, Hillman & Phillips, 1973).

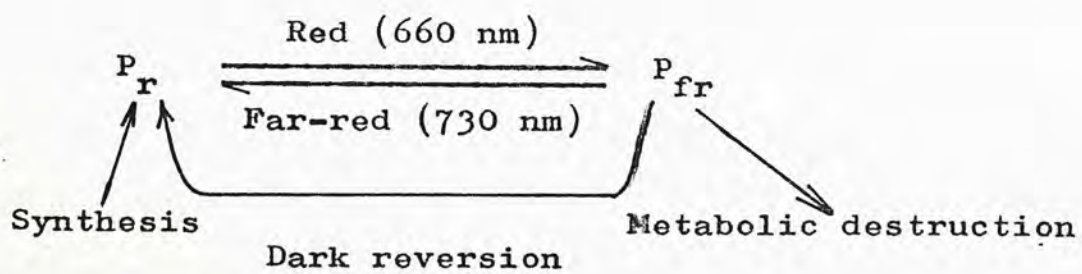
Lettuce seed will not germinate in soil at high temperature (25-30°C). Seeds held in darkness for several days at a temperature too high for germination become dormant and then will not germinate when transferred to a lower temperature that previously would have favored germination. The prolonged treatment at the high temperature changed the seeds from light insensitive to light requiring (Toole & Toole, 1961; Gutterman, Evenari & Heydecker, 1972).

Effect of light upon seed germination may be a general phenomenon controlling living processes. Caspary, in 1860, was the first botanist to observe the beneficial influence of light on germination. In general, lettuce seed is positive photoblastic.

In 1934, it was found at the United States Department of Agriculture Research Station in Beltsville, Maryland, that red light stimulated the germination of lettuce seed. Three years later, Flint and McAlister at Smithsonian Institute found that blue and far-red wavelengths were inhibitory (Flint & McAlister, 1937). In 1952, the stimulating effect of red light (R) on seed germination was found to be reversed by immediately

following the R treatment with far-red light (FR). By repeatedly alternating R and FR treatments, the Borthwick group found that the light applied last determined whether the seeds germinated or not, R promoting and FR inhibiting the promotion of R (Borthwick, Hendricks, Parker, Toole & Toole, 1952). It was concluded that the phytochrome pigment system (P) being involved in the seed germination of lettuce.

Nearly all of the phytochrome in dark grown plants or germinating seeds is present as the red-absorbing form (P_r). Exposure of the plants tc light causes a large but incomplete conversion to the far-red absorbing form (P_{fr}) and accompanies by a destruction of some of the P_{fr} produced. This destruction goes on in both light and darkness. In some plants, there is also a slow decay of P_{fr} back to P_r even in darkness. The P_r of phytochrome appears to be first synthesized by developing seedlings. This synthesis seems to be inhibited by light, at least in corn seedlings (Salisbury & Ross, 1969; Clayton, 1971; Mitrakos & Shropshire, 1972). The transformation of phytochrome may be summerized as follows:



Dark reversion of phytochrome occurs only when the phytochrome system is sufficiently hydrated (Hsiao & Vidaver, 1971a

& b, and 1973) and it is independent of temperature (Vidaver & Hsiao, 1972).

Gamma rays also have an effect on lettuce seed germination (Miller, Fishere, Lapa & Brezhge, 1972). Stimulation of germination is observed at a dose of 100 R γ-rays with or without R illumination. The inhibiting effect of a dose of 600 R and higher is somewhat mediated by illumination of seeds with R (Miller & Klinte, 1972). By later work, Hsiao et al. also found that sublethal doses of γ-irradiation and FR light have somewhat analogous and R reversible effects on the germination of lettuce seed. However, the mechanism by which γ-irradiation retards germination appears to differ from that of FR light. Compared to the controls, γ-irradiation retarded germination for the first 24 hr, but after 36 or 48 hr of imbibition, germination of treated seeds was higher than that of the controls, whether or not the γ-irradiated seed received R or FR lights. The effects of γ-irradiation are more pronounced in seed containing 15% of water at the time of treatment than in those containing only 7% of water (Hsiao & Vidaver, 1974).

The embryo excised from seed of Grand Rapids lettuce can be cultured in distilled water. Complete digestion of the endosperm and transfer of nutrients from the endosperm to the embryo occur in the germinating seed with fat as the source of food. The fat is utilized for respiration, synthesis of amino acids and to a degree, converted to sucrose. Much of this carbohydrate fraction is incorporated into which is believed

to be cell wall polysaccharides (Park & Chan, 1974).

The following experiment was carried out to study the light effect on seed germination of different lettuce cultivars.

Materials and methods

Seed germination percentages of 21 lettuce cultivars at different light treatments were studied. Most of the exotic cultivars were obtained from the W. Atlee Burpee Co.

1. Light and dark effects

Each lot of 100 lettuce seeds of different cultivars was placed on wetted blotters in petri dish. Two sets of each cultivar were prepared. One set was kept in continuous white light of 30 watt and the other set was kept in darkness at 20°C. After 48 hr of imbibition, the ones that germinated were counted.

2. Effects of red and far-red lights

Each lot of 50 lettuce seeds of different cultivars was placed on wetted blotters in dish. Four sets of each cultivar were prepared. One set was kept in continuous illumination and the other three sets were covered immediately and kept in darkness at 20°C. After 4 hr of imbibition, one of these three sets was irradiated with 1 min of R light, the second was irradiated with 4 min of FR light, and the remained one was treated as the dark control. After irradiation, all seeds were still held in darkness at 20°C. Fourty-eight hours after imbibition, the ones that germinated were counted.

Results and discussion

1. Light and dark effects

The seed germination percentage of different lettuce cultivars under light and dark conditions are shown in Table 5-1.

Table 5-1. Seed germination percentages of 21 lettuce cultivars under light and dark conditions.

Loose Leaf Lettuce Cultivars	Light (%)	Dark (%)	Head Lettuce Cultivars	Light (%)	Dark (%)
A. Black-seeded			A. Black-seeded		
Black-seeded Simpson	98	96	Burpee Bibb	94	88
Burpee Greenhart	0	0	Buttercrunch	94	94
Grand Rapids	96	98	Deer Tongue	88	96
Green Ice	98	92	Fordhook	98	98
Salad Bowl	98	94	Imperial No. 847	0	0
			Tom Thumb	96	90
B. White-seeded			B. White-seeded		
Early Prize Head	100	100	Burpee's Iceberg	88	94
Hong Kong Local	98	97	Dark Green Boston	90	84
Oak Leaf	90	96	Great Lakes	92	92
Ruby	100	94	Imperial No. 44	98	98
			Premier Great Lakes	94	90
			White Boston	96	94

From the results, there were no significant differences in germination percentages between the light and dark treatments for all cultivars tested. It may be due to that most seeds collected were from new harvest. They were active in germination and did not show any light-dependence. Yet, for the cultivars of Imperial No. 847 and Burpee Greenhart, the germination percentages in both light and dark conditions were zero. Seeds of these cultivars has been stored at least for 3-4 years and this may be the main reason for the zero germination percentages.

2. Effects of red and far-red lights

The R and FR lights effects on seed germination of different lettuce cultivars are presented in Table 5-2.

From the results, the light requirement in lettuce seed germination was so diverse. The process of seed germination was light-independent in some cultivars, but it was light-dependent in others. Since this part of experiment was carried out in 3 months after the first trial, the seed germination of some cultivars had been changed into light-dependent, e.g. Great Lakes, Premier Great Lakes and Salad Bowl. Therefore, storage time may affect the light requirements of seed germination of some lettuce cultivars.

In the same way, R and FR lights also had some effects on lettuce seed germination, e.g. Burpee Bibb, Dark Green Boston, Fordhook, Salad Bowl and White Boston, but they had no effect on other cultivars. Therefore, the same dose of the same

light can lead to responses which differ, according to the species or to the strain. These variations can be explained at the molecular level by different processes (Mitrakos & Shropshire, 1972).

(1) The phytochrome content differs.

(2) The P_{fr} requirement is a function of the thickness of the seed coat.

(3) The rate of supply of an unknown partner for P_{fr} is a limiting factor.

(4) The seeds of some strains have the capacity for P_{fr} formation in the dark.

(5) The light requirement can have a genetic origin.

For the 21 lettuce cultivars tested, only the cultivar of Salad Bowl showed a good example of phytochrome-mediated reaction in seed germination. After a period of storage, seed germination percentages of Salad Bowl under both light and dark conditions were dropped. The process had been changed to be light requirement. Red light also showed a stimulating effect on seed germination, whereas, FR had an inhibitory effect in comparison with the dark control.

However, R light can not break the dormancy of Imperial No. 847 and Burpee Greenhart. It may be due to one or all of the above reasons.

Table 5-2. Seed germination percentages of 21 lettuce cultivars at different light treatments.

Cultivars	Light (%)	Dark (%)	R (%)	FR (%)
Loose Leaf Lettuce				
A. Black-seeded				
Black-seeded Simpson	98	98	98	98
Burpee Greenhart	0	0	0	0
Grand Rapids	96	92	98	80
Green Ice	96	98	94	94
Salad Bowl	78	40	60	32
B. White-seeded				
Early Prize Head	94	100	92	100
Hong Kong Local	98	98	94	94
Oak Leaf	92	92	84	84
Ruby	100	98	94	92
Head Lettuce				
A. Black-seeded				
Burpee Bibb	94	90	90	76
Buttercrunch	98	86	96	94
Deer Tongue	90	88	94	92
Fordhook	94	98	98	46
Imperial No. 847	0	0	0	0
Tom Thumb	82	84	88	90
B. White-seeded				
Burpee's Iceberg	90	92	92	94
Dark Green Boston	86	80	94	18
Great Lakes	92	60	82	88
Imperial No. 44	98	98	84	98
Premier Great Lakes	78	92	88	86
White Boston	88	90	82	62

CHAPTER 6

CULTIVARS OF LETTUCE (*LACTUCA SATIVA* L.)

Introduction

Lettuce (*Lactuca sativa* L.) has been developed into many cultivars. Between 1966 and 1968, 300 named "seed stocks" were currently listed in seed suppliers' catalogues in the United Kingdom. In addition, more than 300 other named "seed stocks" mainly from the continental Europe and U.S.A. were scored.

Twenty-one cultivars of lettuce were collected. They were mostly obtained from the W. Atlee Burpee Co. in the United States.

The weight of seeds, the growth and morphology, and the stomatal distribution on the upper and lower epidermis of leaves of different lettuce cultivars were studied.

Materials and methods

Lettuce cultivars collected:-

Loose Leaf Lettuce

Black-seeded	White-seeded
Black-seeded Simpson	Early Prize Head
Burpee Greenhart	Hong Kong Local
Grand Rapids	Oak Leaf
Green Ice	Ruby
Salad Bowl	

Head Lettuce

Black-seeded	White-seeded
Burpee Bibb	Burpee's Iceberg
Buttercrunch	Dark Green Boston
Deer Tongue	Great Lakes
Fordhook	Imperial No. 44
Imperial No. 847	Premier Great Lakes
Tom Thumb	White Boston

1. Weight of lettuce seeds

Seeds of different lettuce cultivars were weighed. Each lot had 50 seeds. Four lots were used for each cultivars. The results are expressed as mg/50 seeds and % of Hong Kong Local cultivar.

2. Growth and morphology

Lettuce seeds, placed on wetted blotters in dishes, were kept in light at 20°C for germination. After 5 days, young seedlings were transferred to sterilizied vermiculite in pots. "Bio" solution was added to the seedlings. Young plants were then transplanted from pots to field four weeks after germination. Fertilizers, mainly urea, were applied half-monthly.

Morphology of different lettuce cultivars as well as the growth pattern in field in autumn are shown and described. The Hong Kong Local cultivar usually requires 90 days for maturity. Flowering branches usually appear in plants of 3 months old. Phyllotaxis of this cultivar under room conditions was studied.

3. Stomatal distribution

Strips of upper and lower epidermis were usually torn off quite easily from the freshly harvested leaves of different lettuce cultivars. These strips were floated on a drop of distilled water and observed under calibrated eye-piece micrometer. Twelve counts of the number of stomata in unit area at random for each cultivar were made. The results are presented as number of stomata per cm^2.

