Research Branch
Technical Bulletin 1997-01

Monograph on Downey Mildew of Crucifers

Saskatoon Research Centre

Canada

Cover illustration
The images represent the Research Branch's objective:
to improve the long-term competitiveness of the Canadian agri-food sector through the development and transfer of new technologies.

Illustration de la couverture
Les dessins illustrent l'objectif de la Direction générale de la recherche : améliorer la compétitivité à long terme du secteur agro-alimentaire canadien grâce à la mise au point et au transfert de nouvelles technologies.

Monograph on Downy Mildew of Crucifers

G.S. Saharan[1], P.R. Verma[2] and N.I. Nashaat[3]

1. Department of Plant Pathology, C.C.S. Haryana Agricultural University, Hisar, 125004, Haryana, India

2. P.R. Verma, Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan, S7N 0X2, Canada

3. N.I. Nashaat, Crop and Disease Management Department, Institute of Arable Crops-Research Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

---

Saskatoon Research Centre
Technical Bulletin 1997-01
Research Branch
Agriculture and Agri-Food Canada
1997

Copies of this publication are available from
Director
Saskatoon Research Centre
Research Branch, Agriculture and Agri-Food Canada
107 Science Place
Saskatoon, Saskatchewan, Canada S7N 0X2

Cat. No. A5A-13/1997-01E
ISBN 0-662-25744-8

CONTENTS

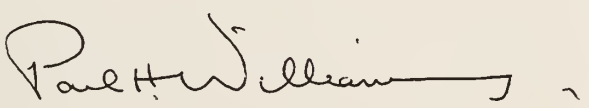
FOREWORD

Crucifer oilseed, vegetables and fodder crops represent an increasing percentage of the agricultural economies of many nations occupying important niches in temperate, cool temperate, continental and subtropical tropical regions of the world.

Vegetable crucifers continue to be a major source of vitamins, fiber, minerals and proteins in the human diet, while crucifer seed oil consumption and industrial utilization increases annually. Substantial efforts are underway to improve the quality of crucifer seed oils through genetic engineering and traditional breeding. With this global expansion in crucifer crop production comes the increasing potential for severe losses due to damage and disease of insect pests and pathogens. This monograph on the downy mildews of crucifers, therefore, is a most timely contribution to our knowledge of a most important global pathogen of crucifers, *Peronospora parasitica* (Pers. ex Fr.) Fr.

One might believe that at this time at the dawn of the 'information age' in which ready access to current information and newly emerging knowledge is increasingly available to all through the internet that there would be little need to bring together all of the relevant information into the format of a printed monograph. I would assert that quite the contrary is the case. This Monograph on Downy Mildew of Crucifers by its inclusiveness, carefully crafted organization and thorough documentation of the existing research and reported literature contextualized to be relevant to both the researcher and the practitioner is a most valuable 'benchmark' publication. In this age of information, this monograph provides the much needed background references and information together with the most current insights and methodologies as to ensure its place as a central document for students, teachers, researchers and practitioners investigating this organism and its hosts.

The authors, G. S. Saharan, P. R. Verma and N. I. Nashaat, bring their lifelong professional interest and expertise to the presentation of this treatise. Together, they have crafted a most useful document in which a wide range of information is logically organized and easily accessed. This is an important contribution in a series including Alternaria diseases and white rust of crucifers that have appeared as Technical Bulletins from the Research Branch of Agriculture and Agri-Food Canada.



April, 1997

Paul H. Williams
Atwood Distinguished Professor of Plant Pathology
University of Wisconsin-Madison
Madison, WI USA

PREFACE

This treatise on downy mildew of crucifers was compiled as the natural extension of a comprehensive literature search published in the form of a bibliography in 1994. Downy mildew is amongst the major devastating diseases of crucifers worldwide. The most common hosts of this disease are high quality edible oil crops (rapeseed-mustard, canola and other rapes), industrial oil crop (crambe and other rapes), common vegetables (cabbage, cauliflower, radish, kohlrabi, broccoli, brussels sprouts, kales, and other Brassica vegetables), and ornamental plants (wallflower and stocks). Weeds are also common hosts.

For convenience of the readers, the information has been arranged into sections and subsections. The sections discuss subjects relating to: i) the disease, its symptoms on different hosts, geographic distribution, yield losses and disease assessment methods; ii) the pathogen's taxonomy, variability, sporulation, perpetuation and spore germination; iii) host-pathogen interactions in the form of seed infection, host range, disease cycle, process of infection and pathogenesis, epidemiology, fine structure, biochemical changes and biochemical compounds; iv) host defense mechanisms; v) techniques to study host-pathogen relationships; and vi) management practices related to cultural control, chemical control, biological control, host resistance and integrated disease management. To stimulate new ideas in downy mildew research, a section on future priorities has been included.

We hope that this monograph on downy mildew will be useful to research scientists, teachers, extension specialists, students, industries and all others who are working with cruciferous crops and striving for crop improvement through disease management.

G.S. Saharan
Hisar, India

P.R. Verma
Saskatoon, Canada

N.I. Nashaat
Harpenden, U.K.

ACKNOWLEDGEMENTS

An undertaking of this nature cannot succeed without the help of many people. It is a pleasure to acknowledge those who have contributed. The original tracking down of many papers was done by Saskatoon Research Centre Librarians, Van Keane and Gail Charabin. Three other people deserve special acknowledgement: Ralph Underwood helped reproduce photographs from original journals and Ila Woroniuk and Jan Korven-Stott spent many hours typing, proof reading and preparing a final version. Any errors, either of commission or omission are, however, our responsibility. We hope they will be brought to our attention.

During the preparation of this monograph, several of the second authors' colleagues, including Dr. P.A. O'Sullivan as Director, Dr. R.K. Downey as Emeritus Scientist, and Dr. G.F.W. Rakow as Chairman of the Oilseed Section, at Saskatoon, have made valuable suggestions and have been very supportive of the work. The authors also thank Drs. K.L. Bailey and L.J. Duczek, Research Scientists, Agriculture and Agri-Food Canada, Research Centre, Saskatoon for critically reviewing the manuscript. Here, we also wish to thank many people at Headquarters Library, Agriculture and Agri-Food Canada, Ottawa, for providing countless photocopies, and to the Translation Bureau, Department of Secretary of State for translation of a series of foreign language papers.

The senior author also thanks Dr. J.B. Chowdhury, Vice-Chancellor, Dr. D.P. Singh, Dean, College of Agriculture and Dr. M.P. Srivastava, Head, Department of Plant Pathology, C.C.S. Haryana Agricultural University, Hisar, India, for permitting him to spend two months in the second author's laboratory to co-author this publication.

1. INTRODUCTION

The term "mildew" was first used in the United States to denote a wide group of Pparasitic fungi with little in common except their appearance as a white or lightly coloured delicate outgrowths caused by the proliferation and fructification of mycelium on the surface of green and necrotic plant tissues. Downy mildew quickly adapted to European conditions when Vine mildew was introduced from North America. Downy mildew or members of the family Peronosporacae are a distinctive group of obligate plant pathogens classified within the Mastigomycotina in the Oomycete order Peronosporales. In the family cruciferae, about 50 genera and more than 100 different species are susceptible to infection by downy mildew pathogen. Originally Gaumann (1918), on the basis of conidial measurements and cross inoculation tests, recognized 52 species of *Peronospora* on crucifer hosts. Later studies by Yerkes and Shaw (1959) concluded that there are no reliable morphological criteria for distinguishing *Peronospora* isolates from different host species, and all collections of downy mildew from the cruciferae are currently grouped in the single aggregate species *P. parasitica* (Pers. ex Fr.) Fr. Constantinescu (1989) later proposed a new genus, *Paraperonospora*, to accommodate several species of *Peronospora* pathogenic on hosts in the family compositae.

There are two different patterns of host colonization: systemic and localized. Systemic infection is characterized by colonization of leaves, stems and sometimes roots, mostly through the infection of the seedlings by primary inoculum. The symptoms vary from chlorotic discolouration to stunting and distortion of the whole plant. Localized infections are characterized by the occurrence of lesions on leaves, surrounded by a conspicuous characteristic white "down" on the abaxial surface (Lucas and Sherriff, 1988).

2. THE DISEASE

It is commonly known as "mildew", "mould", "false oidium or mildew" (French), "Falscher-Mehltau" (German) and more commonly by the name of downy mildew. The disease is caused by the fungus *Peronospora parasitica*.

The upper surface of affected young and older leaves have ill-defined, irregular, pale yellow necrotic lesions, whereas the lower surface is covered by white grey mycelium. The cotyledons and older leaves may be killed prematurely when single lesions coalesce to form large blotches. Attacked pods may be covered with angular brown lesions, or under high humidity, a sparse white-greyish mycelium may develop. Severe attacks may lead to premature ripening. Geographic distribution, economic importance, symptoms on various hosts, host range and disease assessment are discussed below.

a. Geographical distribution

Downy mildew on cultivated *Brassica* species and other cruciferous host species is prevalent in widely separated localities in numerous countries throughout the world (Channon, 1981; Verma et al., 1994). World records of *P. parasitica* causing downy mildew disease on crucifers are given in Table 1. The names of various hosts in this table are as reported in the original papers.

b. Economic importance

The economic importance of *Peronospora parasitica* (downy mildew) has been adequately documented over the years. This pathogen, alone or in combination with *Albugo candida* (white rust), is responsible for causing severe losses in yield of several temperate and tropical Brassicaceae crops, particularly rapeseed and mustard. Yield losses due to downy mildew infection alone is very difficult to estimate, since in most cases it is always associated with white rust.

i) Brassica oilseeds: Hypertrophied host tissues termed as staghead are often observed in association with a mixed infection of *A. candida* and *P. parasitica* particularly at the flowering stage. Yield losses in *B. rapa* var. *toria* (Toria) due to such combined infections is estimated to be about 34%, when the average length of individual hypertrophied racemes is 10 cm (Kolte, 1985). The combined infection with both pathogens on *B. juncea* may cause 37-47% and 17-32% reduction in siliques formation and seed production respectively (Bains and Jhooty, 1979). Others have reported 23-55% yield loss in the same host species due to the mixed infection with both pathogens (Saharan, 1984, 1992a). Kolte (1985) suggested the following formula for estimating the yield loss due to infection with white rust or downy mildew alone, or for combined infections: $Q = \frac{A-(BxC)}{A} \times 100$

where: Q = percentage yield
A = average actual or expected yield of a healthy plant
B = average or expected yield from the affected raceme, which is equal to the actual average yield from the corresponding length of the healthy raceme
C = number of affected racemes per plant.

ii) Brassica vegetables: During 1911-1912, downy mildew infection in cabbage near Lahore, Pakistan caused more than 50% yield loss (Butler, 1918). Vasileva (1976) reported that under favourable conditions, *P. parasitica* may infect up to 50-60% of cabbage seeds and reduces yield by 16-20%.

Table 1. World records of *Peronospora parasitica* on crucifers (Verma et al., 1994)

Location	Recording Year	Host	Reference
Argentina	1939	Cabbage, Radish, Swede	Lindquist, 1946
Australia	1924	Cauliflower, Cabbage	Samuel, 1925
Austria	1969	Cabbage	Glaeser, 1970
	1987	Radish, Chinese Cabbage	Bedlan, 1987
	1989	Cabbage	Bedlan, 1989
Bavaria	1936	Horseradish	Boning, 1936
Bermuda	1939	Stock	Waterston, 1940
Borneo	1962	Chinese Cabbage	Anonymous, 1962
Brazil	1943	Cabbage & Broccoli	Viegas & Teixeira, 1943
Britain (U.K.)	1948	*Capsella-bursa-pastoris*	Foister, 1948
	1959	Broccoli, Brussels sprouts, cabbage, Cauliflower, Kale, Kohl-rabi, Marrow-stem Kale, Rape, Turnips, Radish, Horseradish, Swede, Stock, Wallflower, Watercress	Moore, 1959
Brunei	1981	Crucifers	Channon, 1981
Bulgaria	1979	Turnip	Khristov, 1979
Canada	1944	Cauliflower	Jones, 1944
	1961	Rape, Crucifers	Downey & Bolton, 1961
Chile	1960	Crucifers	Mujica & Vergara, 1960
China	1925	Rape	Porter, 1926
	1957	Cabbage	Pai, 1957
Costa Rica	1967	Crucifers	McGuire & Crandall, 1967
Cuba	1973	Crucifers	Fernandez, 1973
Cypress	1981	Crucifers	Channon, 1981
Czechoslovakia	1968	Crucifers	Rydl, 1968
Denmark	1924	Crucifers	Gram & Rostrup, 1924
	1949	Stock	Anonymous, 1949
Dominica	1972	Crucifers	Anonymous, 1972a
Ethiopia	1981	Crucifers	Channon, 1981
Fiji	1969	Chinese Cabbage	Anonymous, 1969
Finland	1981	Crucifers	Channon, 1981
France	1943	*B. napus*, turnip, *Camelina sativa*, *Sinapsis alba*	Darpoux, 1945
Germany	1938	Colza	Klemm, 1938
	1939	Rape	Raabe, 1939
	1955	Cabbage	Neumann, 1955
Greece	1981	Crucifers	Channon, 1981
Guatemala	1950	*Brassica* spp.	Muller, 1950
Haiti	1972	Crucifers	Anonymous, 1972a
Holland	1924	Cabbage	Thung, 1926a
Hong Kong	1962	*B. alboglabra* Chinese Kale	Johnston, 1963

Hungary	1957	Stock	Lehoczky, 1957
Iberica (Spain)	1924	*B. napus, B. oleracea*	Gonzalez, 1924
India	1918	*Brassica* spp., Crucifers	Butler, 1918
	1940	*B. campestris, B. napus,* Radish *Eruca sativa, Maledmia africana, Sisymbrium iris*	Thind, 1942
	1968	*Capsalla bursa - pastoris*	Rao, 1968
	1976	*Cardamine impatiens*	Sharma & Munjal, 1977
	1980	Kohlrabi, Kale	Puttoo & Choudhary, 1984
	1981	*B. pekinensis*	Karwasra & Saharan, 1982
	1982	Cabbage	Gupta & Choudhary, 1987
Iran	1989	Radish	Etebarian, 1989
Iraq	1981	Crucifers	Channon, 1981
Ireland	1970	Cauliflower	McKee, 1971
Israel	1953	Cabbage, Cauliflower	Peleg, 1953
Italy	1961	Crucifers	Ciferri, 1961
Jamaica	1967	*B. oleracea*	Leather, 1967
Japan	1934	*Brassica* spp., Crucifers	Hiura & Kanegae, 1934
Kampuchea	1969	Crucifers	Soonthronpoct, 1969
Kenya	1957	Kale	Anonymous, 1957
Korea	1972	Crucifers	Anonymous, 1972b
	1981	Chinese Cabbage	So et al., 1981
Libya	1981	Crucifers	Channon, 1981
Ludlow	1929	Swedes	Preston, 1929
Malawi	1972	Crucifers	Peregrine & Siddiqi, 1972
Malaysia	1949	*B. rapa*	McIntosh, 1951
Malta	1981	Crucifers	Channon, 1981
Mauritius	1950	Cabbage	Orian, 1951
Mexico	1983	Rapeseed	Ponce & Mendoza, 1983
Montpellier	1941	Stock	Kuhnholtz & Gastaud, 1943
Moravea	1928	Radish	Baudys, 1928
Morocco	1981	Crucifers	Channon, 1981
Mozambique	1948	Crucifers, Cabbage	De Carvalho, 1948
Nepal	1966	Crucifers	Bhatt, 1966
Netherlands	1926	Crucifers, Cabbage	Thung, 1926b
New South Wales (Australia)	1938	Cauliflower, Mustard, Kohlrabi, Turnip	Anonymous, 1938
	1955	Cabbage	Anonymous, 1955
	1959	Brussels Sprouts	Anonymous, 1960b
	1966	Stock	Bertus, 1968
New Zealand	1963	Crucifers	Channon, 1981
Norway	1969	Cabbage, Chinese Cabbage, Kohlrabi, Kale, Red Cabbage, Rape, Turnip, Radish	Semb, 1969
Palestine	1935	Cauliflower	Rayss, 1938; Chorin, 1946
Panama	1967	Crucifers	McGuire & Crandall, 1967
Pakistan	1969	Brassica, Crucifers	Perwaiz et al., 1969
Papua New Guinea	1981	Crucifers	Channon, 1981
Philippines	1925	*B. juncea, B. pekinensis*	Ocfemia, 1925
Poland	1970	Crucifers	Zarzycka, 1970
Portugal	1953	Cabbage	Da Costa & Da Camara, 1953

Puerto Rico	1972	Crucifers	Channon, 1981
Queensland	1948	*Caronopus didymus*	Langdon, 1948
Romania	1930	*B. napus, B. nigra, Capsella*	Savulescu and Rayss, 1930
	1948	Wallflower	Savulescu, 1948
Russia	1989	Radish	Timina et al., 1989
Sabah	1962	Crucifers	Anonymous, 1962
Samoa	1975	Crucifers	Firman, 1975
Saxony	1927	Wallflower, Stocks	Wiese, 1927
South Africa	1934	Cabbage, Cauliflower, Turnips, Radish, Kohlrabi	Dippenaar, 1934
Spain	1924	*B. napus, B. oleracea*	Gonzalez, 1924
Sri Lanka	1932	Crucifers	Park, 1932
Sweden	1931	Radish	Hammarlund, 1931
	1944	Colza, White mustard	Bjorling, 1944
	1952	*Camelina sativa*	Borg, 1952
Switzerland	1923	*Brassica* spp., Crucifers	Gaumann, 1923
Taiwan	1961	Crucifers	Lo, 1961
Tanzania	1981	Crucifers	Channon, 1981
Thailand	1962	Crucifers	Chandrasrikul, 1962
Trinidad and Tobago	1922	Cabbage	Stell, 1922
Turkey	1981	Crucifers	Channon, 1981
Uganda	1981	Crucifers	Channon, 1981
USA	1883	*Brassica* spp., Crucifers	Farlow, 1883
	1889	*Sisymbrium* spp., *Lepidium*	Swingle, 1890
	1903	Cauliflower	Schrenk, 1905
	1918	Turnip	Gardner, 1920
	1923	Cabbage	Harter & Zones, 1923
	1927	Watercress	Davis, 1929
	1932	Cabbage, Crucifers, *Brassica* spp.	Weber, 1932
	1940	Horseradish	Kadow & Anderson, 1940
	1942	Cabbage	Snyder & Baker, 1943
	1954	Radish	Thompson & Decker, 1955
	1960	*Brassica* spp., Crucifers	Anonymous, 1960a
Uruguay	1955	Crucifers	Koch & Boasse, 1955
USSR	1955	Cabbage	Pimenova & Maslennikov, 1955
Venezuela	1981	Crucifers	Channon, 1981
Vietnam	1966	Crucifers	My, 1966
Yugoslavia	1954	Cabbage	Sutic & Klijajic, 1954
	1961	Horseradish	Macek, 1961

Downy mildew disease can significantly affect the yield and developmental characters of radish (Achar, 1992b). Variables affected are the size and weight of silique, number of silique/plant, number of seeds/silique and weight of seeds. Seed yield loss can be as high as 58%. Infection also adversely affects the size and weight of roots. Disease loss assessment have been estimated according to the following equation:

$$\text{Yield loss (\%)} = \frac{\{\text{Mean yield of healthy plants - Mean yield of diseased plants}\}}{\text{Mean yield of healthy plants}} \times 100$$

c. Host range

Few detailed studies have been made to determine the extent of the host ranges affected by downy mildew. In earlier work these fungi were inoculated on mature host tissues and then scored for the presence or absence of disease symptoms. The results suggested that downy mildew fungi had a very restricted host range (Gaumann, 1918). *Peronospora* on crucifers was originally examined with this assumption in mind. As a result a large number of species were created, mostly based on their occurrence on a particular crucifer genus. This process continued until Yerkes and Shaw (1959) called attention to the remarkable morphological similarity of the *Peronospora* species which attack crucifers. Following an extensive biometric study they reduced over eighty species names to synonymy with *P. parasitica* (Pers. ex Fr.) Fr. More recent work, involving examination of the reactions of crucifers seedlings to infection, has led to an even wider host range being established for *P. parasitica* (Foster, 1947a; Davison, 1967; McMeekin, 1969). Wide variation can be encountered in the reaction of seedlings of different crucifer species to the isolates of *Peronospora* from *Brassica* and *Raphanus* (Tables 2, 3), but the pathogen can grow well enough to sporulate on several species, apart from the original host (Dickinson and Greenhalgh, 1977). *P. parasitica* can infect a wide range of *Brassica* and other cruciferous species. Gaumann (1923) listed over 80 cruciferous species as susceptible to infection by the numerous species of *Peronospora* which are now regarded as all being *P. parasitica*. Among the common hosts of economic importance are rapeseed-mustard, cabbage, Chinese cabbage, cauliflower, broccoli, brussels sprouts, marrow, stem kale, kohl rabi, turnip, turnip rape, swede, oilseed rape (canola), mustard, radish, horseradish, collards, rutabaga, watercress, stock and wallflower (Channon, 1981; Verma, et al., 1994; Nashaat, 1997). Apart from these, the inventory of hosts reported to be infected by *P. parasitica* are given in Table 4.

d. Symptoms

Downy mildew *(P. parasitica)* is the most frequently recorded disease on horticultural and agricultural members of the genus *Brassica*. The disease mainly affects young plants that may, in severe cases, be stunted or killed. Infection at later stages results in the debilitation and reduction in performance and quality of the host plant.

i) Brassica oilseeds: Rapeseed-mustard: The disease appears on all above-ground plant parts but its symptoms are usually more conspicuous on leaves, stems and inflorescences. At the seedling stage on cotyledons and the first few true leaves, small angular translucent light-green lesions appear. These lesions later enlarge and develop

Table 2. Reaction of seedling cotyledons of members of the Cruciferae to inoculation with *Brassica* and *Raphanus* forms of *P. parasitica.* (Reactions were scored: 1 = no symptoms; 2 = necrotic flecking; 3 = extensive necrosis + slight sporulation; 4 = heavy sporulation; 1/2, etc., indicates intermediate reactions.) (Reprinted from C.H. Dickinson and J.R. Greenhalgh. 1977. Host range and taxonomy of *Peronospora* of crucifers. Trans. Brit. Mycol. Soc. 69: 111-116, by permission of the authors and the publisher British Mycological Society)

Host		Pathogen	
		Brassica form	*Raphanus* form
Brassica oleracea L. subsp. *oleracea* L.	Wild cabbage	3/4	3
B. oleracea L.	Cultivated brassicas	4	3
B. nigra (L.) Koch	Black mustard	1	1
B. juncea (L.) Czern	Brown mustard	3/4	2
B. rapa L.	Turnip	3	3
B. pekinensis (Lour.) Rupr.	Chinese cabbage	3	2
Sinapis alba L.	White mustard	2	2
Raphanus raphanistrum L.	Wild radish	2	4
R. maritimus Sm.	Sea radish	2	4
R. sativus L.	Cultivated radish	2	4
Crambe maritima L.	Seakale	1	1
Cakile maritima Scop.	Sea rocket	1	1
Lepidium sativum L.	Garden cress	1	1
Isatis tinctoria L.	Woad	2/4	3
Iberis amara L.	Wild candytuft	3/4	3
I. umbellata L.	Garden candytuft	3/4	3
I. sempervirens L.	Perennial candytuft	3	3
Thlaspi arvense L.	Field pennycress	2	3
T. rotundifolium (L.) Gaudin	---	2	3
Aethionema grandifloria R. Br.	---	1	1
Capsella bursa-pastoris (L.) Medic.	Shepherd's purse	1	1
Lunaria annua L.	Honesty	2	1
Alyssum saxatile L.	Golden aylssum	2	2
A. maritimum (L.) Lam.	Sweet Alison	2	2
Draba pyrenaica L.	---	1	1
Arabis alpina L.	Alpine rock cress	2	1
A. caerulea (All.) Haenke	---	1	1
Rorippa nasturtium-aquaticum (L.) Hayek	Watercress	1	1
Aubretia deltoidea (L.) DC.	Aubretia	1	1
Matthiola incana L. (R. Br.)	Stock	3	2
M. bicornis (Sibth. & Sm.) DC,	Night-scented stock	2	2
Malcolmia maritima (L.) R. Br.	Virginia stock	1/2	1/2
Hesperis matronalis L.	Dame's violet	2	1
Cheiranthus cherei L.	Wallflower	3	2
Camelina sativa (L.) Crantz	Gold of pleasure	1	1

into grayish-white, irregular necrotic patches on the upper surface of the leaf while downy fungal growth appear on the under surface. In a severe attack, diseased leaves dry up and shrivel. On cotyledons, necrotic lesions are more pronounced in *B. rapa,* whereas on true leaves, lesions are conspicuous on *B. juncea* (Figs. 1, 2).

Symptoms of mixed infection of downy mildew and white rust are common on leaves and inflorescence of *B.* rapa and *B. juncea.* On leaves, downy growth of the fungus appears in or around the white rust pustules (Fig. 3). On malformed inflorescences, sporulation of the downy mildew fungus is predominant in the form of white granular canidia and conidiophores (Fig. 4) (Saharan, 1992a). According to Butler (1918) owing to very frequent co-existence of white rust and downy mildew, it is not easy to separate their effects, but white rust produces the greatest deformities in the stem and flowers (Awasthi et al., 1995, 1997). Stem swellings may be limited often with abrupt bending of the stalk, or swelling may be several inches long. The axis of the inflorescence is equally susceptible to deformity. The leaves and flowers are not often swollen, except for the young ovary, which may be transformed to a twisted body about two or three inches in length. More often, the floral buds are atrophied with all the parts (i.e. sepals, petals, stamens, and pistal) being shrunken and almost colourless. If the attack is late, the buds/silique may be partly normal, partly deformed or atrophied, and a single bud may similarly be affected in part only. There is never any trace of the violet colour produced in downy mildew infections which often occur with white rust.

Systemically mixed infected plants with *P. parasitica* and *A. candida* have stunted and thickened growth of the whole plant which bears profuse sporulation of both pathogens. Hypertrophy of the affected cells, which is mainly attributed to infection with *A. candida,* causes thickening of the stem and inflorescence. The hypertrophied tissues tend to attract infection by *P. parasitica* because their relative susceptibility to this pathogen is much higher than normal tissues (Awasthi et al., 1995, 1997). The pith of the stem has more hypertrophied tissue than the cortex. The affected inflorescence either bears no silique, or produces abnormal silique which are often curled without seeds. In the initial stages, an affected inflorescence does not show typical symptoms of infection such as the presence of oospores and downy growth on the surface. But at the later stages, conidial fungal growth of downy mildew and sporangiophores blisters of white rust occurs on the surface of affected tissue and formation of oospores takes place in the tissue as it dries. Necrotic lesions bearing downy growth of the fungus may also be observed on well developed silique (Awasthi et al., 1995, 1997; Kolte, 1985; Saharan, 1992a, b; Vasudeva, 1958).

The internal changes due to infection by *Peronospora* differ from those caused by *Albugo* in many respects. With *Peronospora* infections, the palisade cells of the leaf are not changed. In the deeper layers of the cortex, endoderm and pericycle, new cell layers

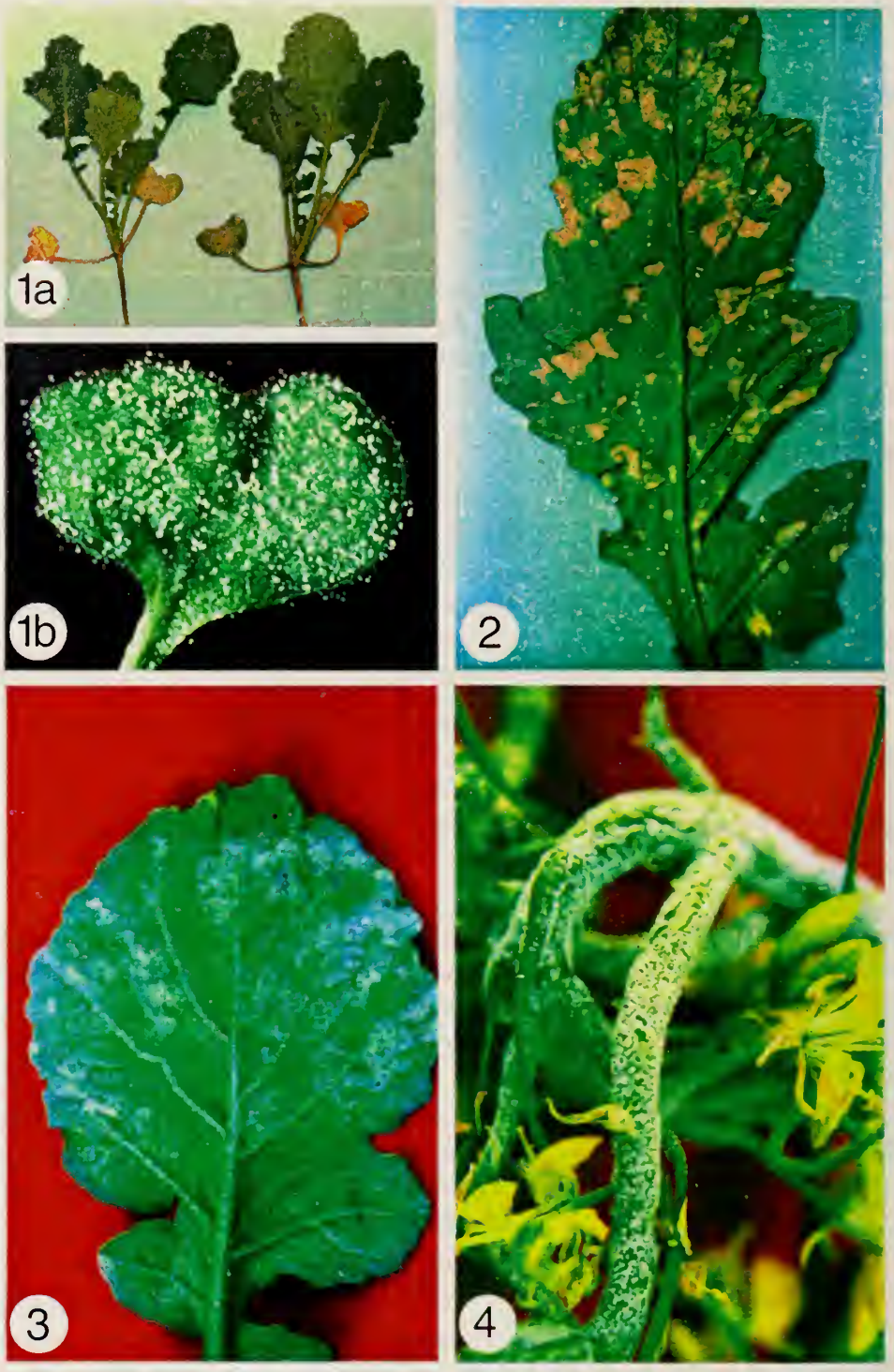


Fig. 1 Irregular, necrotic lesions of downy mildew (1a), and white growth of conidia and conidiophores of *Peronospora parasitica* 1b) on cotyledons of *B. juncea* (Courtesy: Mehta, 1993).

Fig. 2 Irregular, necrotic brown lesions of downy mildew on leaf lamina of *B. juncea* (Courtesy: Mehta, 1993).

Fig. 3 Downy growth of *Peronospora parasitica* in or around the white rust (*Albugo candida*) pustules on the lower surface of *B. juncea* leaf (Courtesy: Mehta; 1993).

Fig. 4 White growth of conidia and conidiophores of *Peronospora parasitica* on *Albugo candida* - induced malformed inflorescence of *B. juncea* (Courtesy: Mehta, 1993).