Results and discussion

1. Weight of lettuce seeds

Weight of lettuce seeds of different cultivars are outlined in Table 6-1. From the results, White Boston had the heaviest seed-weight and Green Ice was the lightest. In general, black-seeded lettuce had heavier seed-weight than the white-seeded ones. The black-seeded lettuce of 11 cultivars had an average of 54.65 mg/50 seeds, whereas, the 10 white-seeded cultivars had the average of 53.95 mg/50 seeds.

2. Growth and morphology

Morphology of different lettuce cultivars (Burpee, 1974):-

(1) Loose Leaf Lettuce: non-heading, leaves not folding or overlapping.

A. Black-seeded (Plate 6-1)

1. Black-seeded Simpson (A)

Matured in 45 days. Grown extensively for early spring use. Crisp and supposed of exceptionally

good flavor and very appetizing. With broad, light green frilled and crumpled outer leaves. Heart leaves blanching almost white.

2. Green Ice (B)

Matured in 45 days. Grown best in cool weather. Used for salads, sandwiches and garnishes. Rich in vitamins A, B and C. Savory, crisp textured, dark glossy green leaves with blisters, and leaf margins wavy and fringed.

3. Salad Bowl (E)

Matured in 45 days. Large rosettes of deeply lobed medium green leaves. Slow bolting.

4. Grand Rapids (F)

Matured in 45 days. Plant large but loose. Leaves colored light green and wrinkled.

5. Burpee Greenhart (G)

Matured in 45 days. Good for winter greenhouse forcing. Tender, crispy leaves suitable for marketing and salad mixture pack. Plant smaller. Leaves light green in colour and margins finely frilled and deeply cut.

B. White-seeded (Plate 6-2)

1. Oak Leaf (C)

Matured in 40 days. Grown better in summer than in winter. Tender and keeping its excellent quality late in summer after other cultivars

turning bitter. Mostly heat resistant. Form a tight rosette of medium green leaves, deeply lobed.

2. Ruby (I)

Matured in 50 days. Sweet and succulent. Crisp, bright, green and frilled leaves with intense red shading.

3. Early Prize Head (J)

Matured in 45 days. A tasty, quick-growing and productive lettuce. Excellent flavor. Medium large, tender loose heads of bright green, curled and crimped leaves shaded with brownish red.

4. Hong Kong Local (K)

Matured in 90 days. Medium large, loose heads of crisp and bright green leaves.

(2) Head Lettuce: head forming, when sown at the right time. Head round.

A. Black-seeded (Plate 6-3)

1. Imperial No. 847 (H)

Matured in 85 days. Head medium large, extremely compact. Leaves dark green and leathery.

2. Burpee Bibb (Q)

Matured in 65 days. Slow bolting and less subject to tip burn than other cultivars. Of outstanding quality and far superior to regular Bibb. Grown by many commercial growers for extra early forcing

under glass. Recommended for spring or fall use. Small, loose folded leaves dark green. Heart leaves usually blanching to golden yellow.

3. Buttercrunch (R)

Matured in 75 days. More heat resistant than Bibb. Small leaves forming tight rosettes. Head usually larger, spread more, very compact and heavy. Inner leaves blanching to bright yellow.

4. Deer Tongue (S)

Matured in 80 days. Heat resistant. Leaves oblong to triangular with round tip. Slightly savory. Head usually compact and upright.

5. Fordhook (T)

Matured in 75 days. Upright growing. Its outstanding flavor and quality superior to other cultivars. Loosely folded, crisp thick leaves usually flashy and heavily crinkled. Leaves deep green in color.

6. Tom Thumb (U)

Matured in 65 days. Good for indoor or window box use. Head small and compact.

B. White-seeded (Plate 6-4)

1. Great Lakes (D)

Matured in 90 days. Grown well under many adverse conditions. Heavy, large, and well-folded heads of extra good quality. Leaves large, dark green

and fringed.

2. Imperial No. 44 (L)

Matured in 85 days. With small rosette of light green leaves. Heads usually medium large, well-formed, slightly flattened, extremely compact, dark green and heavily savory.

3. Dark Green Boston (M)

Matured in 80 days. Delicate flavor. Tightly folded heads with smooth, tender and dark green leaves.

4. Burpee's Iceberg (N)

Matured in 85 days. Standing hot weather. Similar to the loose leaf type. Leaves with light red shading. Heads usually compact, medium size, with tender hearts blanched to an attractive silvery white. Leaves light green, wavy, fringed and heavily savory.

5. Premier Great Lakes (O)

Matured in 80 days. An early maturing cultivar, plants resistant of tip burn and heat. Leaves larger, leathery, dark green, fringed at the margin. Heads usually compact, large, uniform, solid and practically round.

6. White Boston (P)

Matured in 80 days. High quality and fine texture. With small rosette of yellowish green,

smooth leaves. Heads usually tightly folded with solid hearts that blanched to an attractive bright creamy yellow.

However, due to the comparatively warm and dry winter in Hong Kong which is not suitable for head formation and the soil conditions here being rather poor, most cultivars of head lettuce performed differently and similar to those of the loose leaf type.

Three months' plants of different lettuce cultivars were measured both by height and width in cm. Results are shown in Table 6-2.

In general, the mature plant of Hong Kong Local cultivar had 29 pieces of leaves. Phyllotaxis under room conditions is presented in Table 6-3.

3. Stomatal distribution

Number of stomata per cm^2 on both upper and lower epidermis of different lettuce cultivars are shown in Table 6-4.

Stomatal distribution on both upper and lower epidermis of different lettuce cultivars were quite diverse. The cultivar of Buttercrunch had the most stomata per unit area in comparison with other cultivars. Whereas Ruby had the least stomata on the upper epidermis. In general, leaves have more stomata on the lower epidermis than on the upper one. However, Deer Tongue and Salad Bowl had more stomata on the upper epidermis than those on the lower one.

Table 6-1. Weight of lettuce seeds

Loose Leaf Lettuce

Cultivars	mg/50 seeds	% of Hong Kong Local cultivar
	Average	
A. Black-seeded	52.53±4.21	
Black-seeded Simpson	57.75±2.85	111.59
Grand Rapids	54.70±2.82	105.70
Burpee Greenhart	52.78±0.94	102.03
Salad Bowl	50.90±1.41	98.36
Green Ice	46.50±1.56	89.85
B. White-seeded	54.16±4.12	
Early Prize Head	59.78±0.68	115.55
Ruby	54.57±1.10	105.41
Hong Kong Local	51.73±1.01	100.00
Oak Leaf	50.53±1.72	97.68

Head Lettuce

A. Black-seeded	56.42±3.47	
Tom Thumb	61.35±0.49	118.55
Fordhook	58.30±1.13	112.66
Deer Tongue	58.20±0.28	112.46
Buttercrunch	54.70±1.27	105.70
Imperial No. 847	54.10±0.35	104.54
Burpee Bibb	51.85±1.91	100.19
B. White-seeded	53.81±4.66	
White Boston	62.25±2.47	120.29
Burpee's Iceberg	54.53±1.70	105.41
Imperial No. 44	54.35±0.07	105.02
Great Lakes	53.85±1.05	104.10
Premier Great Lakes	52.15±0.23	100.77
Dark Green Boston	49.05±1.20	94.78

Table 6-2. Growth of different lettuce cultivars.

Loose Leaf Lettuce

Cultivars	Width (cm)	Height (cm)	Ratio (W/H)
A. Black-seeded			
Grand Rapids	27.10±2.23	9.03±0.55	3.01
Burpee Greenhart	15.17±4.79	6.50±1.29	2.34
Black-seeded Simpson	24.43±1.41	10.95±0.97	2.23
Salad Bowl	14.65±2.25	10.00±0.75	1.46
Green Ice	15.00±3.61	11.60±3.37	1.29
B. White-seeded			
Early Prize Head	24.47±6.22	7.40±1.40	3.31
Oak Leaf	18.07±3.52	7.23±2.66	2.50
Ruby	22.25±1.77	9.40±0.85	2.36
Hong Kong Local	20.50±0.71	10.00±0.50	2.05

Head Lettuce

A. Black-seeded			
Burpee Bibb	21.50±6.42	6.28±2.02	3.41
Imperial No. 847	22.00±4.16	8.00±2.00	2.75
Tom Thumb	13.12±1.21	5.95±1.49	2.18
Fordhook	19.10±4.63	10.60±4.93	1.80
Deer Tongue	19.90±4.24	11.73±4.39	1.69
Buttercrunch	18.63±5.38	11.83±7.95	1.58
B. White-seeded			
Dark Green Boston	18.53±5.92	6.33±2.49	2.94
Premier Great Lakes	28.08±5.66	10.40±1.82	2.70
White Boston	18.90±3.01	7.32±1.71	2.59
Imperial No. 44	15.07±1.50	5.93±1.01	2.56
Burpee's Iceberg	14.75±3.18	6.75±0.35	2.18
Great Lakes	24.77±1.63	11.57±1.59	2.14

Table 6-3. Phyllotaxis of Hong Kong Local cultivar under room conditions.

Leaf order	Initiation day	Leaf order	Initiation day
1 st	8	16 th	65
2 nd	12	17 th	68
3 rd	16	18 th	70
4 th	25	19 th	72
5 th	30	20 th	75
6 th	35	21 st	76
7 th	38	22 nd	77
8 th	43	23 rd	78
9 th	46	24 th	79
10 th	48	25 th	81
11 th	50	26 th	83
12 th	53	27 th	84
13 th	56	28 th	87
14 th	60	29 th	90
15 th	63	Flowering branch	

Table 6-4. Stomatal distribution (Stoma No. / cm^2) on upper and lower epidermis of different lettuce cultivars.

Cultivars	Upper	Lower	Ratio (L/U)
Ruby	2,440± 170	5,510± 370	2.3
Imperial No. 44	4,970± 650	8,710± 780	1.8
Dark Green Boston	3,020± 930	5,090±1,020	1.7
Burpee Bibb	9,180±1,280	15,390± 640	1.7
Imperial No. 847	4,770± 560	7,500± 820	1.6
Early Prize Head	5,580± 300	8,450± 950	1.5
Black-seeded Simpson	4,840± 700	7,060± 570	1.5
Buttercrunch	11,500± 700	16,250±1,020	1.4
White Boston	3,040± 540	4,180± 640	1.4
Tom Thumb	2,640± 920	3,490± 800	1.3
Premier Great Lakes	5,430± 820	6,000± 510	1.1
Green Ice	7,240± 580	7,700± 880	1.1
Grand Rapids	3,800± 630	4,020± 540	1.1
Fordhook	5,170± 640	5,470± 780	1.1
Burpee's Iceberg	8,490± 720	8,900± 670	1.1
Great Lakes	5,880± 636	6,130± 880	1.0
Hong Kong Local	10,260±1,300	10,440±1,220	1.0
Oak Leaf	4,460± 500	4,430± 540	1.0
Deer Tongue	5,060± 560	3,950± 640	0.8
Salad Bowl	8,980±1,020	6,380± 760	0.7