Table 3. Performance of the *Brassica* form of *P. parasitica* on cultivars of *B. olerace* (Reprinted from C.H. Dickinson and J.R. Greenhalgh. 1977. Host range and taxonomy of *Peronospora* of crucifers. Trans. Brit. Mycol. Soc. 69: 111-116, by permission of the authors and the publisher British Mycological Society)

Host Variety	Sporulation intensity*	Mycelial development in cotyledon (% grid squares covered)	Occurrence of pathogen (% seedlings positive)			
			Cotyledons	Hypocotyl	Main Root	Lateral Root
var. *capitata* L. (cabbage)						
cv. Red Drumhead	+++	76	100	100	90	25
cv. Savoy Drumhead	+++	33	100	95	0	0
cv. Flower of Spring	++	31	100	100	0	0
cv. Standby	++	47	100	100	25	0
cv. Greyhound	++	26	100	95	5	0
cv. Harbinger	++	30	100	100	45	0
cv. Primo	++	31	100	100	35	0
cv. January King	+	11	100	60	0	0
var. *botrytis* L. (cauliflower/broccoli)						
cv. Veitch's Self Protecting	+++	63	100	100	50	0
cv. Roscoff Early	+++	56	100	100	5	0
cv. Calabrese	+++	53	100	100	40	5
cv. All the Year Round	++	50	100	100	15	0
cv. Veitch's Autumn Giant	++	45	100	100	25	5
cv. June	++	23	100	100	0	0
cv. Snowball	++	50	100	100	0	0
cv. Majestic	++	24	100	95	0	0
var. *gemmifera* Zenker, (brussel sprouts)						
cv. Masterman	++	33	100	100	10	0
cv. British Allrounder	++	37	100	100	5	0
cv. Cambridge No. 5	++	51	100	100	15	5
cv. Jade Cross	++	18	100	100	0	0
cv. Exhibition	++	21	100	90	0	0
cv. Cambridge No. 1	++	34	100	75	0	0
var. *gongylodes* L. (kohlrabi)						
cv. Green Vienna	++	16	100	95	0	0
cv. Purple	++	20	100	100	0	0
var. *acephala* D.C. (Borecole)						
cv. Tall Green	++	42	100	95	5	0
cv. 1000-headed	++	38	100	90	0	0
subsp. *oleracea* (wild cabbage)	+++	60	100	100	60	10

* + = sparse sporulation; ++ = moderate sporulation; +++ = heavy sporulation

Table 4. Host species of *P. parasitica* (Channon, 1981; Verma, et al., 1994)

Host		Reference
Arabidopsis spp.		Koch & Slusarenko, 1990
Armoracia rusticana	Horseradish	Moore, 1959
Arabis spp.	Rockcress	Anonymous, 1960
Aubretia spp.	Aubretia	Moore, 1959
Brassica alba	White mustard	Anonymous, 1960a
B. arvensis	Wild mustard	Anonymous, 1960a
B. alboglabra	Chinese kale	Johnston, 1963
B. chinensis	Chinese cabbage	Hiura & Kanegae, 1934
B. juncea	Mustard	Gaumann, 1926
B. kaber	White mustard	Anonymous, 1960a
B. hirta	White mustard	Anonymous, 1960a
B. fructiculosa		Gaumann, 1926
B. napus	Rape	Moore, 1959
B. napus var. *napobrassica*	Swedes	Moore, 1959
B. nigra	Black mustard	Gaumann, 1926
B. oleracea var. *acephala*	Marrow stem kale	Moore, 1959
B. oleracea var. *botrytis*	Cauliflower	Moore, 1959, Ramsey, 1935
B. oleracea var. *capitata*	Cabbage	Moore, 1959, Ramsey, 1935
B. oleracea var. *caularapa*		Moore, 1959, Ramsey, 1935
B. oleracea var. *gemmifera*	Brussels sprouts	Moore, 1959, Thung, 1926
B. oleracea var. *gengyloides*	Kohlrabi	Johnston, 1963
B. pekinensis	Chinese cabbage	Chang et al., 1963, Ocfemia, 1925
B. campestris var. *rapifera*	Turnip	Moore, 1959
B. campestris var. *yellow sarson*	Yellow sarson	Saharan, 1992a
B. campestris var. *brown sarson*	Brown sarson	Saharan, 1992a
B. campestris var. *toria*	Toria	Saharan, 1992a
Chenopodium album	Bathu	Saharan, 1996
B. tournefortii		Gaumann, 1926
Barbarea		Anonymous, 1960a
Camelina sativa		Darpoux, 1945
Cheiranthus allioni		De Bruyn, 1935b
C. cheiril	Wallflower	Moore, 1959, Wiese, 1927
Capsella brusa-pasteris		Farlow, 1883
Cardamine impatiens		Sharma & Munjal, 1977
C. rhomboidea		Farlow, 1883
Coronapus didymus		Langdon, 1948
C. squamatus		Dias & Da Camara, 1953
Crambe maritima	Crambe	Moore, 1959
Dentaria spp.		Anonymous, 1960a
D. laciniata		Farlow, 1883
Descurainia spp.		Anonymous, 1960a
Draba spp.		Anonymous, 1960a
D. caroliniana		Farlow, 1884
Eruca sativa	Taramira	Gaumann, 1926
Hesperis spp.		Anonymous, 1960a

Erysimum cheiranthoides	Warmseed mustard	Anonymous, 1960a
Iberis amara	Candytuft	Anonymous, 1960a
Lepidium sativum	Garden cress	Anonymous, 1960a
L. intermedium		Swingle, 1890
L. graminifolium		Nicolas & Aggery, 1940
L. virginicum		Farlow, 1883
Lobularia spp.	Koniga	Anonymous, 1960a
Malcolmia africana		Thind, 1942
Matthiola incana	Stock	Moore, 1959; Wiese, 1927
Nasturtium officinale	Watercress	Moore, 1959
Raphanus sativus	Radish	Moore, 1959
R. raphanistrum	Wild radish	Gaumann, 1926
Radicula nasturtium-aquaticum		Davis, 1929
Rorippa		Anonymous, 1960a
Sinapis alba	White mustard	Gaumann, 1926
S. arvensis	Wild mustard	Gaumann, 1926
Sisymbrium officinale	Hedge mustard	Anonymous, 1960a
S. irio	Tumbling weed	Thind, 1942

may be formed by normal cell division. Only the cells around the vascular bundles become enlarged and thin-walled; the rest of the interfascicular sclerenchyma remains unaltered. There is no interfascicular cambium in these host plants. The cambium of the vascular bundles remains active whereas the xylem and phloem vessels become enlarged and separated by radial bands of parenchyma when hyphae have penetrated. There are no accessory bundles. In general, the effect on the cells seems to be more destructive than in *Albugo*, the chlorophyll content is diminished and the cell contents more rapidly used up. There is no tendency for chlorophyll accumulation in unusual places as with white rust. In general, the effect of downy mildew on the cell seems to be more destructive than white rust (Butler, 1918).

The protoplast of epidermal cells respond differently to haustorial development than protoplast of the mesophyll cells (Chau, 1970). The epidermal cells result in a severe disruption of the protoplast. The central vacuoles contract and probably undergo fragmentation, the plasmalemma is broken down or detached from the wall and numerous vesicles are formed from it. The cytoplasm is either dislocated and aggregated into a vacuolated blob or completely dispersed to the extent that its identity cannot be discerned. Consequently, haustoria in epidermal cells are not, in most cases, surrounded by a clearly defined layer of host cytoplasm. Haustoria formation in a mesophyll cell cause less disruption. The host cytoplasm is merely invaginated by the invading haustorium, while the tonoplast and plasmalemma apparently remain intact.

ii) Brassica vegetables: Plants can be infected at any time (Sherf and Macnab, 1986). In seed beds, the cotyledons and first leaves are invaded. The adaxial surface

of the leaf bears small, pale-yellow, angular spots, which may grow together to form irregular brown patches. On the abaxial surface the corresponding areas are covered with a light-gray fungus formed by multi-branched conidiophores bearing conidia. Young leaves and cotyledons may drop off as they yellow. Older leaves usually persist, and affected areas enlarge becoming papery and tan coloured. Severe infection may cause the death of the whole leaves. Minute necrotic flecks covering the leaf surface may often form resembling peppery leaf spot caused by bacteria.

When the fungus enters the stalk at the leaf base of an old head of cabbage, a grayish-black discolouration of the stalk occurs (Ramsay and Smith, 1961). In some storage lots of cabbage this discolouration has been found extending up through the stalk to the innermost bud leaves. On cabbage heads, the pathogen may cause numerous sunken black spots, varying in size from minute dots to an inch or more in diameter (Sherf and Macnab, 1986). A similar blackening occurs on cauliflower curds. The infection is evident as brown to black streaks in the vascular system of the upper portion of the main stalk and branches leading to the florets. The fleshy roots of turnips and radishes have an internal irregular region of discolouration extending from the root crown downward or beginning on the side at soil level. The flesh is brown to black or shows net necrosis. In advanced stages the skin can be roughened by minute cracks, and the root can split open (Sherf and Macnab, 1986).

According to Butler (1918) the fungus is visible as a thin, grayish-white, downy growth, occurring in scattered patches on the under surfaces of the leaves in cabbage, cauliflower and turnip, and on the leaves, stem and inflorescence in radishes. The upper surface of the leaf is marked by white spots corresponding to the downy growth below. In severe attacks, the spots may be so crowded that the leaf dries up, shrivels, and tears easily. In seedlings, the whole under surface may be evenly covered, and total infection of the young inflorescence is also found. Occasionally the roots of radish and Swedish turnip are attacked in Europe. The tissues blacken and rot near the surface, oospores occur within the tissues and conidiophores form if exposed to the air.

iii) Broccoli: Downy mildew appears first on the lower leaves of broccoli plants (Natti et al., 1956). Leaf infection may occur soon after the plants are set in the field or may take place later in the season. Older leaves appear to be more susceptible than newly developed leaves. When the surface of the foliage is wet, the downy white mycelium of the fungus is readily observed on the under surface of the leaves. The first symptoms of leaf infection are small water-soaked spots surrounded by a halo of light green tissue on the under surface of the leaf. Under conditions favourable for development of infection, the spots enlarge to form indefinite yellow areas. Later, the tissues within these infected areas collapse and become light brown and parchment-like. The mildew lesions vary in size and shape. The largest lesions usually are bounded by leaf veins. The initial spots of infection may also remain localized. The tissues of the

spot collapse to form a small brown lesion. Systemic infections are usually confined to the upper portion of the main stalk and to the branches leading to the florets of the head. Infected tissues develop brown to black netted lesions, and in others as long strands of discoloured tissues. In some plants systemic invasion of the head can be detected by diffuse blue to purple areas on the stalk and branches of the head.

iv) Wallflower *(Cheiranthus)*: On the diseased plants the upper surface of the leaves show pale yellowish patches, while the corresponding parts of the undersurface are covered with a grayish or white fungal growth (Gram and Weber, 1952). The infected stems and flowers are swollen and often twisted. Diseased flower buds do not develop.

v) Stock *(Matthiola)*: The disease is more common on young plants before they are transplanted but may also appear later, especially on crop grown indoors (Gram and Weber, 1952). On the upper surface of the leaves there are pale spots, while on the corresponding parts of the undersurface is a whitish layer of the fungus. Stalks and flower heads may also be attacked. The diseased parts show various kinds of distortion. According to Jafar (1963) the disease appears as light green areas on the upper surface of leaves. The corresponding under surface is chlorotic with white growth of fungal conidia and conidiophores. Infected areas turn yellow, and become necrotic leading to premature leaf fall. The flowers of infected plants frequently fail to open and often die. Fructification appear on cotyledons and seedlings may be killed.

e. Disease assessment

Different scales have been used for classifying leaf infection by downy mildew pathogen. Natti et al., (1967) and Sadowski (1987) used scales ranging from 0-5, where:

0 = no symptoms
1 = spots, necrotic flecks or streaks, but no sporulation
2 = spots, necrotic flecks or streaks, with sparse sporulation confined to necrotic tissue
3 = systemic infection and sporulation in
4 = systemic infection and sporulation in increasing degree
5 = systemic infection and sporulation in increasing degree

Plants with ratings 0 to 2 are considered resistant. Ebrahimi et al. (1976) has rated downy mildew resistance in *B. juncea* lines on a scale of 1 to 5 where, 1 = indicates no sporulation, 2 = very sparse sporulation, and 5 = heavy sporulation. A similar scoring scale of 1 to 4 with slight modification has been used by Dickinson and Greenhalgh (1977). Use of a 0 - 9 scale has been suggested by several workers (Knight and Furber, 1980; Nashaat and Rawlinson, 1994; Saharan, 1992b; Williams, 1985). It

is described as follows:

0 = no symptoms or signs of *P. parasitica*
1 = very minute to larger scattered necrotic flecks under the inoculum drop, no or small amounts of necrosis on the lower cotyledon surface, no sporulation
3 = very sparse sporulation, one to a few conidiophores on the upper or lower surfaces, necrotic flecking often present, tissue necrosis present
5 = sparse scattered sporulation on either or both cotyledon surfaces, tissue necrosis
7 = abundant to heavy sporulation mainly on lower surfaces, light to scattered sporulation on upper surfaces; tissue necrosis and chlorosis may be present
9 = abundant sporulation; leaf or cotyledon collapsed.

A disease index (DI) was calculated using the formula:

$$DI = \sum_{i=0}^{9} (i \times j)/n$$

where n = total plants, i = infection phenotype class, and j = number of plants per class. Genotypes are categorized as resistant (0 to 1), partially resistant (3 to 5) and susceptible (7 to 9).

Kruger (1991) suggested use of 1-9 scale in the form of diagrams to estimate disease on leaves of oilseed rape (Fig. 5); scores 3, 5, 7, and 9 respectively represent 7, 27, 65, and 100 percent of the leaf area infected. If larger leaves are concerned, those in Fig. 5 should be enlarged by 2 to 5 times to get a comparable shape and size to the leaves found in the field.

Brophy and Laing (1992) assessed disease severity using an image analyser to determine logarithmic rating scales of percentage leaf area infected for both cotyledons and primary leaves of cabbage. They found that the maximum area infected can be 100% in cotyledons but in primary leaves it rarely exceeds 25%. In order to integrate the two components, transformation of the data is necessary. Percentage disease severity (PDS), expressed as a function of cotyledon and primary leaf infection is calculated using the formula PDS = (C + x P)/2, where C is the percentage cotyledon area infected with a maximum value of 100%; x is the inverse of the maximum measured percentage primary leaf area infected, and P is the percentage primary leaf area infected of treated plants.

3. THE PATHOGEN

Downy mildew of crucifers is caused by an obligate pathogen, *Peronospora*

Fig. 5. Disease assessment (1 to 9) on leaves of oilseed rape (Reprinted from W. Krüger. 1990. A review on assessment of diseases in oilseed rape - comparison of various methods. IOBC/WPRS Bulletin, Number 14: 91-111, by permission of the author and the publisher).

parasitica (Pers. ex. Fr.) Fr., Sum. Veg. Scand. 493, 1849. Extensive synonymy is given by Yerkes and Shaw (1959). Sometimes it is referred to as *Peronospora brassicae*.

a. Taxonomy and morphology

The earliest reference of downy mildew on crucifers is by Persoon (1796) who ascribed the cause of the disease on *Thlaspeos bursa-pastoris (Capsella bursa-pastoris)* to the fungus *Botrytis parasitica* Pers. In 1849, Fries (Gaumann, 1918) transferred the fungus to the genus *Peronospora* which had been established in 1837 by Corda in his description of *Peronospora ramicis* (Corda, 1837). At that time all isolates obtained from cruciferous hosts were ascribed to *P. parasitica* (Pers. ex. Fr.) Fr. However, Gaumann (1918) named isolates of *Peronospora* affecting plants of *Brassica* species as *P. brassicae* Gaum. He considered that the various isolates obtained from different hosts should be classified as separate entities, and on this basis recognized 52 species of *Peronospora*. His conclusions were based largely upon conidial dimensions and the results of cross-inoculation tests. The value of conidial dimension as a taxonomic criterion has since been questioned because size may vary according to environmental conditions (Thung, 1926a). Yerkes and Shaw (1959) reported remarkable morphological similarity of *Peronospora* species which attack crucifers. Measurements of conidia (Tables 5, 6), an inability to associate conidiophores types with particular host genera, and the uniformity of oospores led Yerkes and Shaw (1959) to conclude that there is no reliable morphological basis to distinguish different species of *Peronospora* affecting the crucifers. Following an extensive biometric study, over 80 species names were reduced to one synonym and now a single species, i.e., *P. parasitica*, has been recognized on cruciferae hosts (Dickinson and Greenhalgh, 1977; Haura and Kanegae, 1934; Waterhouse, 1973; Yerkes and Shaw, 1959). However, in view of the apparent differences in the antheridial structure in the isolates of *Peronospora* on *Capsella bursa-pastoris* (Wager, 1889) and on *B. oleracea* (McMeekin, 1960) the merits of some separate speciation must not be ruled out. The phylogeny of the Peronosporales (Fig. 6) has been shown by Shaw (1981).

b. Reproduction and reproductive structures

The general morphology and infection cycle of *P. parasitica* is similar to that of other members of the family Peronosporaceae.

i) Asexual phase: Mycelium and haustoria: The mycelium is hyaline and coenocytic. It grows intercellularly in the host tissues and produces haustoria to penetrate the host cells. The haustoria are large, lobed, elongated or club shaped (Butler, 1918; Fraymouth, 1956; Holliday, 1980). They branch extensively and can nearly fill the entire cell. In the leaf of Japanese radish, the mycelia turn and twist irregularly in the intercellular spaces of the spongy parenchyma, and usually develop

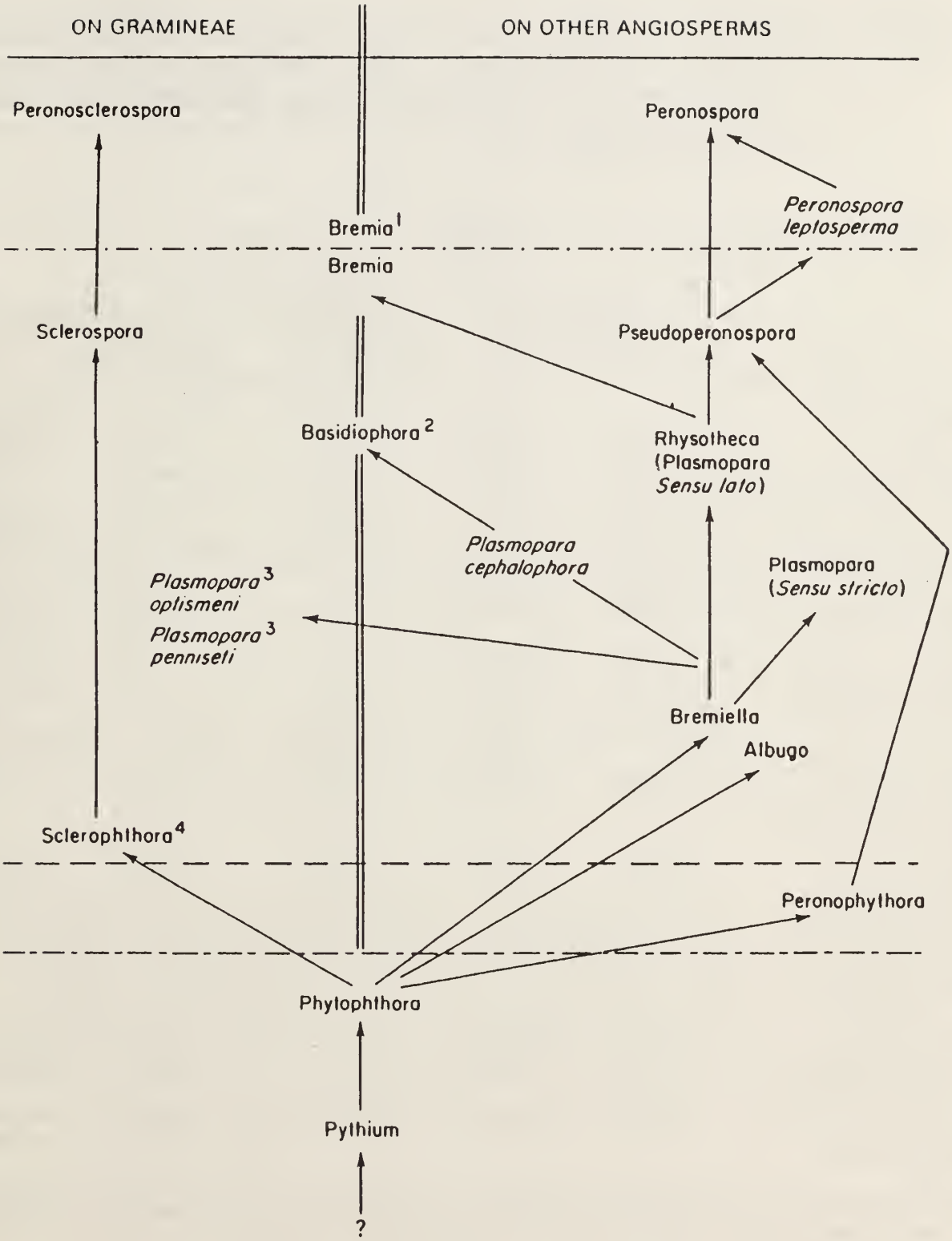


Fig. 6. Phylogeny of the Peronosporales (Reprinted from C.G. Shaw. 1981. Taxonomy and evolution. IN. The downy mildews, D.M. Spencer (editor) Chapter 2: 17-29, by permission of the author and the publisher Academic Press Limited, London).

Table 5. Measurements of *Peronospora* conidia on Crucifers (Reprinted from W.D. Yerkes and C.G. Shaw. 1959. Taxonomy of the *Peronospora* species on cruciferae and chenopodiaceae. Phytopathology 49:499-507, by permission of the authors and the publisher American Phytopathological Society)

	Length (μ)				Quotient length/width)			Width (μ)				No. spores measured + no. collections
	Means			Range	Means			Means			Range	
	Min.	Grand	Max.		Min.	Grand	Max.	Min.	Grand	Max.		
Sisymbrium altissimum L.		(15.66)		11.9-19.9		(1.32)			(11.89)		9.6-15.3	100 (1)
Arabis hirsuta		(16.12)		11.0-23.0		(1.16)			(13.92)		8.0-20.0	1,000 (1)
Arabis laevigata (Muhl.) Poir.		(16.89)		10.3-16.1		(1.24)			(13.63)		12.6-19.9	100 (1)
Arabis hirsuta (L.) Scop.	16.99	(17.62)	18.11	13.4-22.6	1.23	(1.30)	1.37	13.02	(13.54)	14.48	11.1-19.2	220 (3)
Cardamine bulbosa (Schreb.) BSP.		(17.72)		13.4-23.0		(1.25)			(14.22)		11.5-16.1	100 (l)
Draba caroliniana		(17.64)		11.0-24.0		(1.12)			(15.81)		9.0-21.0	1,000 (1)
Rorippa palustris (L.) Bess.		(18.75)		15.3-24.9		(1.31)			(14.27)		11.5-18.0	100 (1)
Cardamine parviflora L.		(18.90)		14.2-23.0		(1.28)			(14.79)		11.5-17.2	100 (1)
Nasturtium officinale R. Br.	18.32	(19.50)	20.29	14.6-26.8	1.17	(1.21)	1.25	15.33	(16.19)	17.37	12.3-21.1	140 (3)
Cardamine pennsylvanica Muhl.		(19.62)		15.3-23.8		(1.35)			(14.56)		12.6-16.9	100 (1)
Brassica nigra (L.) Koch		(19.77)		15.7-23.7		(1.16)			(17.00)		14.5-21.4	100 (1)
Brassica arvensis (L.) Ktze.		(19.97)		15.3-27.2		(1.25)			(16.04)		11.5-20.7	100 (1)
Raphanus sativus		(20.01)		14.0-26.0		(1.10)			(18.17)		14.0-22.0	1,000 (1)
Draba caroliniana Walt.	19.53	(20.19)	21.37	14.6-26.4	1.21	(1.22)	1.25	15.68	(16.58)	17.66	11.9-20.7	220 (3)
Nasturtium officinale		(20.32)		16.0-27.2		(1.19)			(17.07)		12.8-20.8	101 (1)
Dentaria laciniata Muhl.	20.23	(21.17)	22.87	15.3-27.6	1.26	(1.29)	1.31	15.69	(16.45)	17.41	11.5-21.8	140 (3)
Capsella bursa-pastoris	20.87	(21.39)	21.90	12.0-35.0	1.15	(1.18)	1.20	18.21	(18.26)	18.30	11.0-24.0	1,200 (2)
Capsella bursa-pastoris (L.) Medic.	20.79	(23.10)	28.40	14.6-36.8	1.26	(1.35)	1.44	15.86	(17.14)	19.70	11.9-23.8	1,200 (13)
Raphanus sativus L.		(23.54)		18.0-29.5		(1.26)			(18.72)		14.2-22.6	100 (1)
Sisymbrium canescens		(26.38)		17.0-32.0		(1.36)			(13.25)		12.0-24.0	1,000 (1)
Sisymbrium canescens Nutt.		(27.13)		19.9-33.3		(1.77)			(15.33)		11.9-18.4	100 (1)
Lepidium virginicum		(27.52)		19.0-37.0		(1.50)			(18.35)		14.0-24.0	1,000 (1)
Lepidium apetalum Willd.	28.94	(29.19)	29.38	19.1-38.3	1.46	(1.47)	1.48	19.44	(19.79)	20.14	15.3-25.3	120 (2)
Lepidium virginicum L.		(29.46)		23.0-38.3		(1.50)			(19.68)		15.7-26.8	100 (1)

Table 6. Measurements of *Peronospora* conidia on Chenopodiaceae (Reprinted from W.D. Yerkes and C.G. Shaw. 1959. Taxonomy of the *Peronospora* species on cruciferae and chenopodiaceae. Phytopathology 49:499-507, by permission of the authors and the publisher American Phytopathological Society)

	Length (μ)				Quotient length/width)				Width (μ)				No. spores measured + no. collections
	Means			Range	Means				Means			Range	
	Min.	Grand	Max.		Min.	Grand	Max.		Min.	Grand	Max.		
Spinacia oleracea		(24.30)		17.6-32.0		(1.22)				(19.90)		12.8-25.6	500 (1)
Beta vulgaris L.	21.54	(24.81)	27.69	17.5-32.5	1.25	(1.30)	1.36		17.13	(18.94)	20.88	13.7-23.7	375 (15)
Chenopodium murale		(24.83)		16.0-32.0		(1.14)				(21.63)		12.8-28.8	500 (1)
Chenopodium hybridum		(24.98)		17.6-32.0		(1.46)				(17.11)		8.0-24.0	500 (1)
Chenopodium bonus-henricus		(26.01)		17.0-34.0		(1.13)				(22.86)		16.0-31.0	500 (1)
Chenopodium sp.	24.88	(26.55)	28.72	20.0-35.0	1.30	(1.34)	1.40		19.09	(20.09)	20.46	16.2-26.2	100 (4)
Chenopodium leptophyllum Nutt.		(26.68)		20.0-33.7		(1.38)				(19.28)		15.0-23.7	25 (1)
Spinacia oleracea L.	24.43	(26.92)	28.99	20.0-37.5	1.26	(1.37)	1.47		18.08	(19.61)	20.74	15.0-25.0	750 (30)
Chenopodium bonus-henricus L.	26.23	(26.95)	27.67	22.5-33.7	1.28	(1.30)	1.33		20.49	(20.70)	21.32	16.2-23.7	125 (5)
Chenopodium hybridium L.	23.94	(27.53)	33.44	16.9-40.2	1.30	(1.38)	1.50		17.11	(19.88)	22.23	15.3-25.7	150 (6)
Chenopodium gigantospermum Aeller		(28.58)		26.2-36.5		(1.46)				(19.57)		17.5-21.2	25 (1)
Chenopodium murale L.		(29.09)		25.0-35.0		(1.31)				(22.17)		16.2-26.2	25 (1)
Chenopodium album		(29.10)		17.2-40.2		(1.53)				(19.04)		12.2-27.2	500 (1)
Chenopodium album L.	23.83	(29.65)	33.53	20.0-40.0	1.31	(1.41)	1.58		17.77	(20.98)	23.17	14.6-27.5	825 (33)

only one haustorium for each host cell (Ohguchi and Asada, 1990). However, in root tissues where parenchyma cells are large and much closer together, the mycelia are smooth and one to several haustoria are formed in the infected host cell. Mycelial growth patterns in petioles and hypocotyls are similar to those in root tissue. Prior to haustorium formation a leaf like structure is formed from the intercellular mycelium in the narrow spaces between root parenchyma cells. It is flat, 6 μm thick and covers the surface of host cell. The leaf like structure forms various type of haustoria, ranging from 0-25 number in one cell.

In turnip and radish roots, the haustoria are initially spherical to pyriform, but later become cylindrical or clavate, and often dichotomously or trichotomously branched (Chu, 1935). In cabbage, some haustoria are large irregular vesicles while others are bilobed and regular in shape. In cauliflower, they are single, globose and uniform in size.

Variations in shape and size of haustoria of *P. parasitica* occur in hosts other than *Brassica* spp., such as *Matthiola incana*, *Cheranthus cheiri*, *Capsella bursa-pastoris*, *Diplotaxis muralis* and *Rhynchosynapis manensis* (Fraymouth, 1956). Penetration of the haustorial branch occurs through a hole 1-2 μ diameter in the cell wall which may form a collar-like structure round the base of the primordial haustorium. As the haustorium enlarges, invagination of host plasmalemma occurs and a sheath, possibly of callose, forms round the intrusive organ (Fraymouth, 1956). Moderately high temperatures of 20-24°C favour the most rapid development of the haustoria (Felton and Walker, 1946).

Conidiophores and conidia: After vegetative growth of the mycelium, erect conidiophores singly or in groups emerge vertically through stomata on the abaxial surface of the host leaves during a period of darkness. The conidiophores are hyaline and measure 200-300 μ. Condiophores are uniform with a flattened base and stout main axis. At 8°C, the rate of elongation reaches 100-200 μ h^{-1} and the whole process from emergence to spore formation takes approximately 4-6 h (Davison, 1968b). They are dichotomously branched, 6-8 times, tips bifurcate, branching acute and slightly thickened above each fork. The terminal branches are long, slender, pointed and end in a single conidium. The sterigmata are slender and acutely pointed (Butler, 1918; Holliday, 1980; Channon, 1981).

The conidia are hyaline, broadly elliptic, or nearly globose, measure 24-27 x 15-20 μ and are delimited from sterigmata by cross-walls at maturity. A single conidium is borne at the tip of each branch and is deciduous (Butler, 1918; Holliday, 1980). Detachment of conidia is possibly caused by hygroscopic twisting of the conidiophores which in turn is related to fluctuations in humidity (Pinckard, 1942). Conidia germinate in free water by a lateral germ-tube, not by zoospores. Infection occurs both by direct penetration of the epidermis and through stomata (Butler, 1918). In cauliflower leaves conidia form appressoria in the junction areas between the anticlinal walls of adjoining epidermal cells (Preece et al., 1967).

ii) Sexual phase: Sexual organs, gametogenesis, fertilization and oospore formation: During sexual reproduction *P. parasitica* forms spherical oogonia and paragynous antheridia. Oogonia are pale yellow, irregularly round, and swollen into crestlike folds (Butler, 1918; Holliday, 1980). Antheridia are tendril-like and are produced on separate hyphae. Wager (1900) observed that the protoplasm of the oogonium becomes differentiated into a central vacuolated ooplasm and a peripheral multinucleate granular periplasm. A receptive thin-walled papilla forms on the oogonium at the point of contact with the antheridium. A fertilizing tube grows from the antheridium through the receptive papilla towards a "central body" in the ooplasm,

to discharge a single "male" nucleus. Meanwhile, a single "female" nucleus detaches itself from the periplasm and also migrates towards the central body. The two nuclei fuse and initiate the uninucleate oospore. During ripening of the oospore the periplasm is deposited on its wall as an exosporial layer. The oospores are formed in the host tissues at late stage of sporulation. They have also been found in the cavity of the ovary on hyphae emerging between the cells of the inner epidermis of the carpels. The oospore lies inside, almost filling the cavity. The mature oospore is thick-walled, yellow-brown and globose or spherical, and measures 30-40 μ in diameter (Butler, 1918; Holliday, 1980). Oospore formation is favoured by conditions which induce senescence of the host tissues such as a deficiency of N, P, or K (McMeekin, 1960). Germination of oospores is by a germ-tube (Butler, 1918).

c. Electron microscopy and ultra structures

Electron microscopy in association with physiological, biochemical and genetical studies have provided information which helps in understanding the complex host-parasite relationship of this disease.

i) Host penetration: Penetration and haustorial formation in epidermal cells begins 6h after inoculation (Fig. 7B, C) (Chou, 1970). Appressoria, which look like swollen discs 7-10 μ across form at the junction of epidermal cells (Fig. 7B). At this stage, the appressoria and haustoria appear densely granulated, as the spores empty their contents during the process of germination and infection. The penetrating hyphae lay in between the anticlinal cell walls of the two epidermal cells (Fig. 7C) along with the formation of one or two haustoria, reaching to the adjacent mesophyll cells. After 45h, intercellular hyphae ramify through more cells and reach the opposite epidermis. At this stage, the haustoria appear broad and conspicuous, reaching 20μ in length. The intercellular hyphae are about 7μ across. Sometimes a sheath can be observed enveloping a fully grown haustorium (Fig. 7D). The earliest detectable stage of penetration is the formation of penetration hyphae which are as long as the vertical depth of the entire epidermal cell. The thick wall of the appressorium, continuous with the wall of the penetration hypha (Fig. 7A, 9A), has only a thin peripheral layer of cytoplasm. The cell contents migrate into the newly formed penetration hypha. In some cases, the appressorium can be seen to be embedded in an electron-dense, vacuolate material (Fig. 9B) appearing to be a mucilaginous sheath. This sheath is bound by an outer membrane which adheres to the cuticle of the host epidermis with the exception of in the penetration region where it is slightly separated (Fig. 9B). The penetrating hyphae wedge into the middle lamella between the anticlinal walls of two epidermis cells. The hole in the wall through which the fungus penetrates is 4-5μ across. After entering the host, the hypha expands to a diameter of 7-8μ. There is no clearing zone or dissolution of wall material in the immediate vicinity of the penetrating hypha. The penetrating hypha is always seen to be embedded in a moderately electron -