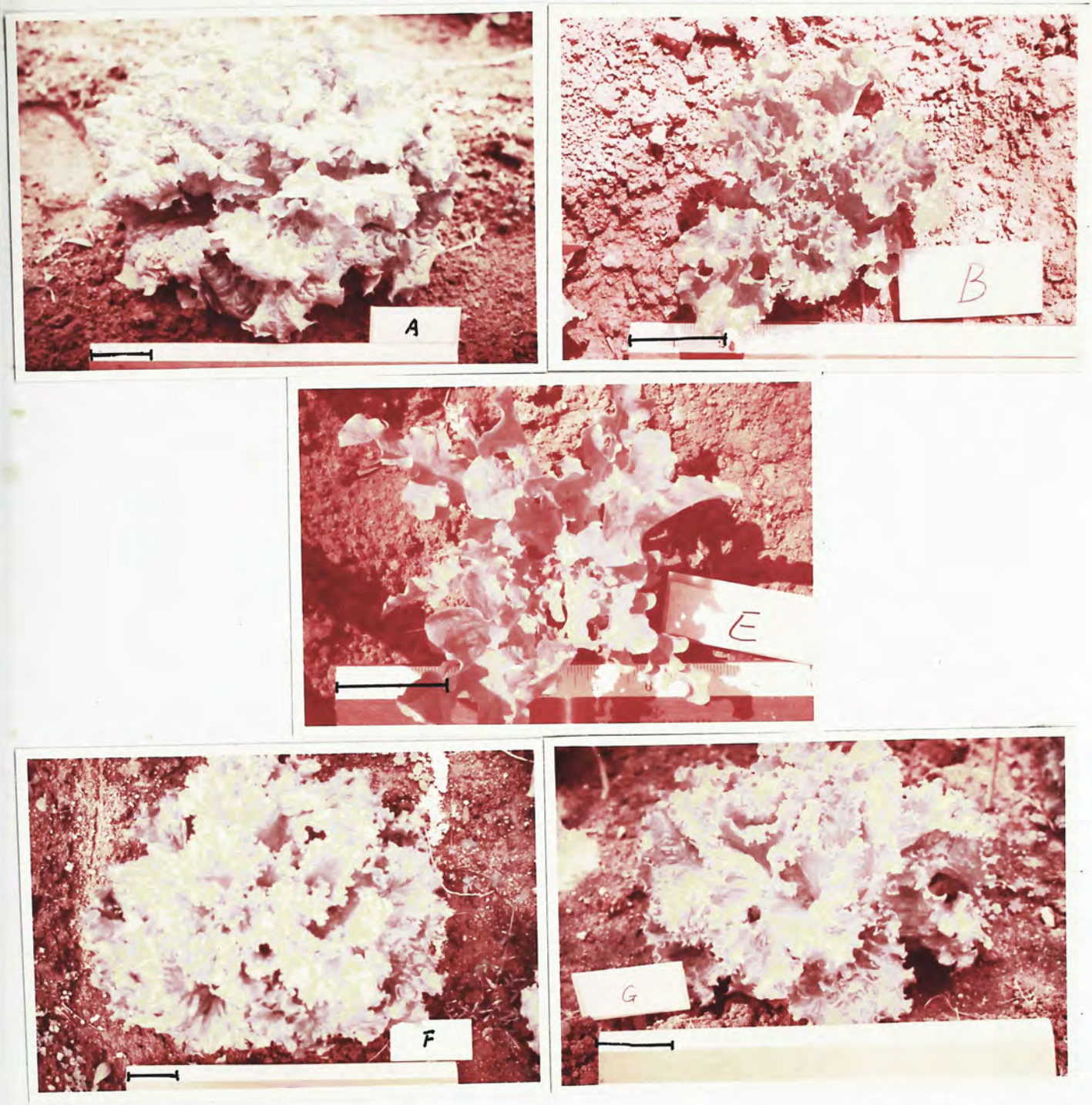


Plate 6-1. Morphology of black-seeded cultivars of loose leaf lettuce in autumn field. A. Black-seeded Simpson; B. Green Ice; E. Salad Bowl; F. Grand Rapids; G. Burpee Greenhart. Scale on the lower left hand corner is 5 cm.

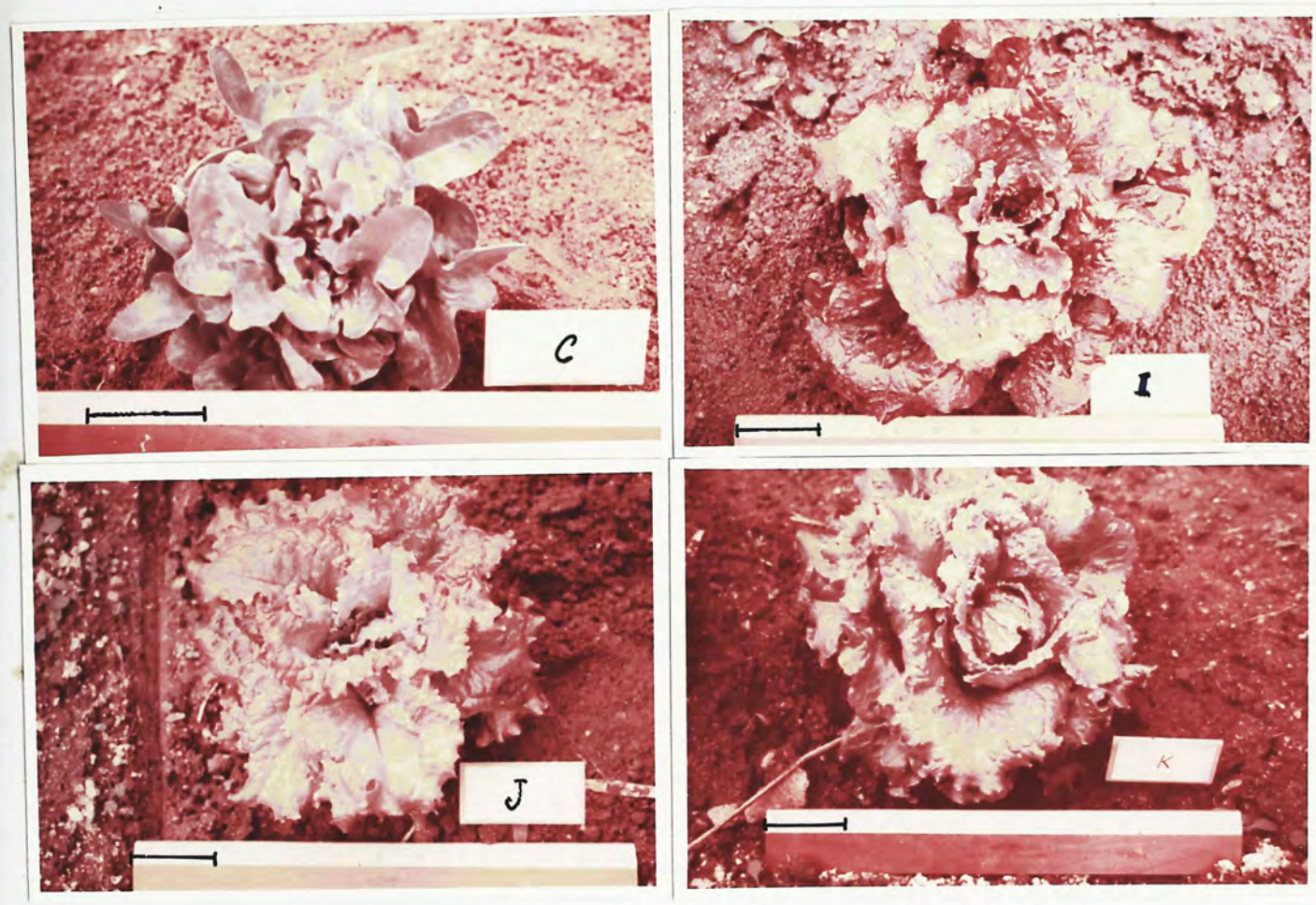


Plate 6-2. Morphology of white-seeded cultivars of loose leaf lettuce in autumn field. C. Oak Leaf; I. Ruby; J. Early Prize Head; K. Hong Kong Local. Scale on the lower left hand corner is 5 cm.

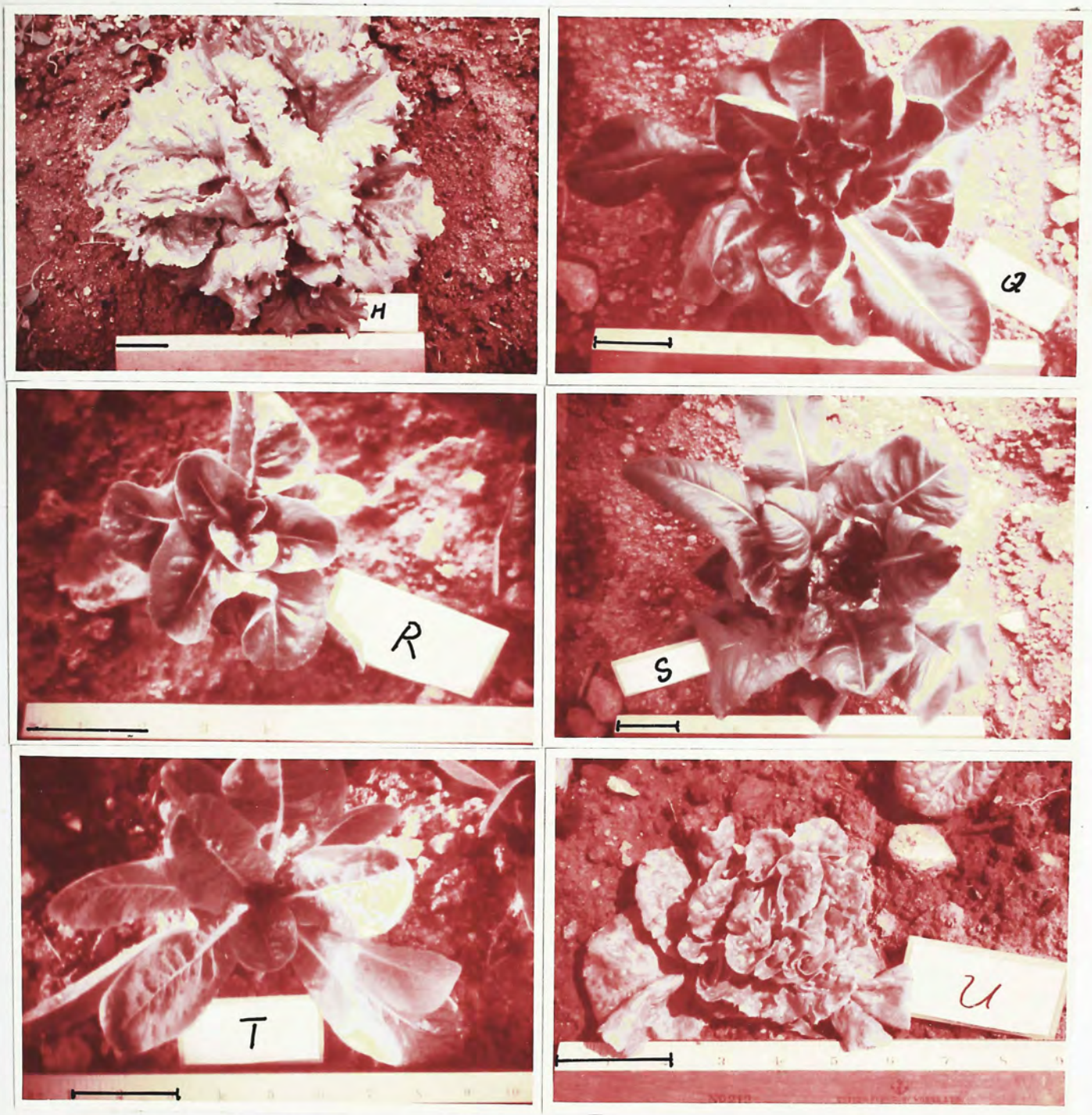


Plate 6-3. Morphology of black-seeded cultivars of head lettuce in autumn field. H. Imperial No. 847; Q. Burpee Bibb; R. Buttercrunch; S. Deer Tongue; T. Fordhook; U. Tom Thumb. Scale on the lower left hand corner is 5 cm.

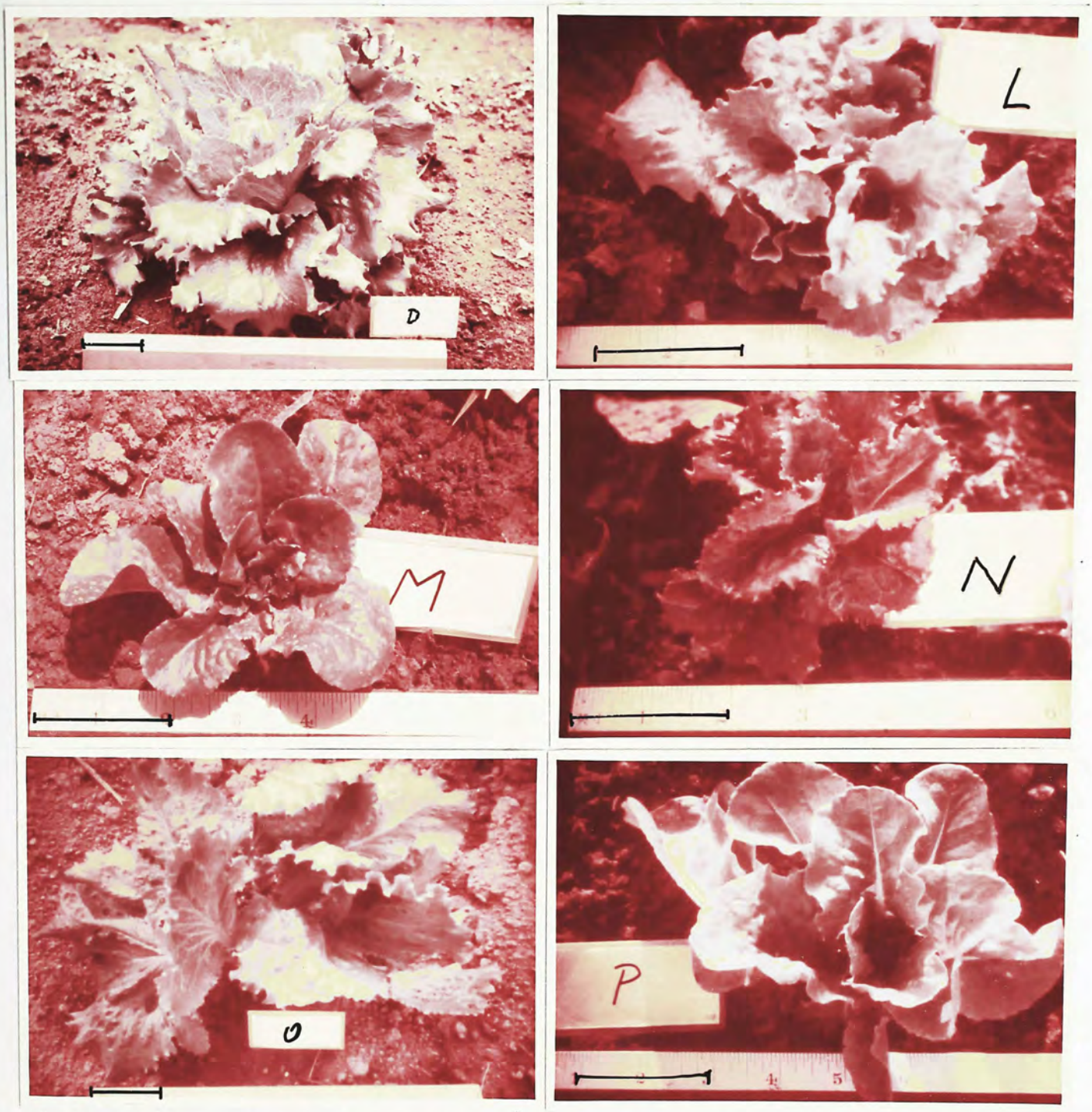


Plate 6-4. Morphology of white-seeded cultivars of head lettuce in autumn field. D. Great Lakes; L. Imperial No. 44; M. Dark Green Boston; N. Burpee's Iceberg; O. Premier Great Lakes; P. White Boston. Scale on the lower left hand corner is 5 cm.

CHAPTER 7

STOMATAL MOVEMENT WITH REFERENCE TO TREATMENTS OF RED AND FAR-RED LIGHTS

Introduction

The stomatal apparatus controls not only transpirational water loss but also the exchange of gases between the interior and exterior of leaves. Stomatal opening and closing are the consequence of turgor changes in the guard cells that are influenced by a number of environmental factors including CO_2 and O_2 tensions, water supply, wind velocity, atmospheric pressure, humidity, temperature and light. Of these controlling factors, the influence of light is perhaps the least understood (Devlin, 1969).

Intensity, quality and periodicity of light influence stomatal opening. Viewed critically, the evidence shows that the photosynthetic production of osmotically active substances is but a small part of a very complex mechanism. It is necessary to take into account that other effects of radiation which may actuate other processes apart from photosynthesis. Several hypotheses have been proposed to explain the mechanism of stomatal opening (Meidner & Mansfield, 1965). They are:-

(1) Reduction of the CO_2 concentration in the leaf tissue.

(2) Mechanisms independent of changes in the CO_2 concentration.

(3) Photosynthetic production of osmotic substances.

(4) Photosynthetic production of glycollic acid.

(5) Changes in the starch-sugar equilibrium and pH effects.

It becomes clear that the evidence relating to the photosynthetic production hypotheses does not indicate that substances synthesized in light, whether carbohydrates, glycollic acid or adenosine triphosphate, play a major role in stomatal mechanisms. An abundance of evidence shows that the most important effect of illumination is to produce a change in the CO_2 concentration in the leaf. Effects of illumination on the sugar-starch equilibrium, leaf temperature, leaf water content and possibly permeability also contribute to the mechanism of guard cell movements.

As well as being affected by the environment, stomatal movements are under the control of endogenous rhythms in light and darkness. Rhythms can produce opening in the darkness and partial closure in light, and hence can modify or overrule the response to external factors. However, the phase of rhythms does come under the control of the environment through a low intensity light reaction. Mansfield first demonstrated a low intensity light effect which caused a phase shift in the rhythm of stomatal opening ability in 1964. The most effective wavelength for induction of such phase shifts was found to be 730 nm (i.e. far-red light), while comparable effects with red light could be obtained only by continuous low intensity

or intermittent high intensity interruption of the dark period. This light reaction is quite distinct from those directly concerned in the productions of stomatal opening. He considered the involvement of phytochrome in the low intensity phase shift phenomenon, but he rejected this hypothesis because of the absence of a reversal of red effects by far-red and the requirement for frequent interruptions of the dark period for a phase shift response to be elicited.

However, from the work of Habermann, the results of her experiments demonstrated a R-FR antagonism in the light dependent opening of both xantha mutant and wild type sunflowers. Stomates of both xantha mutant and wild type sunflowers remained closed in R light. However, both experimental materials responded identically to FR light with a pattern characteristic of low intensity stomatal opening. Long exposure of R preceding FR inhibits opening. Long exposure of FR preceding R promotes opening. These results strongly suggest the involvement of phytochrome and also imply that there is a single mechanism for turgor movement in plants (Habermann, 1972).

The following experiment was to study the light effect on stomatal movement of lettuce (Lactuca sativa L.).

Materials and methods

Lettuce leaves of Hong Kong Local cultivar, were harvested late in the afternoon before next day treatment. They were transferred with petioles in water to dark room at 20°C.

In the next morning, leaf disks of 1.5 cm in diameter were cut with a cork borer. Safe light of green lamp was placed at least 1 meter away from the working surface. Disks were floated on water of 0.5 cm deep and transferred to a light-tight box immediately after cutting.

Samples exposed to various light treatments, i.e. red (R), far-red (FR), R-FR, FR-R, artificial white light (L), as well as dark control (D) were kept at 20°C. The illumination duration were 30 min for R and L, and 25 min for FR.

After illumination, leaf disks were fixed in formalin-propionic acid-50% alcohol (5:5:90, by volume) for one day and then transferred to a freshly prepared clearing fluid composed of 85% lactic acid, chloral hydrate, phenol, clove oil and xylene (2:2:2:2:1, by weight). After one day, leaf disks were transferred with some of the fluid to a slide, covered with cover glass which was supported laterally by two permanently affixed covers and examined with phase-contrast microscope (Herr, 1971). Stomatal aperture was measured under oil immersion with a calibrated ocular micrometer. For estimates of percentages of open stomata at different light treatments, open was defined as having aperture of 2 μm or more and closed as having an aperture of less than 2 μm. Two hundred counts of each light treatment were measured.

Results and discussion

It has been already known that stomatal opening is a light dependent phenomenon. The average percentages of open stomata at different light treatments are presented in Table 7-1.

Table 7-1. Percentages of open stomata at different light treatments.

Treatments	Light	Dark	R_{30}	$FR_{25}R_{30}$	FR_{25}	$R_{30}FR_{25}$
%	55.33 ±7.02	22.00 ±0.00	44.67 ±5.03	44.67 ±8.08	29.33 ±2.31	30.00 ±2.00

From the result, one can speculate on that R light had the stimulating effect on stomatal opening of lettuce, whereas, FR light had the inhibitory effect. A photoreversible effect of R and FR lights had also been shown. The effect of different wavelengths on stomatal opening was only determined by the wavelength applied last. The effects of promotion and inhibition of stomatal opening can be cancelled by the post-irradiation with the antagonistic spectral type, i.e. FR and R lights respectively. Therefore, stomatal opening could be a phytochrome-mediated response.

CHAPTER 8

DEVELOPMENT OF *CERCOSPORA* LEAF-SPOT DISEASE ON LEAVES OF LETTUCE (*LACTUCA SATIVA* L.)

Introduction

The leaf-spot disease of lettuce (*Lactuca sativa* L.), caused by *Cercospora longissima* (Cug.) Sacc., is not a very serious field-disease in Hong Kong. However, diseased plants can be found incidentally in market through the whole year.

Spots, at first, are minute, brown, surrounded by an area of chlorosis. The lesions gradually enlarge into angular to irregular spots and turn to brown or tan color of various shades. Host tissues surrounding lesions are slightly yellow, becoming more obvious in aging. Lesions often become confluent. A fully developed lesion is slightly sunken and water-soaked. Extensive areas of the leaf are killed when spotting is heavy (Plate 8-1a). The disease progresses from older leaves to the younger ones.

Cercospora leaf-spot disease of lettuce has first been recorded by Toro in 1929 in Colombia of South America. The organism found associated with it agrees with *C. lactucae* Stevenson described from the same kind of material in Puerto Rico. Welles also described another *Cercospora* on lettuce and named it *C. lactucae* Welles. Both descriptions agree with that of *C. longispora* Cug. as given by Traverso. *C. longispora* was previously given and Saccardo changed the name to

C. longissima (Cug.) Sacc. (Toro, 1929).

C. longissima being the causal organism of Cercospora leaf-spot disease of lettuce was confirmed by the following methods of Kochs postulates (Riker & Riker, 1936).

(1) The microorganism must be associated in every case with the disease, and conversely the disease must not appear without the microorganism being or having been present.

(2) The microorganism must be isolated in pure culture and its specific characters studied.

(3) When the host is inoculated under favorable conditions the characteristic symptoms of the disease must develop.

(4) The microorganism must be reisolated and identified as that first isolated.

The purpose of this investigation was to obtain information on the nature of host-pathogen relationships of C. longissima and Lactuca sativa.

Materials and methods

Isolate was obtained from infected lettuce leaves by the Agriculture & Fisheries Department of Hong Kong and local markets. Small pieces of diseased host tissue were cut from the advanced margin of the lesion. The infected tissue was washed in running water to remove all debris. Surface sterilization of the tissue was done with 0.2% mercuric chloride for

5 min. It was washed thoroughly in sterile distilled water for 3 times and then placed in PDA for 72 hr. Fascicles of conidiophores usually grew out from the tissue and formed conidia which were picked off with a sterile needle and transferred to agar media. Hyphal tip transfers from the periphery of young colonies were made to CVA and incubated for 10 days at 25°C. The cultures were then stored at 4°C. Subsequent subculture transferring from these stock cultures was made every 6 months.

Sufficient quantities of conidia were usually obtained by the following method. A 7-10 days old culture of the fungus was macerated in a blender for 2 min with 10 ml of sterile water. The conidial suspension was floated on flesh plates of CVA medium and incubated at 25°C.

Seeds of lettuce were sown in vermiculite and watered with 'Bio' solution. When the seedlings were about 4 weeks old, they were transplanted to soil in 10 cm pots. Plants were grown in greenhouse. Fertilizer was applied every half monthly.

Inoculum for pathogenicity tests was prepared by macerating cultures in blender for 2 min. Enough water was added so that the inoculum, including macerated mycelium and detached conidia was in a prescribed concentration.