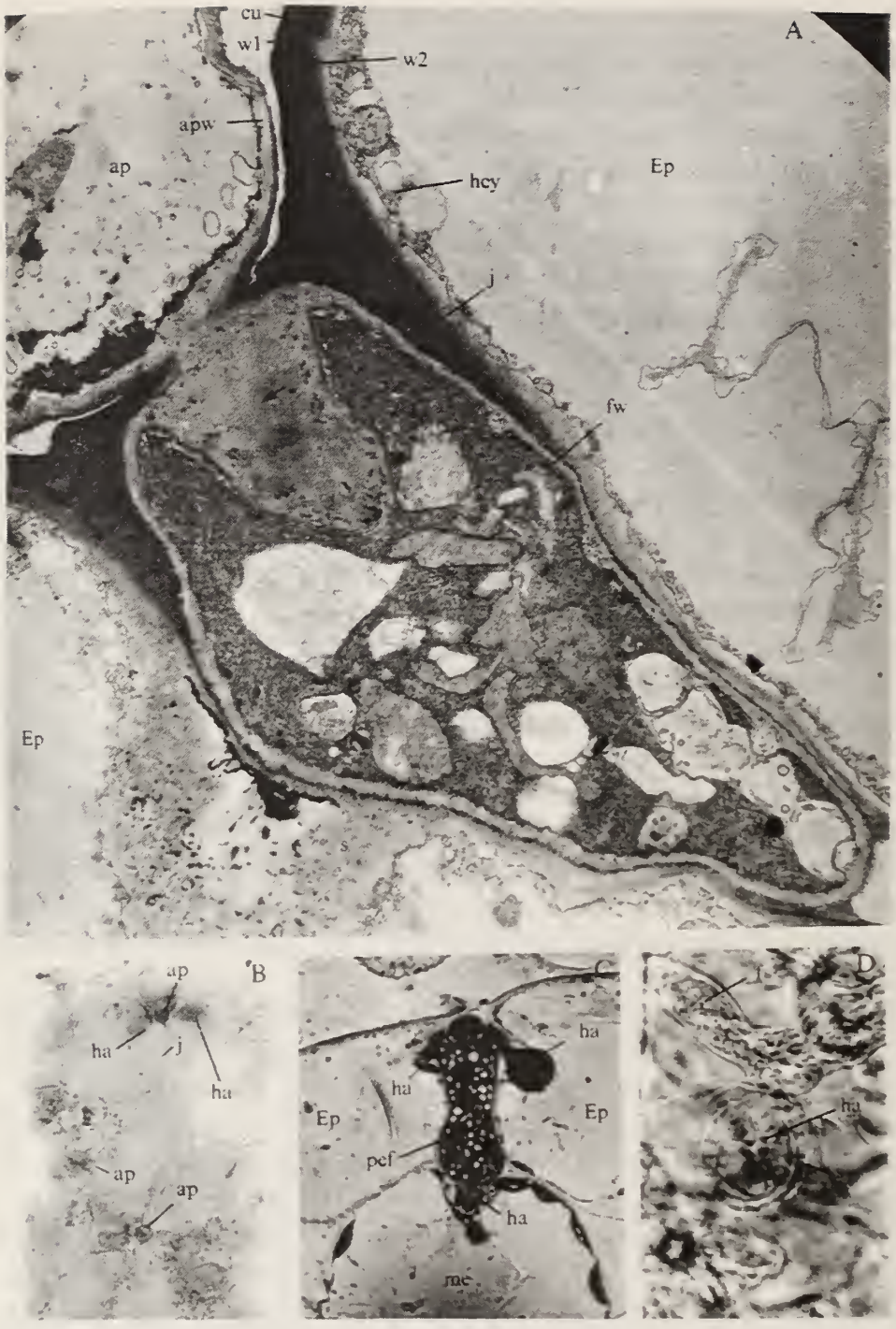


Fig. 7. (A) Electron micrograph of T.S. of epidermal cells of cabbage cotyledon at 6-h after inoculation showing appressorium (ap) and penetrating hypha of *Peronospora parasitica* in between the anticlinal walls (j) of host epidermal cells. In one of the cells a haustorium was formed but the section only shows part of sheath(s). The penetration was cut obliquely and part of the hyphal wall (arrow pointed) is shown. x 8200; (B) Photomicrograph of whole mount of a cleared cabbage cotyledon at 6-h after inoculation showing appressorium (ap) formation predominantly at the junction line of epidermal cells. x 313; (C) Photomicrograph C.T.S. of cabbage cotyledon at 6-h after inoculation showing penetration as in A. x 500; (D) Photomicrograph of whole mount of a cleared cotyledon showing intercellular hypha and haustorium completely ensheathed. x 840 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

Abbreviations for Figures 7-17:

ap = appressorium; apw - appressorium wall; ch = chloroplast; cu = cuticle; cy = cytoplasm; d = dense granules; Ep = host epidermal cell; f = hypha; fw = hyphal wall; h = host; ha = haustorium; hai = haustorium initial; hap = haustorium plasmalemma; haw = hautorium wall; hp = host plasmalemma; ht = host tonoplast; hw = host wall; j = anticlinal wall or junction line of host epidermal cells; lo= lomasome; m = membrane; ma = matrix of dense layer; me = mesophyll cell; mi = mitochondria; mu = mucilaginous sheath or substance; n = nucleus; ne = neck of haustorium; pef = penetrating hypha; s = sheath; smx = sheath matrix; v = vacuole; w1 = cuticular layer; w2 = wall proper; z = dense zone; z1 = outer dense zone; z2 = inner dense zone;

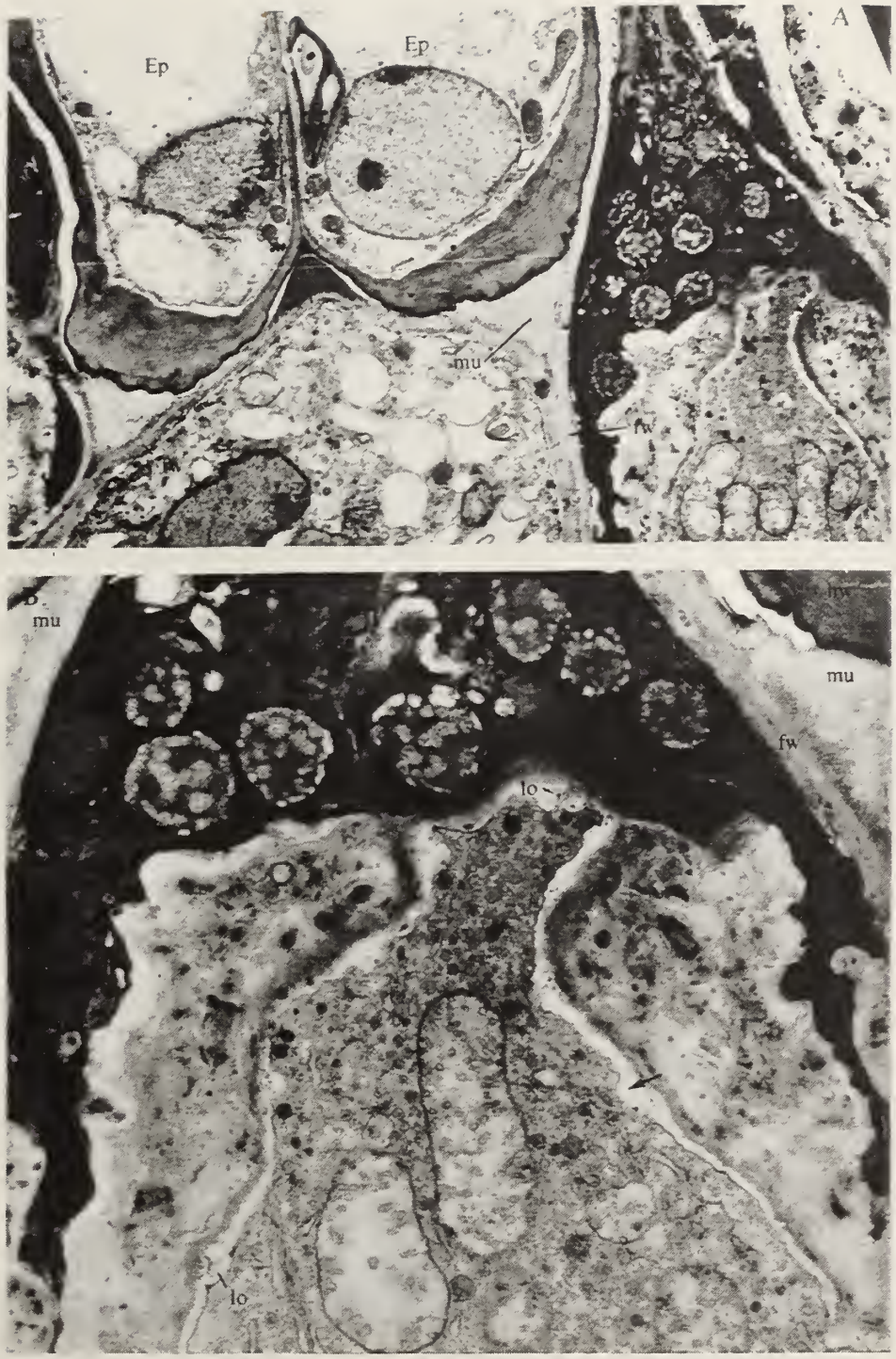


Fig. 8. (A) Electron micrograph of T.S. of epidermal cells of cabbage cotyledons at 8i-h after inoculation showing intercellular hyphae at various stages of penetration to the outside of host epidermis. Arrow points at the spearhead-like thickening of hyphal tip. x 5400; (B) Electron micrograph of part of outgrowing hypha in between two host epidermal cells showing die-back of hyphal tip and walling-off (arrow pointed) of apparently intact cytoplasm. x 18000 (Reprinted from C.K. Chou. Z1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

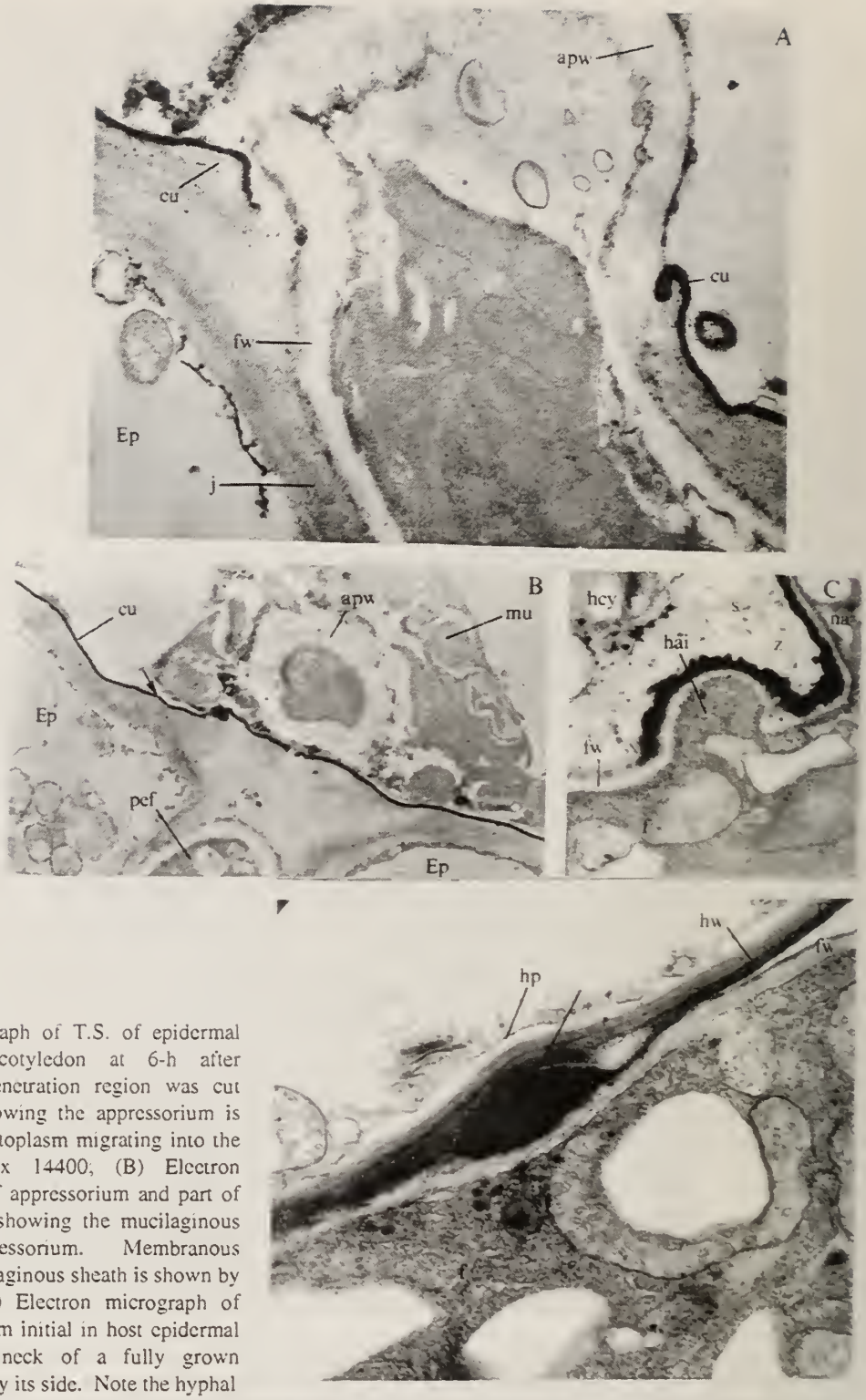


Fig. 9. (A) Electron micrograph of T.S. of epidermal cells of cabbage cotyledon at 6-h after inoculation. The penetration region was cut medianly through showing the appressorium is almost empty with cytoplasm migrating into the penetrating hypha. x 14400; (B) Electron micrograph of T.S. of appressorium and part of host epidermal cells showing the mucilaginous sheath of the appressorium. Membranous boundary of the mucilaginous sheath is shown by arrow. x 6000; (C) Electron micrograph of section of a haustorium initial in host epidermal cell. Part of the neck of a fully grown haustorium is shown by its side. Note the hyphal wall is continuous with wall of the intercellular hypha at this stage. x 13800; (D) Electron micrograph of a section of intercellular hypha and host epidermal cell showing part of host wall in contact with hypha is swollen and partially eroded (arrow). x 17700 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

dense matrix of the middle lamella (Fig 7A, 9A). The cuticle breaks and fits closely around the penetrating hypha. No sign of swelling or change in electron density of the cuticle can be detected in the immediate vicinity of the penetration zone (Chou, 1970).

After 80h, hyphal growth develops conidiophores which may be seen coming out from the epidermal cells (Chou, 1970). The intercellular hyphae appear to aggregate beneath the epidermis and grow either through stomata or in between two epidermal cells to the outside of the host tissue (Fig. 8A). An electron-dense spearhead-like thickening of the hyphal tip is observed to wedge in between two guard cells. This thickening may give rigidity to the hyphal tip for penetration. The hyphae are cemented to each other and also to the host cell walls by an amorphous, moderately electron-dense material, presumably of a mucilaginous nature (Fig. 8A, B). Hyphae penetrating through the junction of epidermal cells invariably show a die-back of the tip (Fig. 8A). A new wall is laid down round the remaining living cytoplasm, while a new growing tip is organised to carry on further growth (Fig. 8B). Large numbers of lomasomes appear around the newly formed walls and numerous dense vesicles approximately 500-1000Å in diameter are concentrated in the walled-off cytoplasm.

ii) Haustorium development: Host penetration by haustoria of the Peronosporales is usually by boring a narrow canal at the point of contact between the hypha and the host cell wall (Fraymouth, 1956). However, according to Chou (1970), it is not possible to find the stage at which the walls of both host and pathogen are perforated prior to haustorial initiation. Localized swelling of the host wall (3X original) is observed in the area of hyphal contact. The swollen area is about 1.5-2μ long, and shows a clearer fibrillar structure, with a partially eroded area (Fig. 9D). The dimension of the swollen region coincides closely with the size of the hole in the host wall made by the haustorium. These observations strongly suggest that the breach of host wall during haustorium initiation is achieved at least partly by chemical means. A dome-shaped protuberance, about 1μ in diameter is formed by the bulging of the wall of intercellular hypha into the lumen of host cell (Figs. 9C, 10A). The host wall is perforated at this stage and the wall of the haustorium primordium is continuous with that of the intercellular hypha (Fig. 9C). The haustorium initial is completely enclosed in a mound-like sheath quite distinct from the host wall in structure as well as density. The perforation made by the invading haustorium measures 1-2μ across. The perforated host wall in most cases remains smooth, but a slight infolding of the wall to form a short collar-like structure is sometimes observed. The external part of the wall of the haustorial initial consists of a very electron-dense layer varying in thickness from 0.1-0.2μ and exhibiting an undulating surface bounded externally by a thickened membrane (Figs. 9C, 10A) which is presumably the invaginated plasmalemma of the host. The primordial haustoria is filled with homogeneous ground-plasm packed with ribosomes. Lomasomes are the only organelles present at this stage. The growth and differentiation of the primordial haustorium is in the form of an elongated neck and