Lettuce leaf disks were cut with a cork borer (1.2 cm in diameter). They were washed with sterile distilled water and placed on 0.4% water agar. Droplets of conidial suspension,

about 4.5×10^4 conidia per ml, were placed on the centre of each leaf disk and spread over the leaf surface. Inoculated leaf disks were incubated at 25-27°C. On the 1st, 2nd, 3rd, 5th and 7th days after inoculation, inoculated leaf disks were removed from the 0.4% water agar and fixed in formalin-propionic acid-50% alcohol (5:5:90, by volume) for one day. Then , they were transferred to a freshly prepared clearing fluid composed of 85% lactic acid, chloral hydrate, phenol, clove oil and xylene (2:2:2:2:1, by weight) for another day (Herr, 1971). Infected leaf disks were stained with 1% tryphan blue in lactophenol for half an hour and rinsed in clear lactophenol to remove excess stain. They were mounted in lactophenol and observed under a microscope (Kelman, 1967).

Results and discussion

1. Germination and penetration

Conidial germination of Cercospora longissima occurred only on leaf surface with a film of water or in near-saturated atmosphere. It took at least 20 hr before protruding of a short germ-tube. Germ-tubes may arise from any or all cells of the conidia, but the basal one usually germinated first, followed by the germination of the terminal cell. The germ-tubes, protruding from the conidia usually extended to a certain length before branching or occasionally they may branch at the germination point in bidirections. The tubes often branched repeatedly as they grew and penetrated the leaves

through stomata without forming appressoria. However, they often passed directly over stomata without any penetration.

2. Development in host tissue

After stomatal penetration, germ-tubes continued intercellular growth within the susceptible tissue rapidly and ramifying through the tissue. Susceptible tissue rapidly became permeated by advancing hyphae. Invaded cells died quickly and formed the necrotic lesions. The hyphae apparently had difficulty in penetrating the large leaf veins. This probably accounts for the vein-delimited lesions on most infected leaves (Plate 8-1b).

3. Sporulation on host tissue

With favorable temperature and humidity, the fungus sporulated on infected leaves. On the 5th day after inoculation, aggregation of mycelium below the host epidermis formed brown conidiophores. More often, the conidiophores were produced in fascicles (Plate 8-2a). On the 7th day after inoculation, the conidiophores matured and bore conidia on newly growing tips. Each conidium was born singly on tip of the conidiophore. Conidia were long, cylindrical to obclavate, straight to curve, multiseptate, with obconically truncate base and tapering obtuse apex, smooth, often guttulate (Plate 8-2b & c). When mature, these conidia were deciduous and separated easily from the conidiophores. Conidial germination also occurred within the mesophyll of the host and produced germ-tubes to incite further infection (Plate 8-2d).

The sequence of disease development has been taken by microphotographs on the 2nd, 3rd, 5th and 7th days after inoculation and is shown in Plate 8-3.

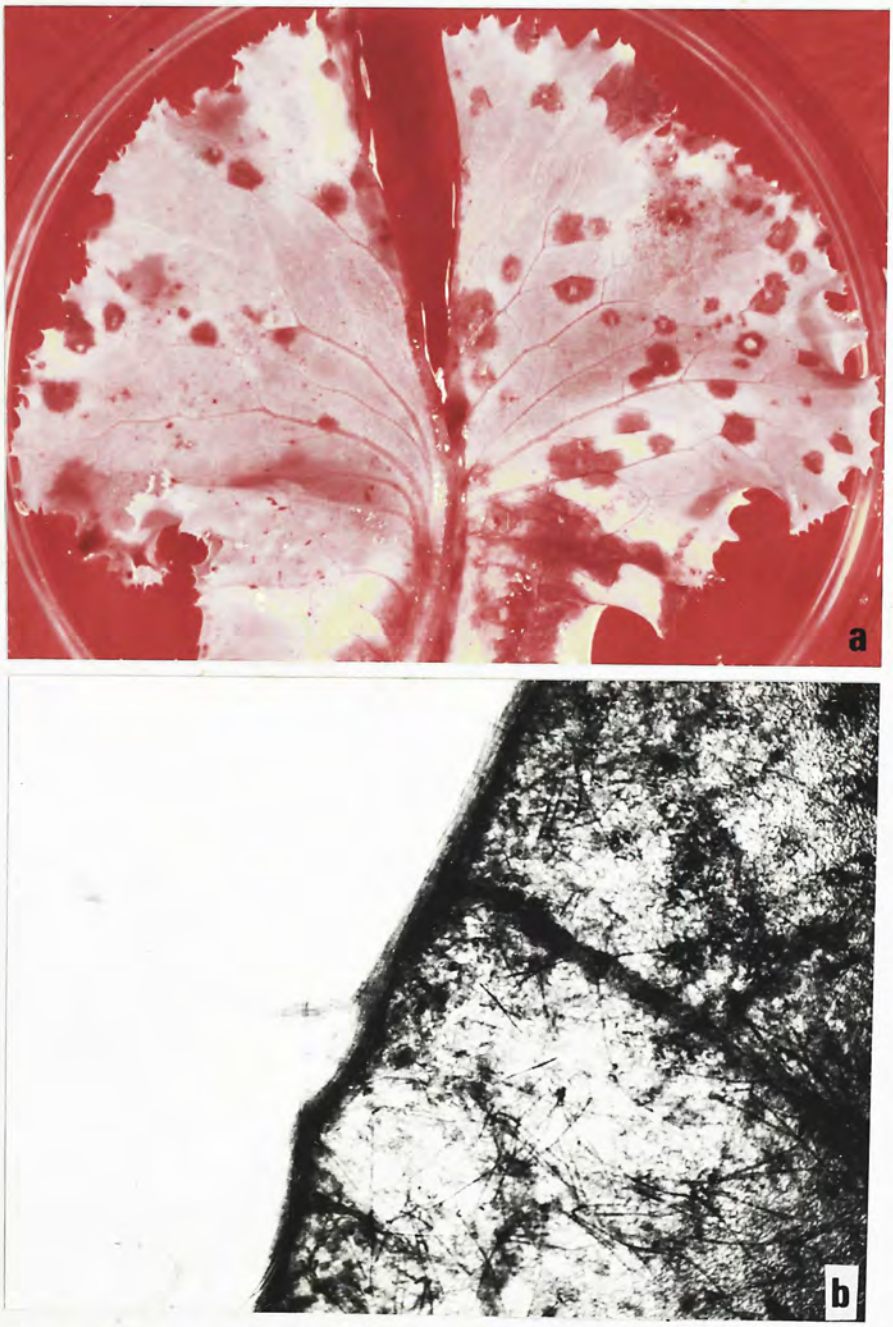


Plate 8-1. (a) Cercospora leaf-spot disease of lettuce. (b) Difficulty in penetrating the larger vein (vertical one as shown in the figure) by vegetative hyphae of Cercospora longissima. (x100)

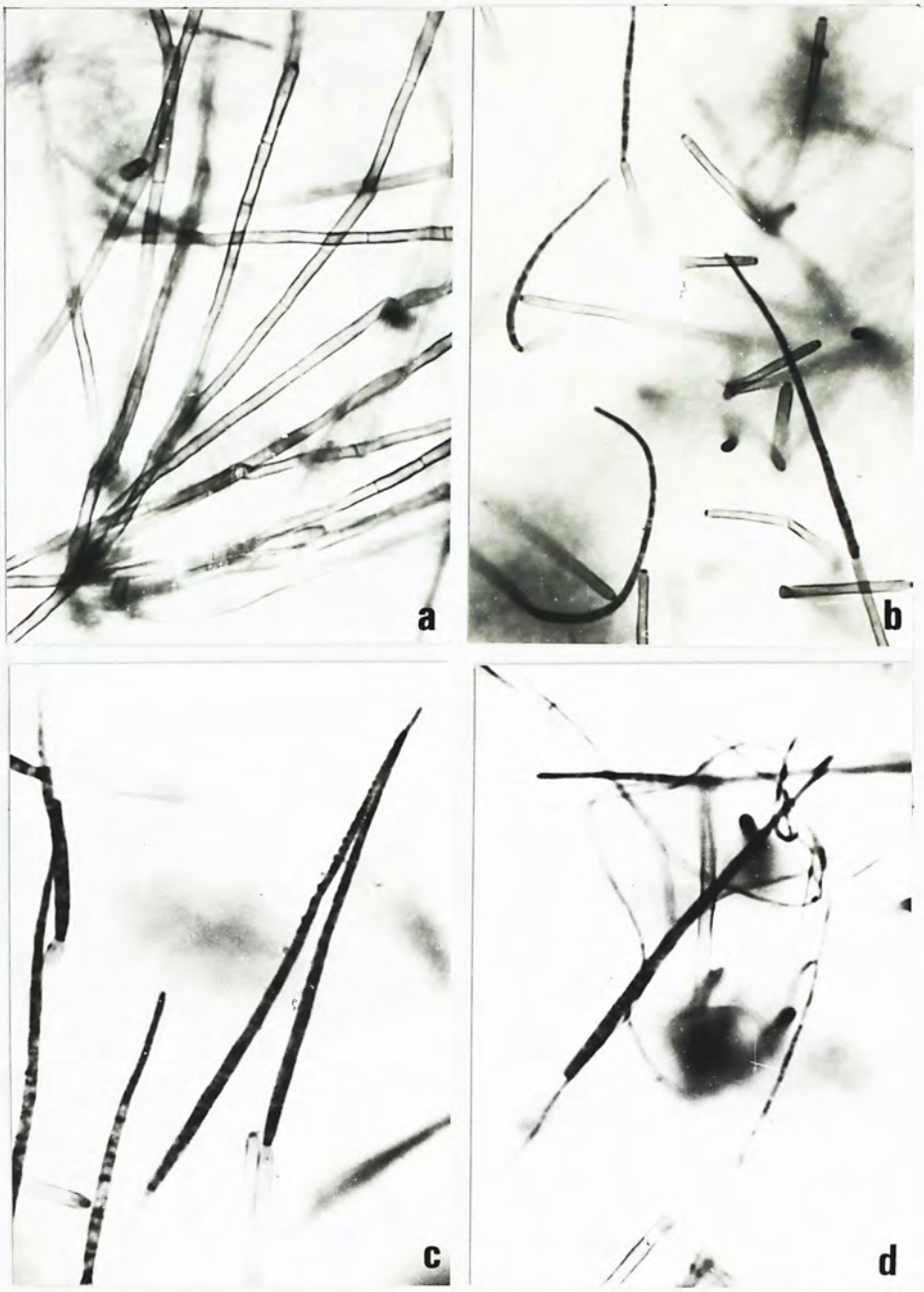


Plate 8-2. Cercospora longissima within the susceptible lettuce leaves. (a) Fasciculate conidiophores. (x500) (b) Curve conidia. (x300) (c) Straight conidia. (x500) (d) Conidial germination within the mesophyll. (x500)

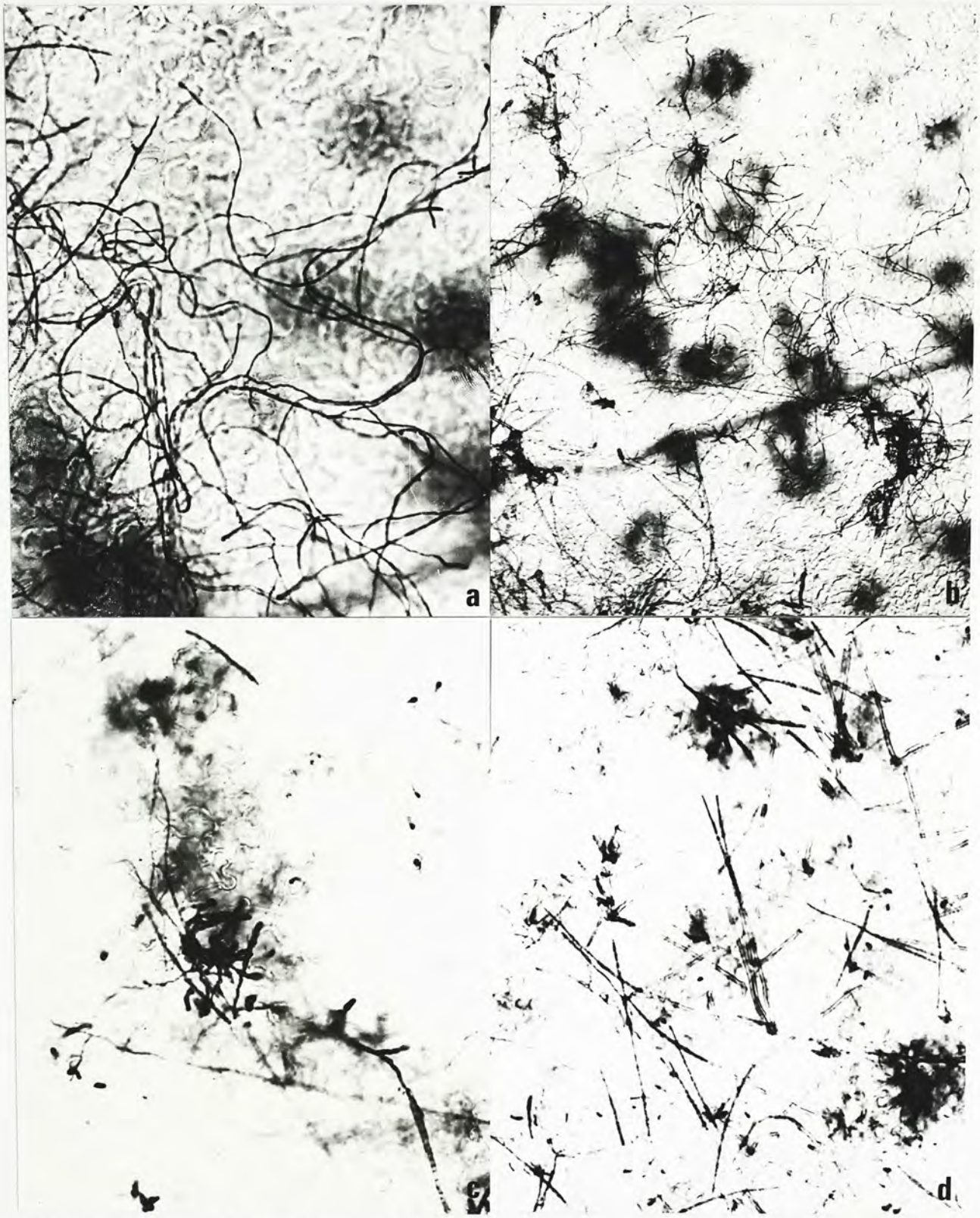


Plate 8-3. Development of Cercospora leaf-spot disease of lettuce. (a) Only with mycelial growth on the 2nd day after inoculation. (x300) (b) Vegetative hyphae permeate the host tissue on the 3rd day after inoculation. (x100) (c) Aggregation of mycelium forms cluster of conidiophores on the 5th day after inoculation. (x300) (d) Sporulation occurs in the host tissue on the 7th day after inoculation. (x100)

CHAPTER 9

FACTORS AFFECTING THE DEVELOPMENT OF *CERCOSPORA* LEAF-SPOT DISEASE

Introduction

As a first approach to the complex of factors which determines the cause of a plant disease, it may be considered to be the outcome of the interplay among the host (H); the agent of a disease (A); and the physical environment (E) as shown in Fig. 9-1.

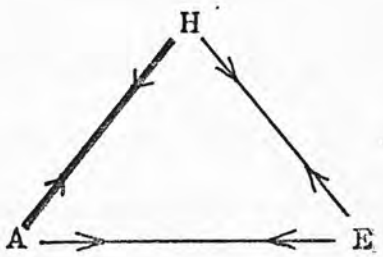


Fig. 9-1. Interplay among the host (H); the agent of a disease (A); and the physical environment (E) in a disease development.

In the case of airborne agents of disease, E refers to the atmospheric microclimate in which the agent develops and the host is attacked. In this fundamental triangle, the interaction between agent and host is the key relationship, primarily a struggle between the virulence of the agent and the capacity of the host to resist. The atmospheric environment may tip the balance of the struggle one way or the other by its repercussions on both agent and host. The host, in its turn, has a considerable influence on the microclimate among the host

individuals. The degree of this influence changes continuously as the vegetation builds up to a maximum and then begins to fade away. The effect of host on environment differs from one kind to another and further among cultivars of the same crop, depending on factors such as relative leafiness, erect or sprawling growth pattern, structure of plants, etc. The agent also should have its effect on the microclimate, but its influence is, at most, indirect and minor (e.g. by premature defoliation) (Bourke, 1970).

1. Some aspects of the host

Infectious plant diseases are the result of interaction of the host and the pathogen. The properties of each of these two organisms are governed by their genes which determine the heritable characteristics of the organism and the apparent uniformity of the organisms within a species. Yet, it is known that hundreds, or even thousands, of cultivars, different from one another in one or more characteristics, exist within a given species of plants. The inheritance of host reaction—degree of susceptibility or resistance—to various pathogen has been known for a long time and has been used quite effectively in breeding and distributing cultivars resistant to pathogens causing particular diseases. When the same variant of a pathogen is inoculated on two appropriated chosen cultivars of a host plant, one cultivar may be susceptible while the other is resistant. This clearly indicates that one cultivar possesses a genetic characteristic that enables it to defend itself

against the pathogen, so that it remains resistant, while the other cultivar does not. Cultivars possessing certain genes of resistance or susceptibility react differently against various pathogen variants and their genes in turn of virulence or avirulence. The property of resistance or susceptibility of a plant against a pathogen is genetically controlled. Lettuce has been developed into many cultivars. The resistance or susceptibility of lettuce cultivars to Cercospora leaf-spot disease was studied.

Apart from its general level of susceptibility to a particular disease, there are other properties of the host which may be significant for epidemology. The general cultivation conditions of the host, the spacings and the cultivation practices are relevant factors in case of many diseases. Resistance of the host plant to disease may differ from organ to organ and may vary from different ages or growth stages of a plant.

The final item recalls the role of wounds in the host which can play in facilitating the entry of pathogen. Wounding damage may involve a weather factor, either directly in the case of strong wing, hail or frost, or indirectly by wing-raised sand or by insect activity favored by calm and sunny weather.

Morphological conditions (e.g. stomatal opening and protective tissues) and physiological conditions (e.g. carbohydrate-nitrogen ratio, osmotic pressure and pH) also play a role in

disease development.

2. Some aspects of the pathogen

An important but usually unknown factor is the initial level of inoculum from which the infection takes place. Most prediction systems are primarily based on the assumption of a uniform starting level of inoculum.

Pathogens also consist of a multitude of physiological variants, each differs from others in its ability to attack certain cultivars of a plant species but not other cultivars.

Thus it has been shown that when a cultivar is inoculated with two appropriately chosen variants of a pathogen, the cultivar may be susceptible to one variant but resistant to the other. This clearly indicated that one variant possesses a genetic characteristic that enables it to attack the plant, while the other does not. The property of virulence or avirulence of the pathogen on a particular cultivar is genetically controlled.

Also, the models of disease prediction accommodate exclusively on weather favorable to the pathogen.

3. Some aspects of the environment

The environment is the atmospheric microclimate in which the agent of a disease develops and the plant is attacked. The main environmental factors affecting disease development are moisture, temperature and light, and the others such as soil pH and soil nutrients.

(1) Moisture

Moisture influences the initiation and development of infections plant diseases in many interrelated ways. The most important influence of moisture seems to be on the germination of fungal spores and on the penetration of the host by the germ-tubes. Moisture also activates the fungi which may then infect the plant and plays an important role on the distribution and spread of the fungus on the same plant or from one plant to another. Finally, moisture affects disease development by increasing the succulence of host plants, thus considerably increasing their susceptibility to certain pathogens.

(2) Temperature

The effect of temperature on the development of a particular disease depends on the particular host-pathogen combination and its minimum, optimum and maximum temperature requirements for developments. Since the two optimum temperatures for the development of the host and the pathogen may be different, the most rapid disease development, i.e. the shortest time required for the appearance of the first symptoms, usually occurs at temperatures at or near the optimum temperature for the development of the pathogen and at temperatures above or below the optimum for the development of the host. At temperatures appreciably below or above the optimum for

the pathogen, and at temperatures near the optimum for the host, disease development is slower. Unfavorable temperature may lengthen the period between infection and the production of a new crop of spores and also influence the number of spore germination for further infection.

(3) Light

Although the effect of light on disease development, especially under natural conditions, is less significant than that of temperature or moisture, yet, several diseases are known in which the intensity and/or the duration of light may either increase or decrease the susceptibility of plants to infection and also the severity of the disease. Intensity and quality of light also affect the germination, growth, or sporulation of most fungal pathogens (Riker & Riker, 1936; Calpouzos & Stallkneoht, 1967; Calpouzos & Chang, 1971; Agrios, 1972; Colhoun, 1973).

The primary purpose of this investigation was to obtain some informations concerning the degree of susceptibility of different lettuce cultivars; pathogenicity of the pathogen; and the effects of temperature and light on development of Cercospora leaf-spot disease of lettuce.

Materials and methods

Pure cultures of Cercospora longissima (Cug.) Sacc. were isolated from infected leaves of lettuce (Lactuca sativa L.) provided by the Agriculture & Fisheries Department of Hong Kong and the local market.

Sufficient quantities of conidia were usually obtained from 7-10 days cultures on CVA at 25°C. Most tests used this isolate as inoculum, except the study of pathogenicity of the pathogen.

Mature leaves were detached and washed twice with sterile distilled water before placed on 0.4% water agar.

Inoculum was prepared by macerating fungal mats in blender for 2 min. Enough water was added so that the inoculum contained mycelial fragments and conidia in a prescribed concentration (about 4×10^4 conidia per ml). Conidial suspension was spread over the upper surfaces of leaves with a small brush. Control plants were applied with sterile distilled water.

1. Susceptibility of lettuce cultivars

Mature leaves of 20 lettuce cultivars were detached, washed and placed on 0.4% water agar. They were Black-seeded Simpson, Burpee Bibb, Burpee's Iceberg, Buttercrunch, Dark Green Boston, Deer Tongue, Early Prize Head, Fordhook, Grand Rapids, Great Lakes, Green Ice, Hong Kong Local, Imperial No. 44, Imperial No. 847, Oak Leaf, Premier Great Lakes, Ruby, Salad Bowl, Tom Thumb and White Boston. After inoculation,

leaves were kept in constant incubator at 25°C. Each lettuce cultivar had 3 replicates in trial and the experiment was repeated five times.

2. Pathogenicity of the pathogen to Hong Kong Local lettuce cultivar

Two variants were obtained from the culture of C. longissima in single spore isolation. One was white and the other was gray. Pathogenicity of these two variants was studied.

Two 7-10 days colonies of each variant were blended with 10 ml of sterile distilled water. These spore suspensions were used as inoculum. Leaf disks of Hong Kong Local lettuce cultivar were used as hosts. Inoculated leaf disks were kept at 25°C. The experiment was repeated thrice.

3. Environmental factors affecting development of disease

(1) Temperature

Effect of temperature in development of Cercospora leaf-spot disease on Hong Kong Local cultivar and Oak Leaf cultivar was studied in incubators at 5°, 10°, 15°, 20°, 25°, 30° and 35°C. Three inoculated leaf disks with the control of the two cultivars were placed at each temperature. The experiment was repeated thrice.