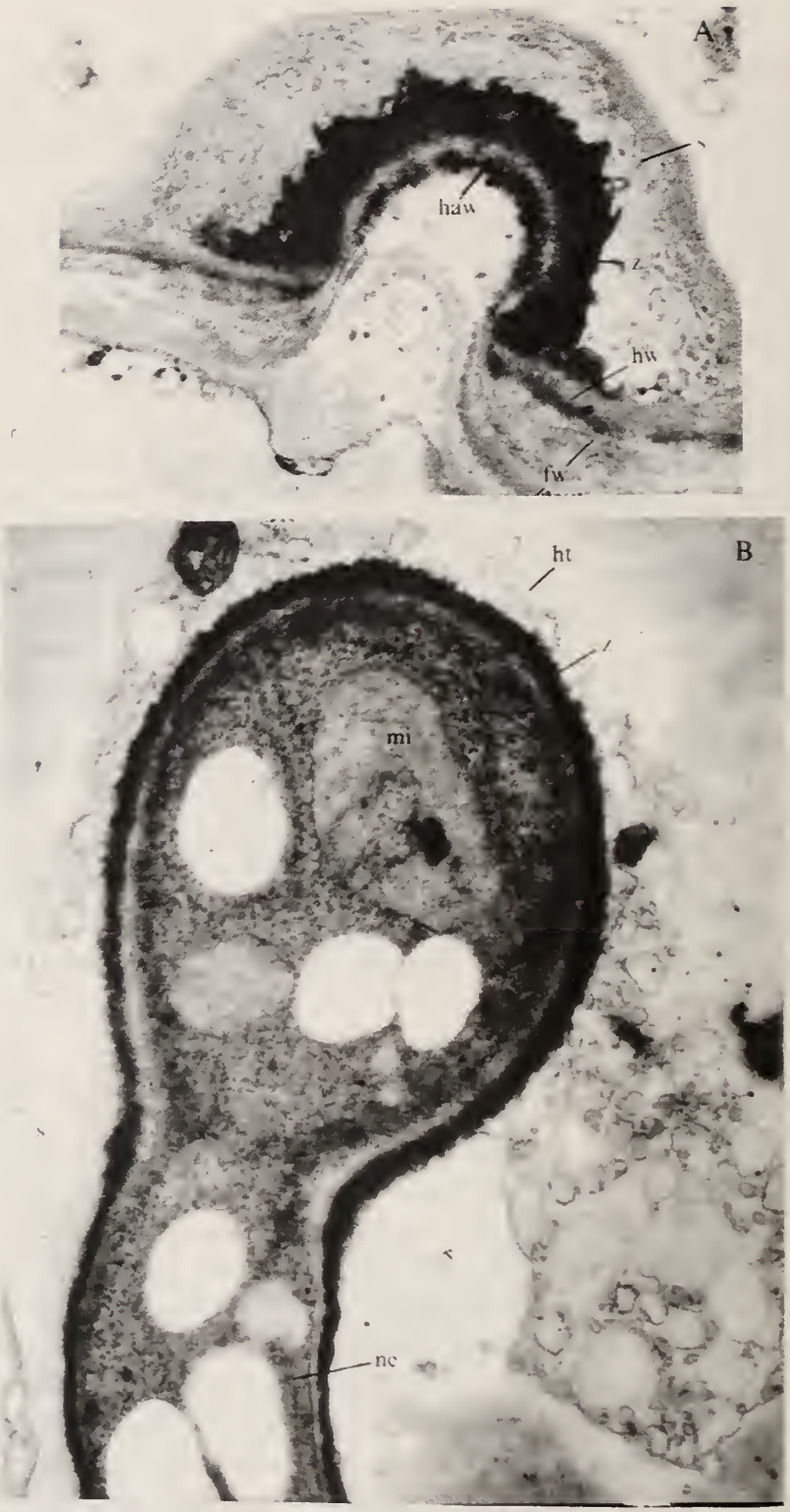


Fig. 10. (A) Electron micrograph of a section of a haustorium initial in host mesophyll cell (section slightly oblique to the penetration zone). x 16500; (B) Electron micrograph of a section of a very young haustorium in host epidermal cell showing breakdown of host cytoplasm into large number of vesicles. x 18000 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

expanding head (Fig. 10B). The sheath seems to burst apart, remaining as a collar-like structure around the neck region (Fig. 10B).

In a young haustorium the contents are invariably dense with a high population of ribosomes, a profuse system of endoplasmic reticulum and relatively few vacuoles (Chou, 1970). The dictyosomes occur more frequently and the mitochondria are strikingly irregular (Figs. 11, 12, 15B). The same pattern of these structures are also present in young penetration hyphae. A complicated membrane system of unknown nature and origin is always present (Fig. 16B, C). One type consists of a complicated system of tubules and vesicles enclosed by a unit membrane. The inter-tubular spaces do not contain ribosomes. This organelle looks like a lomasome except that there is no apparent connection with plasmalemma. Another type consists of whorls of closely packed membranes formed in vacuoles (Fig. 16C). Generally the lomasomes are more or less hemispherical to saucer-shaped, about 0.2-0.3μ in the longer diameter, but occasionally they can extend to 2-3μ in diameter (Fig. 12). The tubules and vesicles of lomasomes range from 15 to 80μm in diameter. The nuclei are about 3-3.5μ in diameter. As many as three sections of nuclei are observed in one haustorium section (Fig. 15A). The nuclei envelope consists of a double membrane interrupted by pores. The envelope is very similar in form to the endoplasmic reticulum and connections between these two are often observed. The endoplasmic reticulum is mainly of the smooth type (Fig. 15A, 12) enlarged in part to form cisternae of various forms. The mitochondria are large (1-2μ in diameter), usually elongated dumb bell shaped or irregularly branched (Figs. 12, 15A, 7A, 9D). Those in old haustoria are roundish with a much less dense matrix (Fig. 15B).

iii) The host - pathogen interface: The external surface of the haustorial walls always appears to consist of very electron dense layer (Figs. 10B, 11, 12, 15A, 16A, 17C, D) which is well-developed at the earliest stage of haustoria development (Chou, 1970). The outer region of the hyphal wall can be further differentiated into a well-defined, very dense and thin outer boundary, about 50-100Å thick, and an inner less dense zone of rather obscure lateral limit (Fig. 14E). The hyphal wall thus appears to be a three-layered structure. The zone of apposition of the haustorial wall consists of a well-defined very dense and thin outer layer approximately 50-100Å thick and a broad, slightly less-dense inner zone without a well-defined boundary (Fig. 14D). Chou (1970) proposed that the zone of apposition should be termed as an outer and inner dense zone being both an integral part of the haustorial wall. The surface of the haustorium neck is covered by a dense layer much thicker than that of the rest of the haustorium. Its surface always appears to be deeply roughened with numerous vesicular and tubular extensions (Figs. 12, 13B, 17B). Dense granular bodies can sometimes be seen lodged between the surface of the dense layer and the invaginated host plasmalemma and in both of the matrix of the dense layer and of the tubular extension (Figs. 13A, 17B). There is a frequent occurrence of a porous substance of uniform pore diameter (about

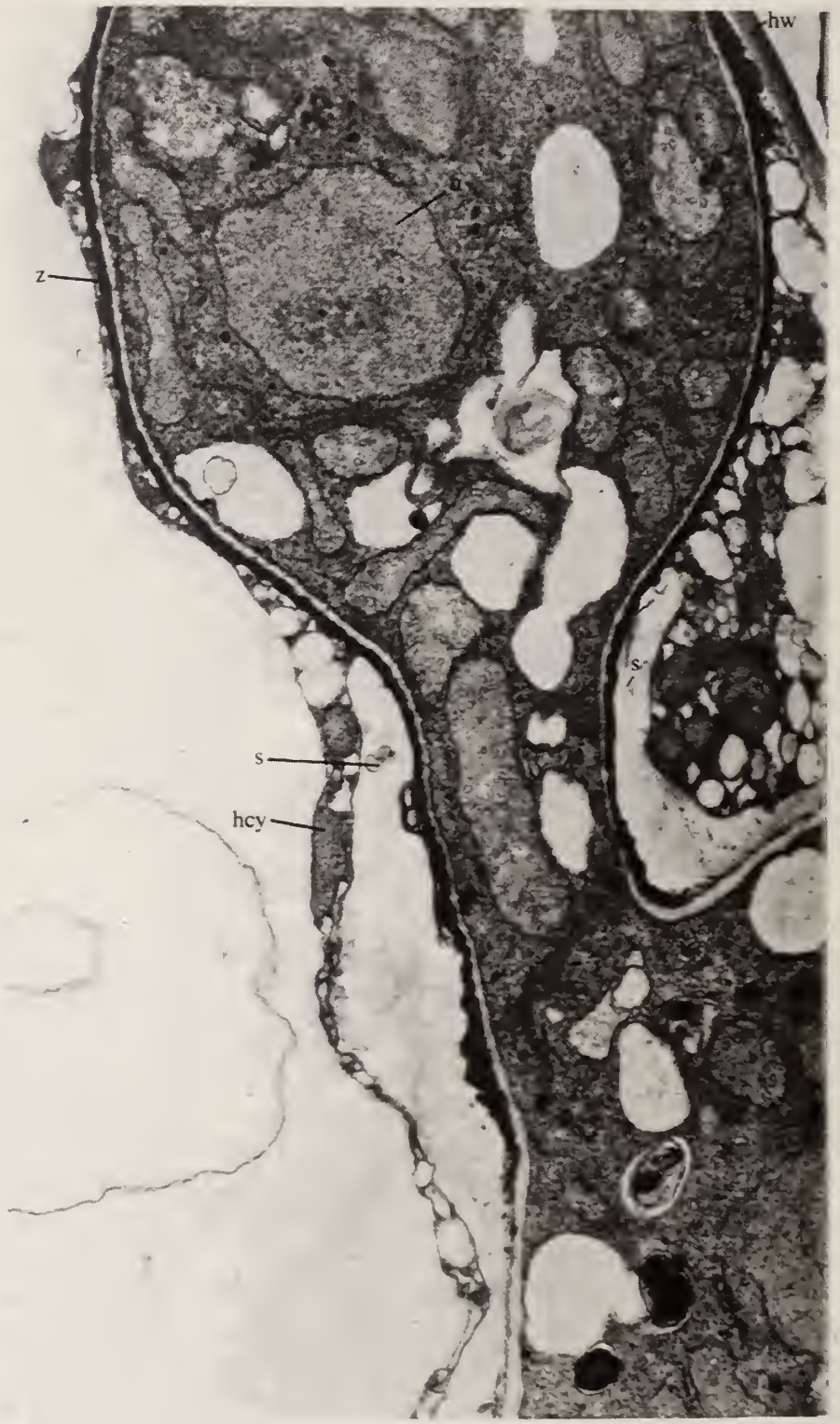


Fig. 11. Electron micrograph of a section of a haustorium in host mesophyll cell at 6h after inoculation. x 12000 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

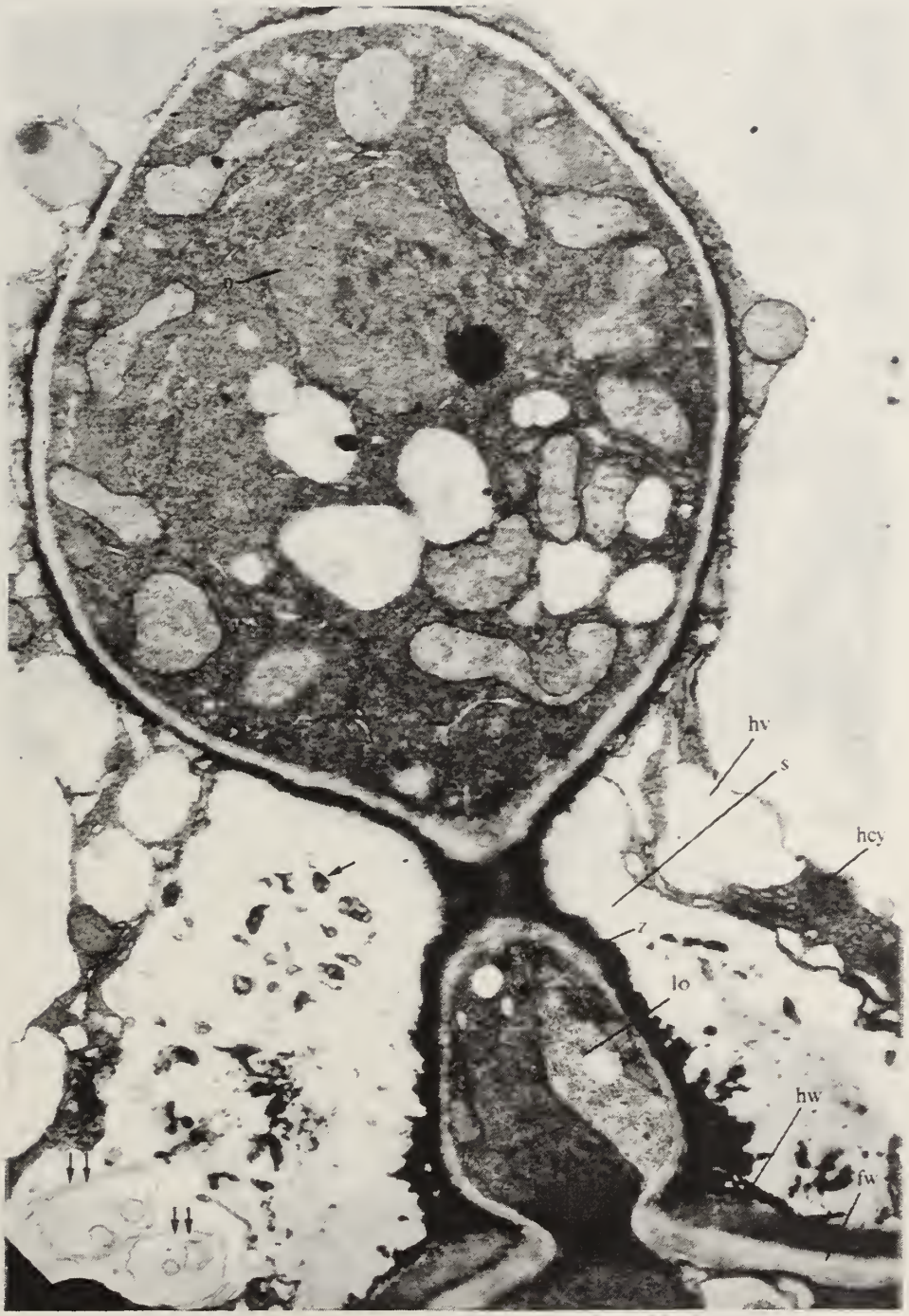


Fig. 12. Electron micrograph of a section of a haustorium in host mesophyll cell at 6h after inoculation, showing the sac-like sheath and numerous vesicles (arrow pointed) and intravacuolar vesicles (pointed out by double arrow) in the sheath matrix. x 8580 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