(2) Light

Effect of light in disease development on Hong Kong Local lettuce cultivar was studied at 25°C. Leaf disks and inoculum were covered immediately after

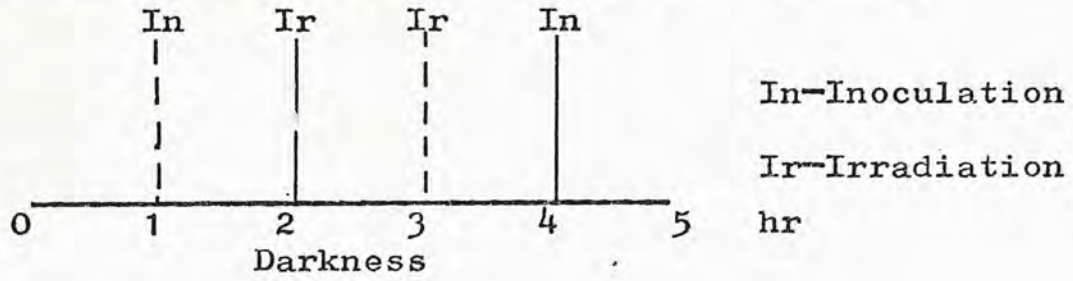
preparation and kept in darkness at 25°C. After an hour, 15 leaf disks were inoculated. Safe light of greenlamp was placed at least a meter away from the working surface. After inoculation, leaf disks remained in darkness for 2 hr before and after illumination. These 15 leaf disks were divided into 5 groups of 3 each. Group 1 was irradiated with R_{30}, group 2 with FR_{25}, group 3 with $R_{30}FR_{25}$, group 4 with $FR_{25}R_{30}$ and group 5 was the light control (C). Another 27 leaf disks which were divided into 3 groups of 9 each and 3 sets of inoculum were kept in darkness for 2 hr. One set of these materials was irradiated with R_{30}, the second with FR_{25} and the third one was the light control (C). Two hr after irradiation, leaf disks were inoculated at the following combinations with the abbreviation of H for the host and P for the pathogen.

H \ P	R_{30}	FR_{25}	C
R_{30}	H_RP_R	H_RP_{FR}	H_RP_C
FR_{25}	$H_{FR}P_R$	$H_{FR}P_{FR}$	$H_{FR}P_C$
C	H_CP_R	H_CP_{FR}	H_CP_C

One hour after inoculation, all leaf disks were removed to room conditions at 25°C. Experiment was repeated thrice.

Procedures were simplified as the following graph

with Trial I (-----) and Trial II (———).



Results and discussion

1. Susceptibility of lettuce cultivars

All tested lettuce cultivars, showed obvious leaf-spot symptoms on the 4th day after inoculation. The cultivars were grouped into 4 classes according to their susceptibilities to the disease. Photographs were taken on the 5th day after inoculation. Lettuce cultivars moderately resistant to Cercospora leaf-spot disease are shown in Plate 9-1; those of moderately susceptible in Plate 9-2; susceptible in Plate 9-3; and highly susceptible in Plate 9-4.

Among the 20 cultivars of lettuce, Early Prize Head and Green Ice were the moderately resistant to Cercospora leaf-spot. Number of leaf-spot and the area of lesion to the whole leaf area of these two cultivars were comparatively smaller than other lettuce cultivars tested. On the other hand, Buttercrunch, Grand Rapids, Great Lakes and Oak Leaf showed the heaviest symptoms. Leaf-spot of these cultivars were numerous. The area of lesions was about 3/4 of the entire leaf area.

In comparison of the reaction of these 20 lettuce cultivars

to the disease with the stomatal distribution on the upper epidermis, there was no direct relationship of stomatal density with susceptibility in lettuce to C. longissima. There were also no relationships between the color and weight of lettuce seeds, and between the type of lettuce to Cercospora leaf-spot disease (Table 9-1). Ruppel found that there was negative relationship of stomatal size and density with resistance in sugar beet to C. beticola (Ruppel, 1972). However, there was a direct relationship between the stomatal movement with resistance of this disease. Germ-tubes of C. beticola entered only through the open stomata (Zelitch, 1965).

2. Pathogenicity of the pathogen to cultivar of Hong Kong Local lettuce

The two variants tested were all pathogenic to the lettuce. However, the white variant was more pathogenic than the gray one. Minute leaf-spots appeared on leaves of Hong Kong Local lettuce inoculated with the white variant on the 4th day after inoculation. Leaf-spot symptoms caused by the gray variant appeared later.

On the 6th day after inoculation, the leaf-spot symptoms caused by the white variant was more severe than that of the gray one. However, lesions of the gray variant produced more conidia than the white one. In this case, the number of conidia may not play an important role in inoculation. However, the real mechanism is unknown.

Table 9-1. Some characters of lettuce cultivars and their susceptibility to Cercospora leaf-spot disease.

Lettuce Cultivars	Reaction to the disease (A)	Seed color (B)	Seed wt. to H. K. cultivar (C)	Stoma No. on upper epidermis (D)	Head type (E)
Early Prize Head	+	W	1.16	5.6	L
Green Ice	+	B	0.90	7.2	L
Deer Tongue	++	B	1.12	5.1	H
Imperial No. 44	++	W	1.05	5.0	H
Imperial No. 847	++	B	1.04	4.8	H
Premier Great Lakes	++	W	1.01	5.4	H
Ruby	++	W	1.05	2.4	L
Black-seeded Simpson	+++	B	1.12	4.8	L
Burpee Bibb	+++	B	1.00	9.2	H
Burpee's Iceberg	+++	W	1.05	8.5	H
Dark Green Boston	+++	W	0.95	3.0	H
Fordhook	+++	B	1.13	5.2	H
Hong Kong Local	+++	W	1.00	10.3	L
Salad Bowl	+++	B	0.98	9.0	L
Tom Thumb	+++	B	1.18	2.6	H
White Boston	+++	W	1.20	3.0	H
Buttercrunch	++++	B	1.06	11.5	H
Grand Rapids	++++	B	1.06	3.8	L
Great Lakes	++++	W	1.04	5.9	H
Oak Leaf	++++	W	0.98	4.5	L

(A) Reaction of lettuce cultivars to Cercospora leaf-spot:
+=moderately resistant ++=moderately susceptible
+++=susceptible ++++=highly susceptible

(B) Seed color: W-white seeded B-black seeded

(C) Seed weight: wt. of 50 seeds/wt. of 50 seeds of H.K. local cultivar

(D) Stoma No. on upper epidermis: No. x 10^3 / cm^2

(E) Head type: L-loose leaf lettuce H-head lettuce

3. Environmental factors affecting development of disease

(1) Temperature

Both the lettuce and C. longissima had different optimum temperatures for development. Lettuce grew best at 10-15°C, whereas the conidial germination, growth and sporulation of C. longissima were the best on CVA at 25°C.

For Cercospora leaf-spot disease of lettuce, the time required for appearance of the first symptoms varied greatly with the prevailing temperature. This was 3 days at 25°C, 5 days at 20°C and 30°C, and 7 days at 15°C. There was no leaf-spot symptoms on leaves incubated at 5-10°C even for a month after incubation. Disease also did not appear on inoculated leaves incubated at 35°C. This was mainly because of thermal destruction of the leaves at such high temperature. For this result, a direct relationship between the conidial germination of C. longissima and the disease development of Cercospora leaf-spot can be found. With reference to Table 4-1 (p. 42), it was shown that conidial germination of C. longissima only occurred at 15-30°C and the optimum temperature for germination was 25°C. As to the temperature for disease development, 25°C was the optimum point for the most rapid advancement. At this temperature, the conidia had the highest germination percentage,

in turn, the highest probability to infect the leaves. Sporulation was also best at this temperature, thus, it was allowed to incite further infection. Leaf-spot symptoms were heavy and the leaves were susceptible at this temperature.

However, very few conidia of C. longissima could germinate at 15°C and their growth and sporulation were limited, therefore, the leaf-spot symptoms were least severe and took longer time for appearance of the first symptoms. The leaves were resistant at this temperature.

(2) Light

Development of Cercospora leaf-spot disease at different light treatments are shown in Table 9-2 and Plate 9-5.

Table 9-2. Light effect on development of Cercospora leaf-spot disease.

H \ P	R_{30}	FR_{25}	C	
R_{30}	+++	++	++++	$(HP)_R$ +++
FR_{25}	++	+	++++	$(HP)_{FR}$ ++
C	+++	+++	++++	$(HP)_C$ ++++

+=least severe ++=severe

+++=very severe ++++=highly severe

Light effect on disease development can be generalized as follows:

For susceptibility of the host (H)

$$H_C \succeq H_R \succeq H_{FR}$$

For pathogenicity of the pathogen (P)

$$P_C \succeq P_R \succeq P_{FR}$$

For severity of disease development

$$H_C P_C \succeq H_R P_R \succeq H_{FR} P_{FR}$$

$$(HP)_C \succeq (HP)_R \succeq (HP)_{FR}$$

It was found that light effects were more significant when irradiation made before inoculation than after. The treatment of $H_R P_R$ showed significant difference in disease development with the treatment of $H_{FR} P_{FR}$. It may be due to the combined light effects on stomatal opening of the host and the conidial germination of the pathogen. With reference to Table 7-1 (p. 76) and Table 4-2 a, b, & c (p. 44-45), it was shown that both stomatal opening and conidial germination were promoted by R_{30} and inhibited by FR_{25}. However, the combined light effects on disease development were not as a switch type, it was not promoted or inhibited completely by R_{30} of FR_{25}. Irradiation after inoculation was different from those when the leaves and the pathogen were irradiated separately. A film of inoculum on the surface

of leaves may affect the light effect on stomatal opening as well as the sensitivity of conidia to light for germination. However, the mechanism was unknown yet.

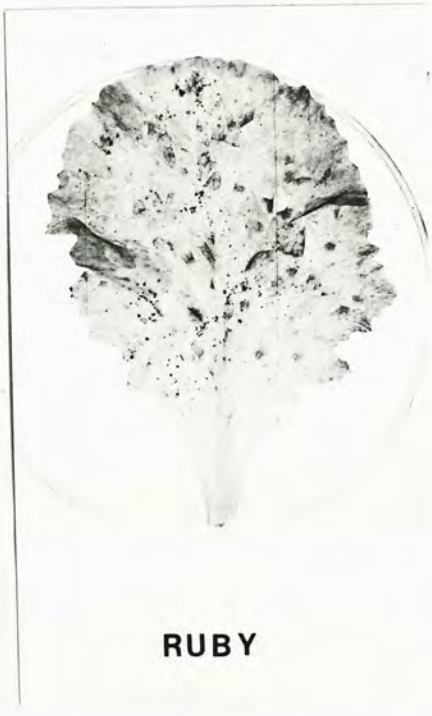
Plate 9-1. Moderately resistant symptoms of lettuce cultivars to Cercospora leaf-spot disease.

DEER TONGUE

IMPERIAL 44

IMPERIAL 847

PREMIER GREAT LAKES



RUBY

Plate 9-2. Moderately susceptible symptoms of lettuce cultivars to Cercospora leaf-spot disease.

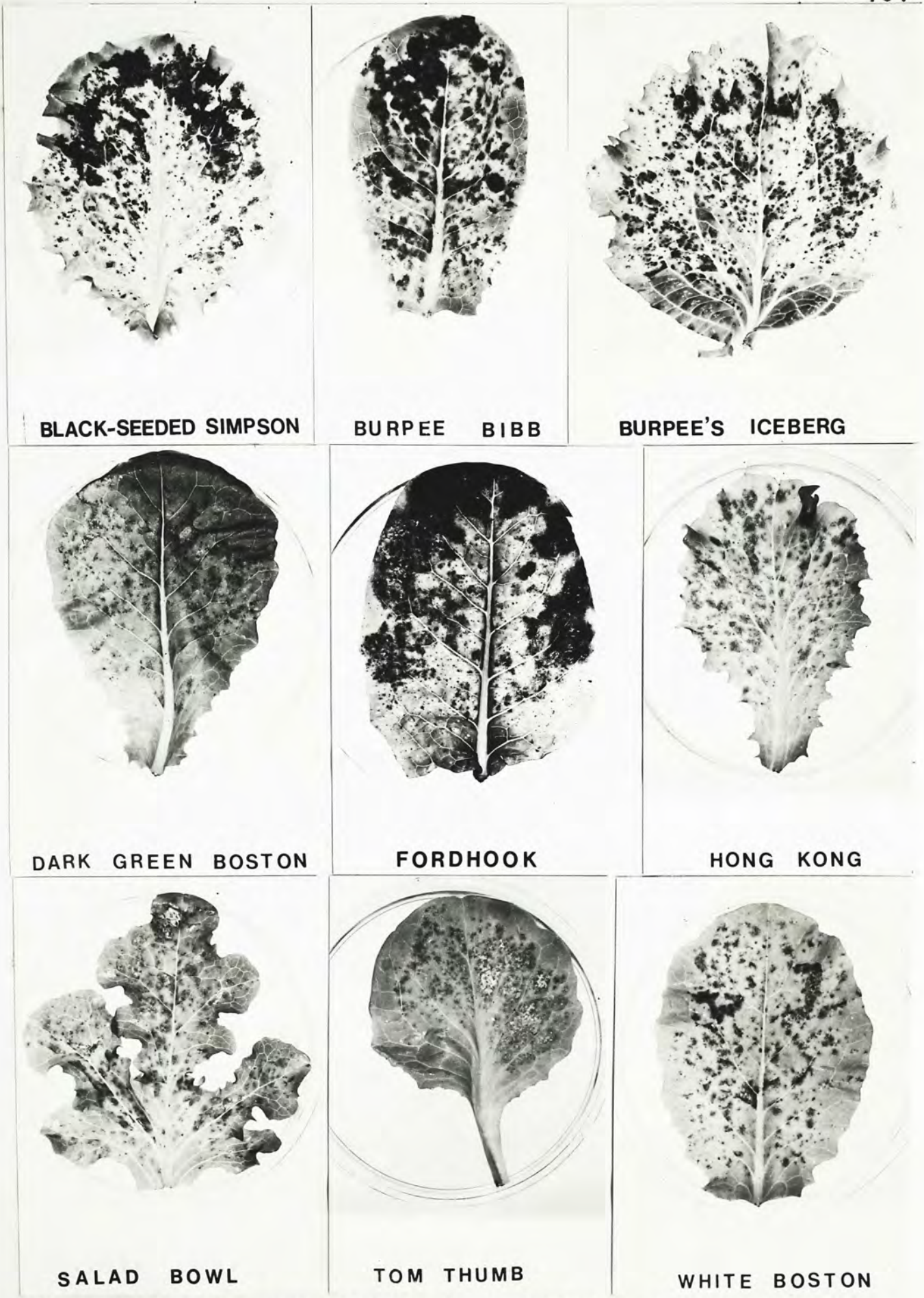


Plate 9-3. Susceptible symptoms of lettuce cultivars to Cercospora leaf-spot disease.

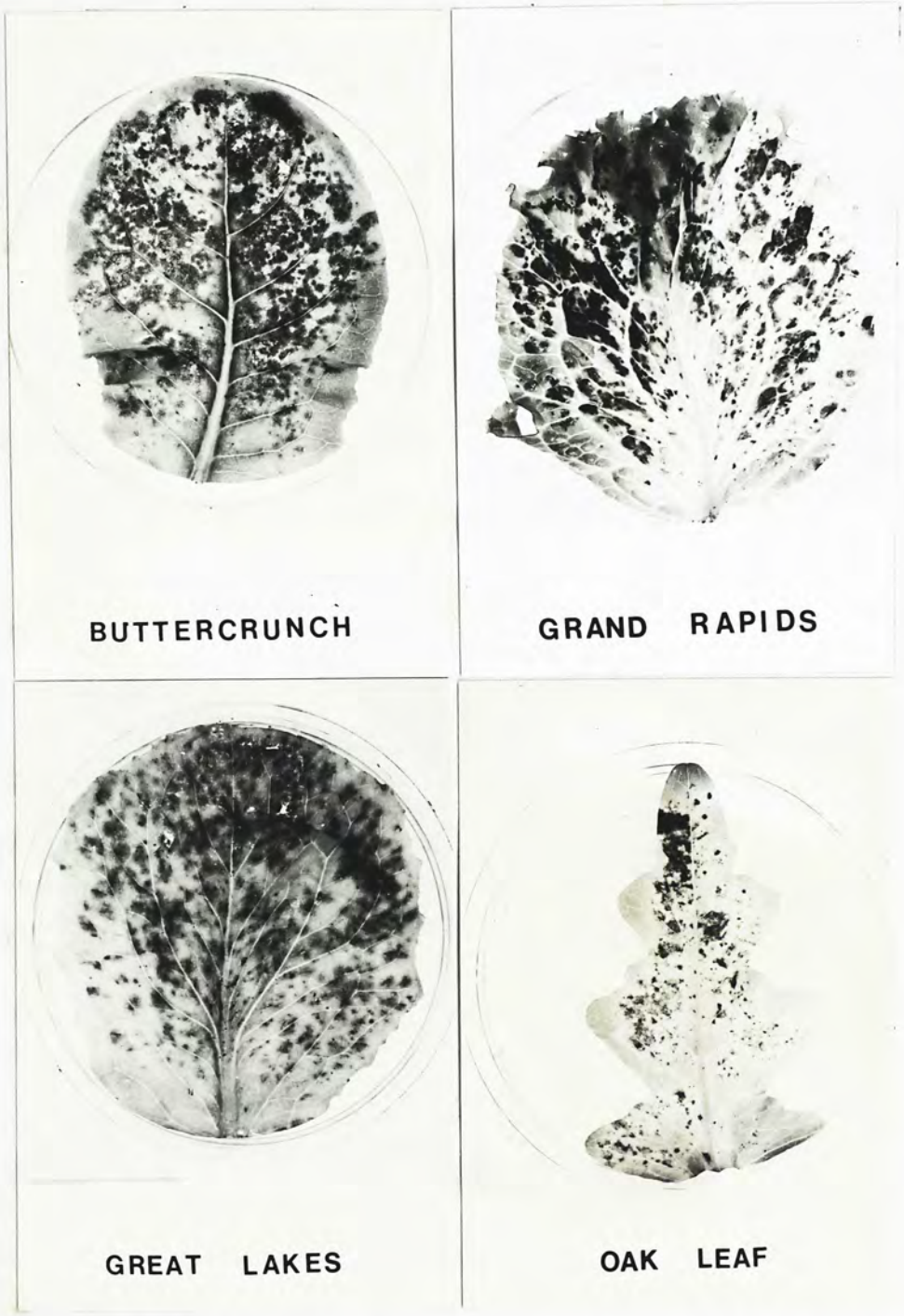


Plate 9-4. Highly susceptible symptoms of lettuce cultivars to Cercospora leaf-spot disease.

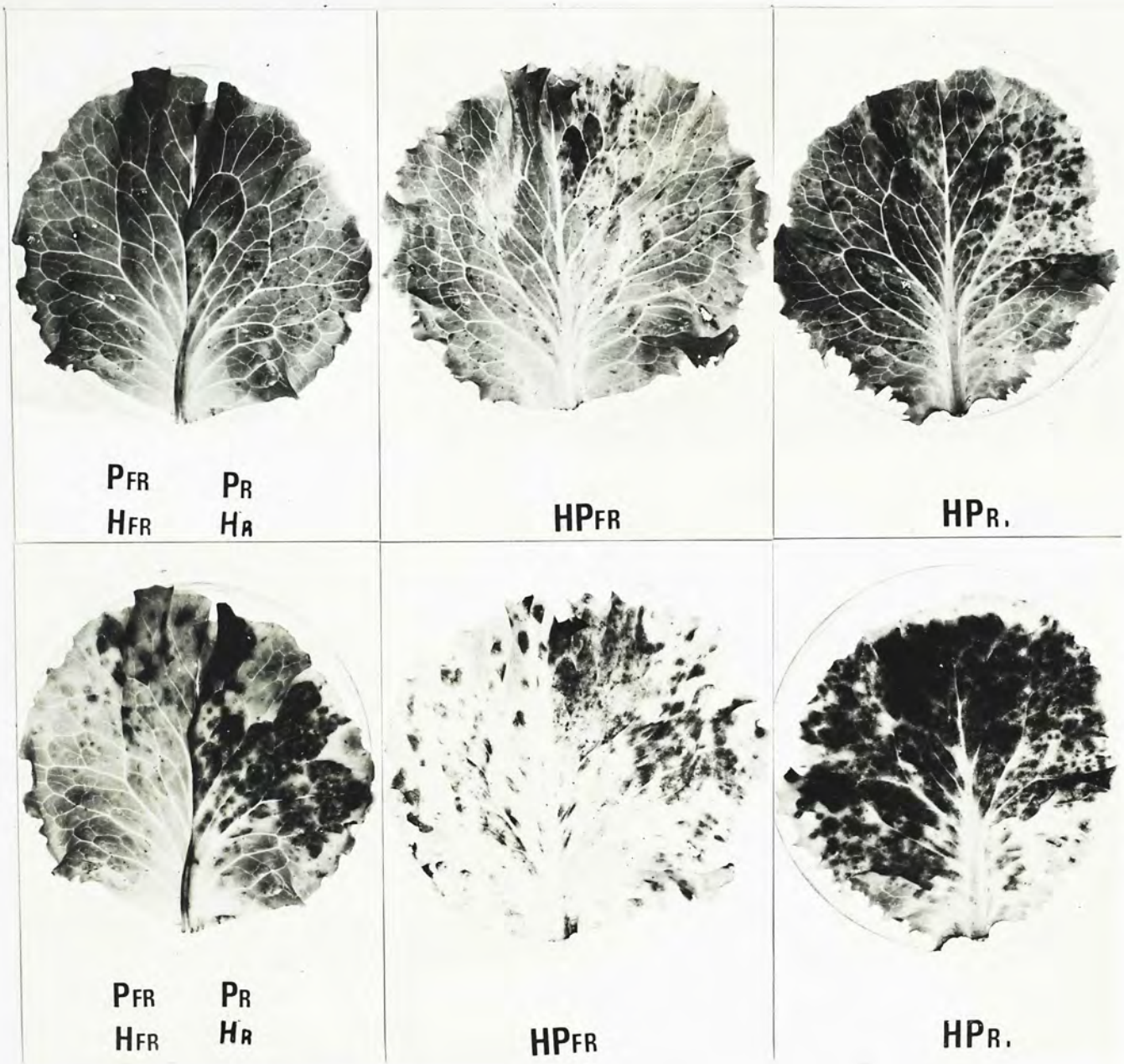


Plate 9-5. Light effect on development of Cercospora leaf-spot disease. Upper: 4 days after treatment. Lower: 6 days after treatment.

SUMMARY

Lettuce (Lactuca sativa L.) is a popular vegetable in Hong Kong as well as other places. Although it is inexpensive, yet, due to its richness in vitamins and iron salts, using as salad or otherwise, quick-growing, good-production and suitable for interplanting with cruciferous vegetables, many farmers prefer to cultivate it. The crop is subjected to a variety of diseases during its growth and distribution. Since it is a leaf vegetable, leaf-spot diseases have therefore some economic importance.

The leaf-spot disease of lettuce, caused by Cercospora longissima (Cug.) Sacc. was first reported by Toro in Colombia of South America in 1929. Since then, there was no other report on this fungus and the disease.

This thesis attempts to give some general information about the environmental factors on cultural growth, sporulation and conidial germination of C. longissima, as well as the lettuce seed germination, collection of lettuce cultivars and light effects on stomatal movement. Infection studies are carried out on the host-pathogen relationship, susceptibility of different lettuce cultivars to Cercospora leaf-spot disease, pathogenicity of the pathogen and environmental factors on its development.

摘　要

生菜（萵苣）是一種普通的蔬菜，雖然售價較廉，但因富含多種維他命和鐵質，可生食或熟食；而且生長迅速，產量高，與十字花科植物間植更爲理想。因此，本港農民多喜種植生菜。在其生長及採收後，生菜常面臨多種病害，因其是葉菜類，所以生菜葉斑病的研究，有其經濟上的價值。

萵苣尾孢霉所引起的生菜葉斑病，自1929年提出後，迄今仍未有任何詳細的資料。

本論文提供有關環境對此病菌的生長、繁殖和孢子萌發的一般影响，並生菜種子的萌發、品種的收集、光對氣孔開閉的影响、及病菌和寄主間的關係、不同品種的感染性、不同菌種的病害性和環境對葉斑病的發生等等有關資料。

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