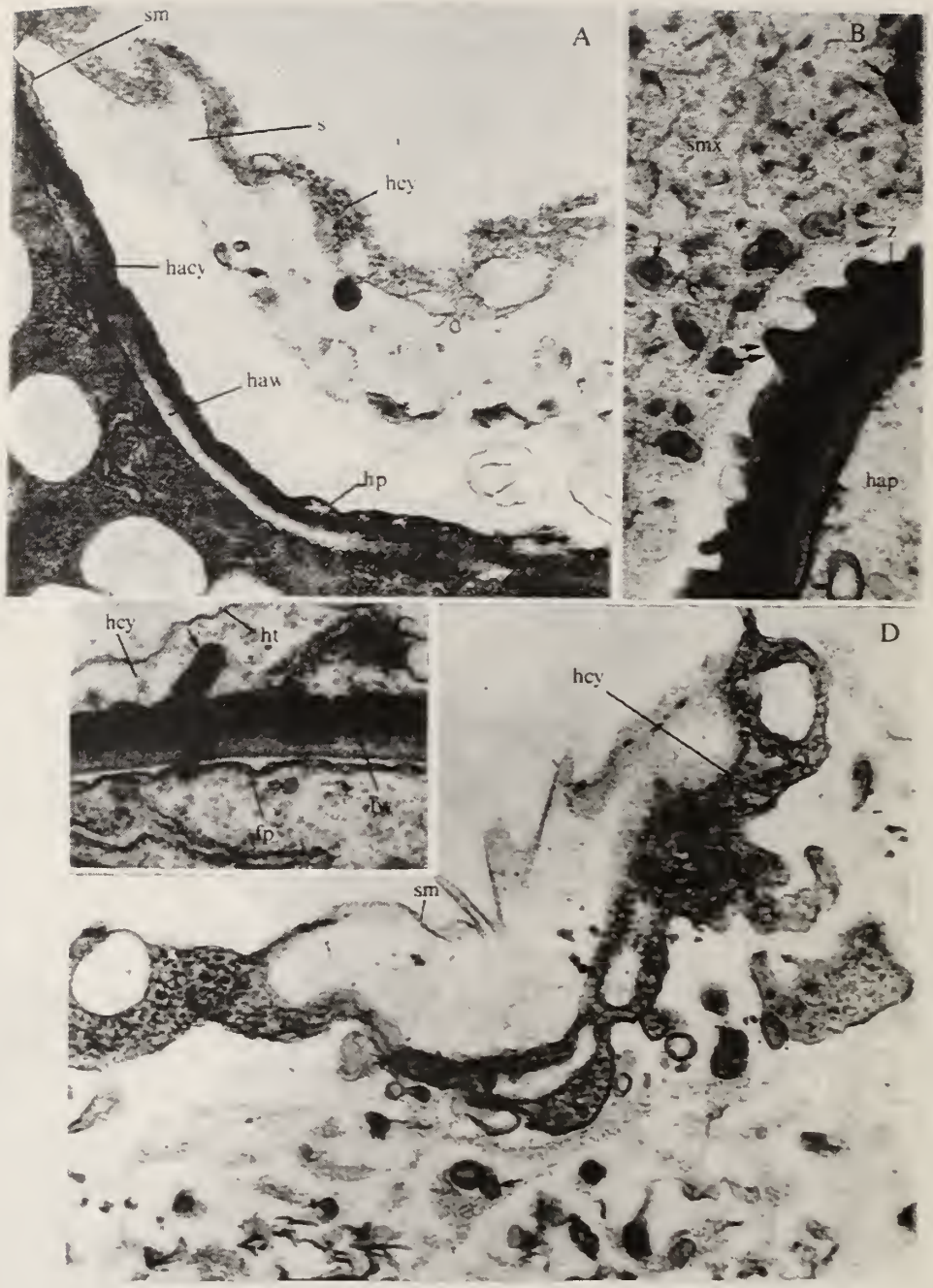


Fig. 13. (A) Electron micrograph of a section of part of haustorium neck and sheath. X 24600; (B) Electron micrograph of a section of part of haustorium neck and sheath showing numerous vesicles (arrow pointed) and dense granules in the sheath matrix (Smx) and the dentate extensions (pointed out by double arrow) of the dense zone (z) of haustorium wall. x 33000; (C) Electron micrograph of a section of the interface between haustorium and host cytoplasm showing a dense vesicle (arrow pointed) like the secretory body. x 33000; (D) Electron micrograph of a section of haustorium sheath showing incorporation of host cytoplasm (arrow pointed) in the sheath matrix and numerous membrane-bounded vesicles both in host cytoplasm and the sheath matrix. x 33000 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London)

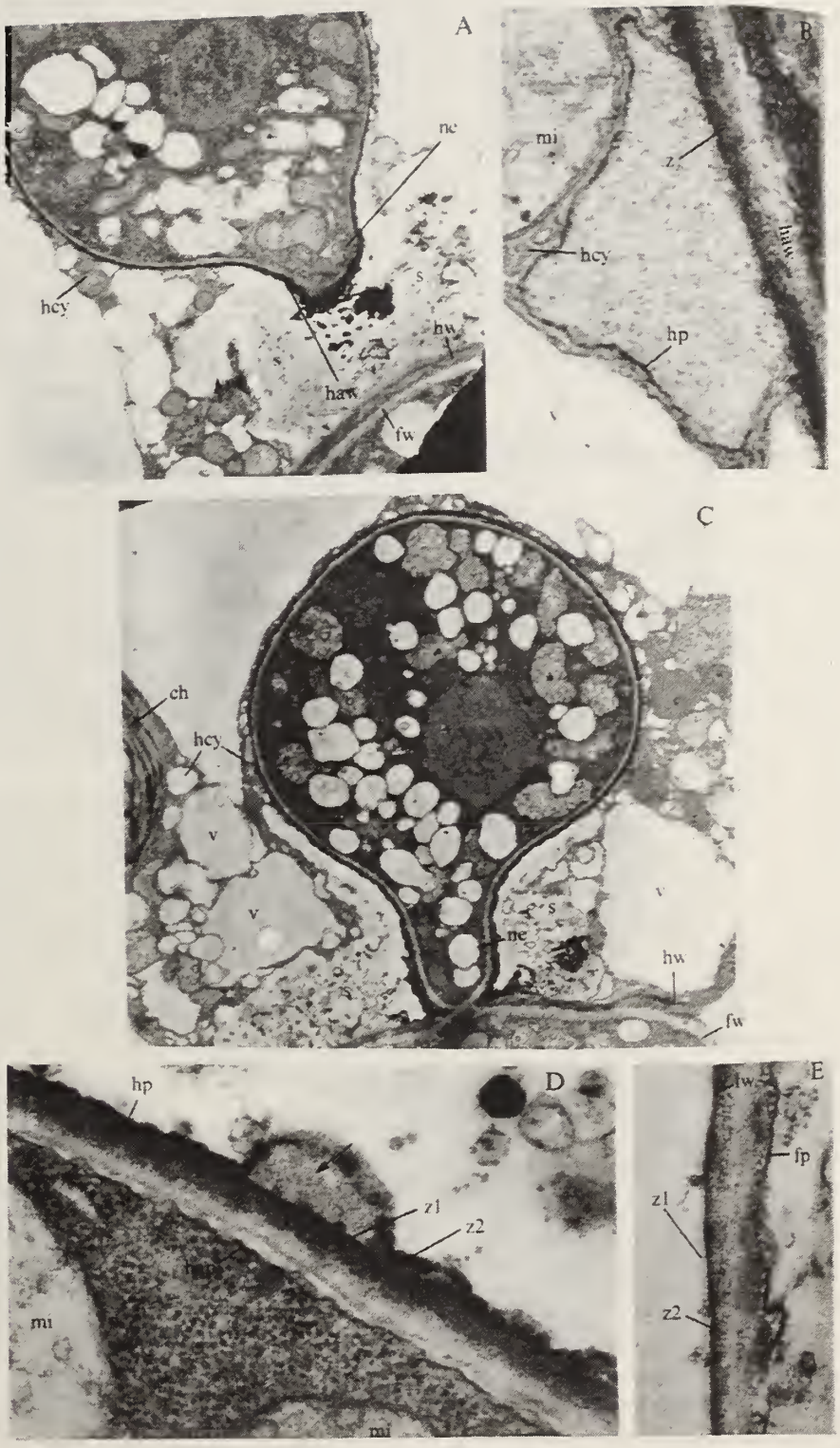


Fig. 14. (A) Electron micrograph of a section of haustorium in host mesophyll cell showing the vacuoles or provacuoles possibly in the process of fusion with each other and also with the sheath (arrow). x 7200; (B) Electron micrograph of a section of the interface between haustorium and host cytoplasm showing vesiculation of the host plasmalemma. x 48000; (C) Electron micrograph of a section of haustorium in host mesophyll cell showing fusion of vacuoles in host cytoplasm and sheath formation. x 7200; (D) Electron micrograph of a section of interface between haustorium and host cytoplasm showing the structure of outer dense zone of haustorium wall distinguished into two well-defined layers (z1) and (z2). x 49500; (E) Electron micrograph of a section of intercellular hyphae showing the hyphal wall also exhibiting a dense outer layer composed of z1 and z2. x 33000 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

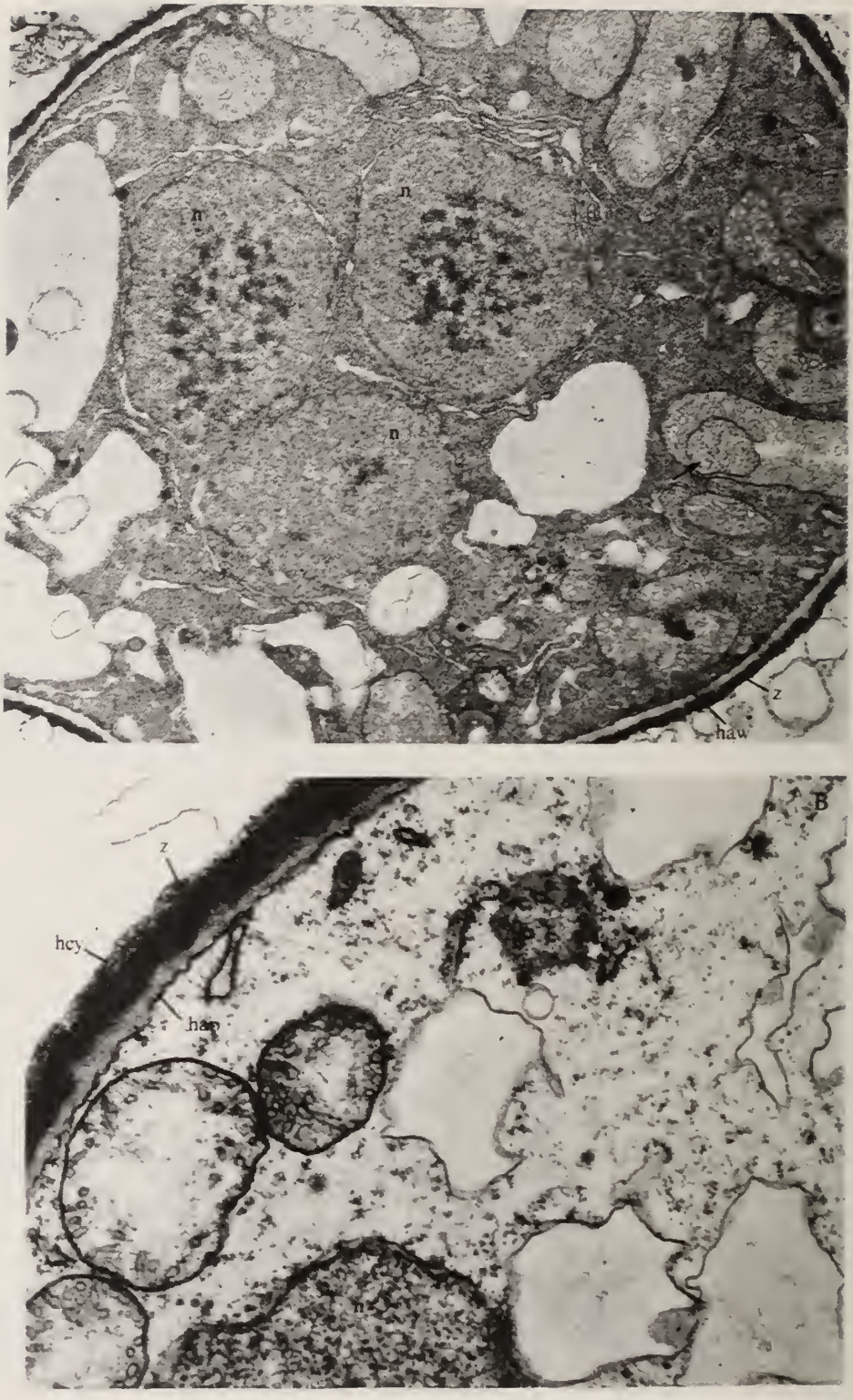


Fig. 15. (A) Electron micrograph of a section of haustorium in epidermal cell at 6h after inoculation showing the typical fine structure of haustorium at this stage. Ring formation in mitochondria pointed out by arrow. x 13200; (B) Electron micrograph section of haustorium in epidermal cell 45h after inoculation. x 24000 (Reprinted from C.K. Chou.1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

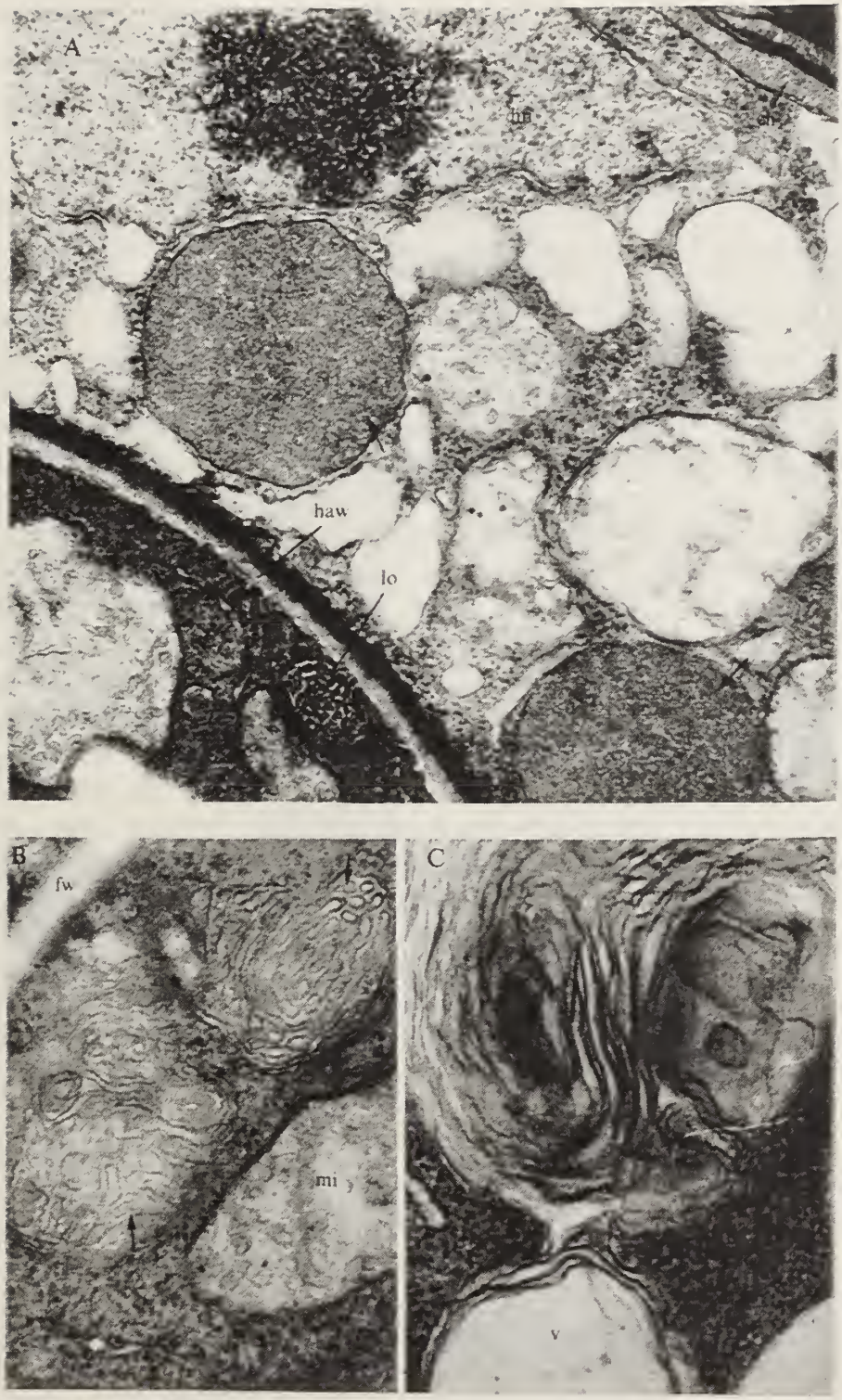


Fig. 16. (A) Electron micrograph of a section of the interface between haustorium and host cytoplasm of mesophyll cell showing sphaerosome-like bodies (arrow pointed) in host cytoplasm. x 33000; (B & C) Electron micrograph of sections of young penetrating hyphae; (B) showing complicated membrane system of unknown nature. (C) showing intravacuolar membrane systems. x 55200 and 36000 respectively (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London).

200Å) covering the entire haustorium surface. The host plasmalemma covering the haustorium surface is often masked due to the accumulation of this substance (Fig. 17A, D).

As soon as the haustorium penetrates the host, the haustorium becomes covered with a layer, moulded to its shape and produced by the host protoplast (Fraymouth, 1956). This layer is named "The Sheath" and appears to be composed of modified cellulose and callose. A sudden increase in the growth rate of the fungus often causes the sheath to burst, remaining as a collar around the base. A morphologically analogous structure enveloping a haustorium initial which has penetrated the host wall (Figs. 9C, 10A) has been detected in the cabbage - *Peronospora* system by Chou (1970). In mature haustoria which have differentiated into a neck and head, the sheath remains as a collar-like structure at the base (Figs. 11, 12) although completely ensheathed mature haustoria are sometimes observed under the light microscope (Fig. 7D). Electron microscope observation revealed that the sheath is a sac-like structure sometimes flattened to a narrow strip (Fig. 11), but in most cases dilated to a broadly conical shape, and quite distinct in texture and electron density from the host wall. The sheath is bounded by a unit membrane which is generally presumed to be the host plasmalemma. No membranous structure can be detected along the sheath/host-wall interface, though the two can be clearly distinguished by their difference in electron density and texture. The sheath matrix is electron transparent, while the host wall is moderately electron dense and often exhibits a fibrillar structure (Fig. 8A). The sheath matrix is always permeated by large numbers of blurred electron-dense granules, and dense vesicles with single or double membranes. These vesicles appear to be of host origin, as they are also found in the adjacent host cytoplasm (Fig. 13B, D, 12). The sheath matrix is also interspersed with host cytoplasm (Fig. 13D) which occurs in isolated packets or as an extension of the adjoining host cytoplasm. The permeation of vesicles into the sheath matrix and the extension of host cytoplasm within it suggest a liquid or semi-liquid state of the sheath matrix (Fraymouth, 1956; Chou, 1970). During penetration, the host cytoplasm adjoining the sheath increase markedly in amount and comes to contain a large number of vacuoles (Figs. 11, 12, 14A, C) (Chou, 1970). At an early stage of haustorium development, coalescence of these vacuoles with the sheath can be seen. Intrusion intravacuolar vesicles in the host cytoplasm and in the sheath can be seen (Figs. 12, 13A, C).

iv) Conidiophore development: Conidiophore development of *P. parasitica* can be divided into five stages (Davison, 1968).

(a) Conidiophore primordia: The emergence of *P. parasitica* from the host cotyledons during sporulation, can be seen as a densely stained region beneath the host stomata. In the substomatal space, a hyphal branch, about 5 μ in diam., grows towards the stoma and then between the guard cells (Fig. 18A). When the

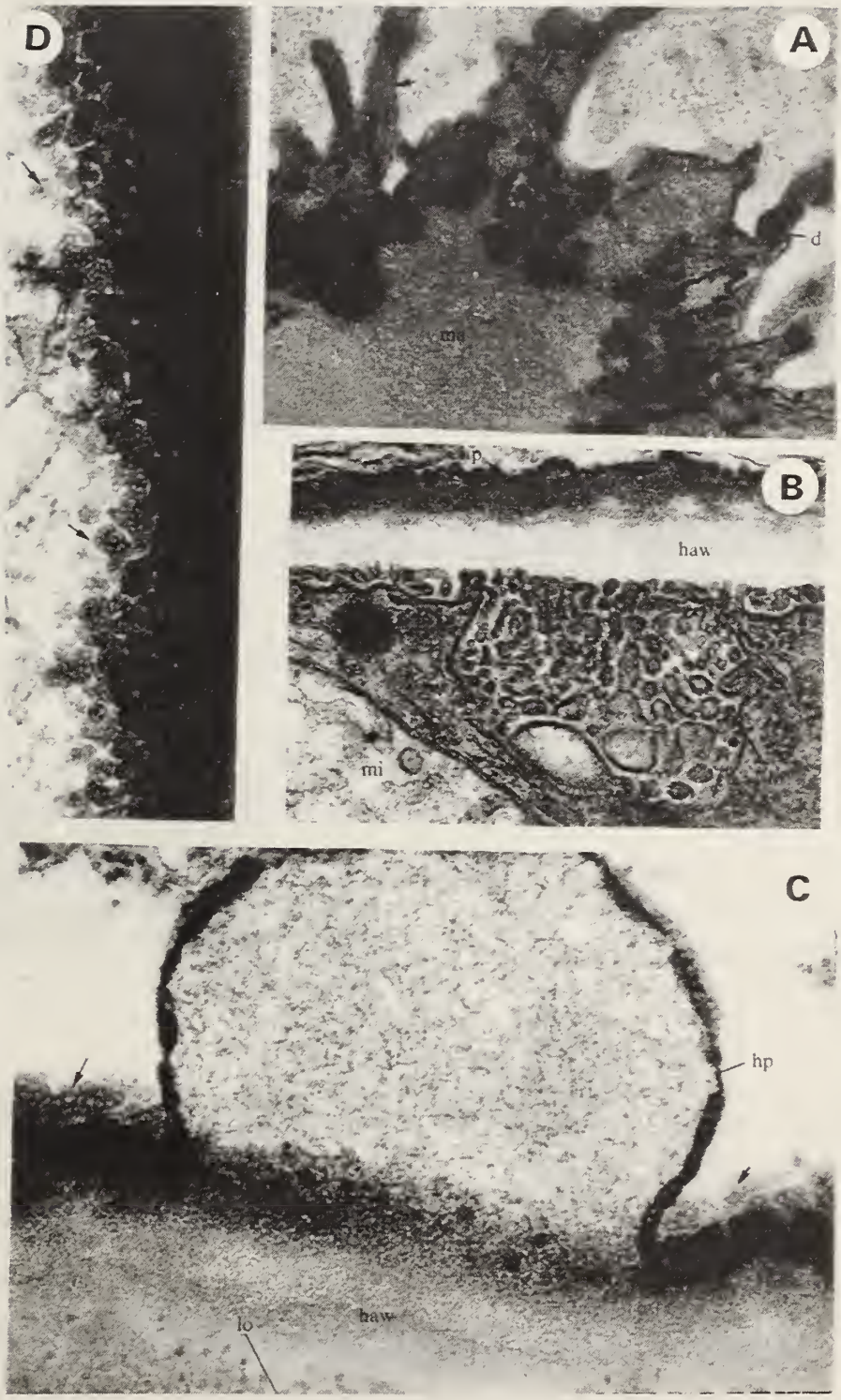


Fig. 17. (A) Tangential section of the dense zone of haustorium neck showing foldings of host plasmalemma (arrow pointed) forming tubular extensions and incorporation of numerous dense granules (d). x 33000; (B) Electron micrograph section of haustorium in host epidermal cell showing lomasome. x 55200; (C) Electron micrograph of a section of haustorium in host epidermal cell showing pinocytotic vesicles formed from host plasmalemma and abundant porous substance (arrow pointed) at the host-parasite interface. x 73200; (D) Electron micrograph section of interface between dense zone of haustorium and host cytoplasm showing the deposition of porous substance (arrow pointed). x 48000 (Reprinted from C.K. Chou. 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204, by permission of the author and the publisher Academic Press Limited, London)

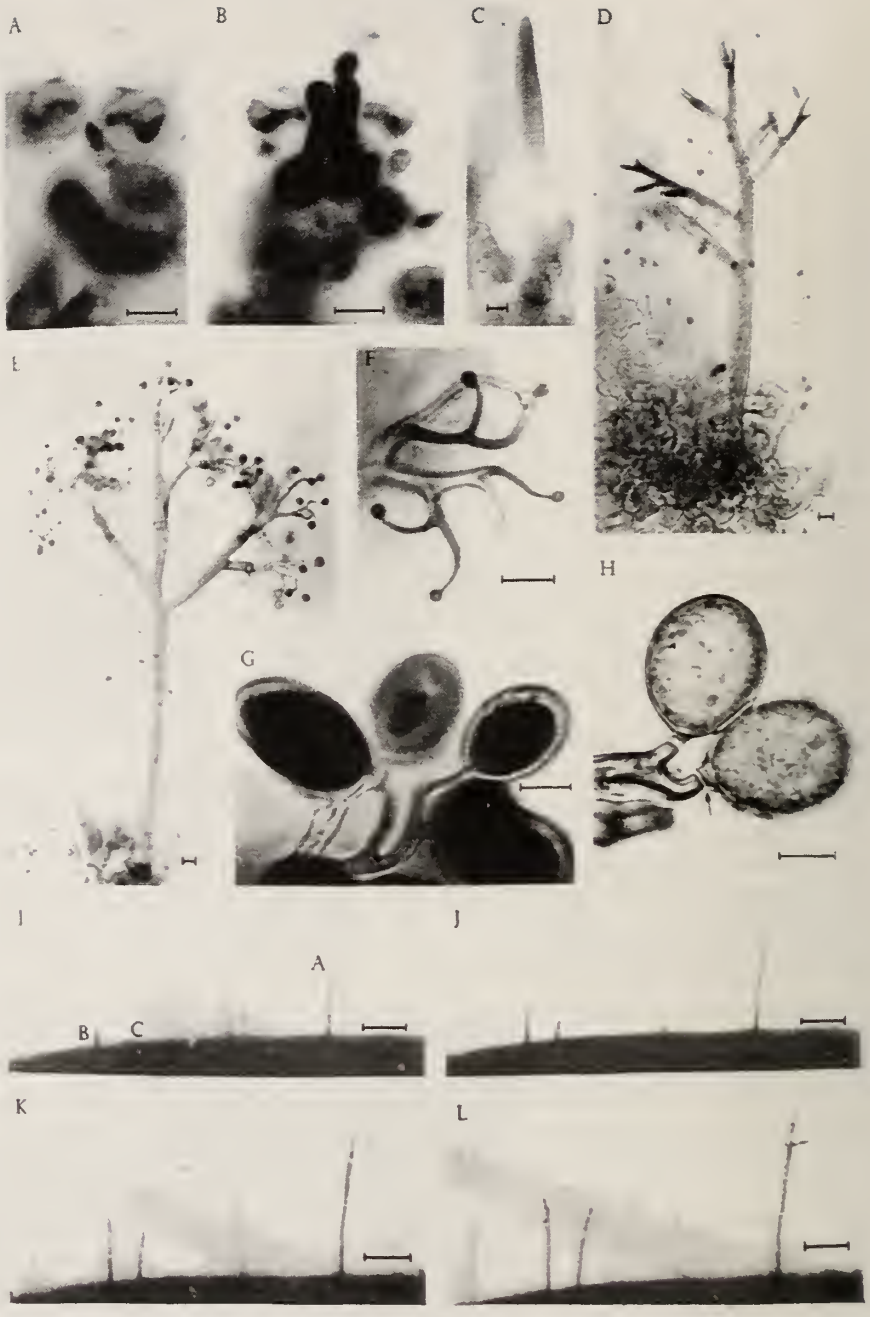


Fig. 18. (A) Section of wax-embedded material showing a hyphal branch growing towards a stoma; (B) section of wax-embedded material illustrating two conidiophore primordia one of which is beginning to grow; (C) stained and macerated preparation of an unbranched conidiophore; (D) stained and macerated preparation of a branched conidiophore; (E) a branched conidiophore with small spores in a stained and macerated preparation; (F) very young spores; (G) mature spores; (H) mature spores delimited by a cross wall (arrow); (I-L) frames from the cine film illustrating the development of conidiophores A, B, and C; (I) incubation time 3h 30 min.; (J) incubation time 3 h 50 min.; (K) incubation time 4h 10 min.; and (L) incubation time 4h 30 min. A-H scale line is 10μ, I-L scale line is 100μ (Reprinted from E.M. Davison. 1968. Development of sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 623-631, by permission of the author and the publisher Academic Press Limited, London).

tip of this hypha is about level with the top of the guard cells it becomes more rounded, and completely blocks the stomatal pore (Fig. 18B). It is referred to as conidiophore primordium. According to Shiraishi et al. (1975) a contracted image is found in the region when the conidiophores develop.

(b) Unbranched conidiophores: It is the earliest stage of conidiophore development visible on the surface of the host, and can be seen about 4h after the cotyledons are placed in a moist, dark environment. From the primordia, unbranched conidiophores may develop immediately or a narrow wall surrounding a "blow out" forms at the apex (Fig. 18B). The basal constriction surmounted by a bulge which is observed in older conidiophores are probably the result of the "blow-out" formation. Developing conidiophores are more or less cylindrical at this stage (Fig. 18C), approximately 10-12 μ in diameter and of varying length.

(c) Production of branches: When the conidiophores reach about two-third of its eventual height, branches are formed one at a time, just behind the conidiophores apex (Fig. 18D). Secondary and tertiary branches are also formed which are narrower than the primary ones. The conidiophore axis is also narrower at the apex, with decrease in branch diameter being proportional to the increase in branch length. The ultimate branches are very slender, often about 1 μ in diameter, and usually curved. Branches form at a projected angle of 55-85° with the major axis, and the number produced is approximately proportional to the condiophore height.

(d) Development of conidia: Young conidia are formed about 2h after initiation of branch production. Initially, conidia are spherical, but as they increase in size they become ellipsoidal (Fig. 18E). Conidia produced on a single conidiophore are of uniform size, but conidia borne by different conidiophores frequently vary in size.

(e) Formation of a cross wall: Conidia are delimited by a cross wall about 2h after the beginning of conidial formation, when they reach about 15 x 20 μ in size (Fig. 18H). However, the cross walls are observed only occasionally since spores are usually detached before cross wall formation.

v) Conidiophore growth: The increase in conidiophore length shows an initial slow period of elongation, just after the fungus has emerged from the host leaf followed by a rapid increase (Figs. 19, 20, 21). During branch formation increase in length is slightly slower and less regular, while just before spore formation conidophore elongation slows down and almost ceases. Once formed, the spores enlarge rapidly, but increase in conidiophore length is only by the enlargement of the apical spore. The most rapid rate of elongation of conidiophores is 100-200 μ/h. As the branches usually begin

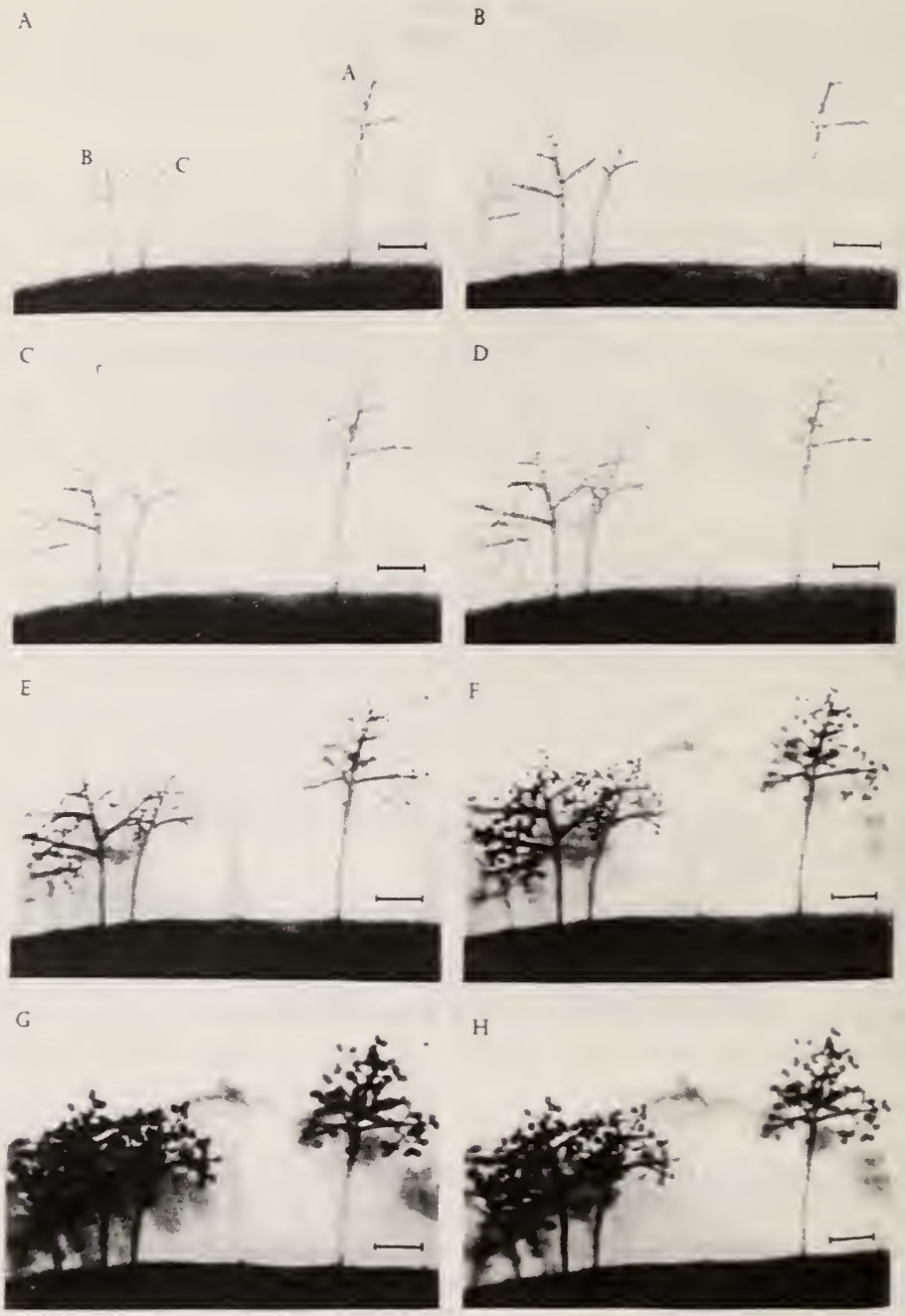


Fig. 19. Continued development of conidiophores (A) incubation time 4h 50 min.; (B) incubation time 5h 10 min.; (C) incubation time 5h 30 min.; (D) incubation time 5h 50 min.; (E) incubation time 6h 10 min.; (F) incubation time 6h 30 min.; (G) incubation time 6h 50 min.; and (H) incubation time 7h 30 min. Scale line is 100μ (Reprinted from E.M. Davison. 1968. Development of sporangiophores of *Peronospora* parasitica (Pers. ex Fr.) Fr. Ann. Bot. 32: 623-631, by permission of the author and the publisher Academic Press Limited, London).

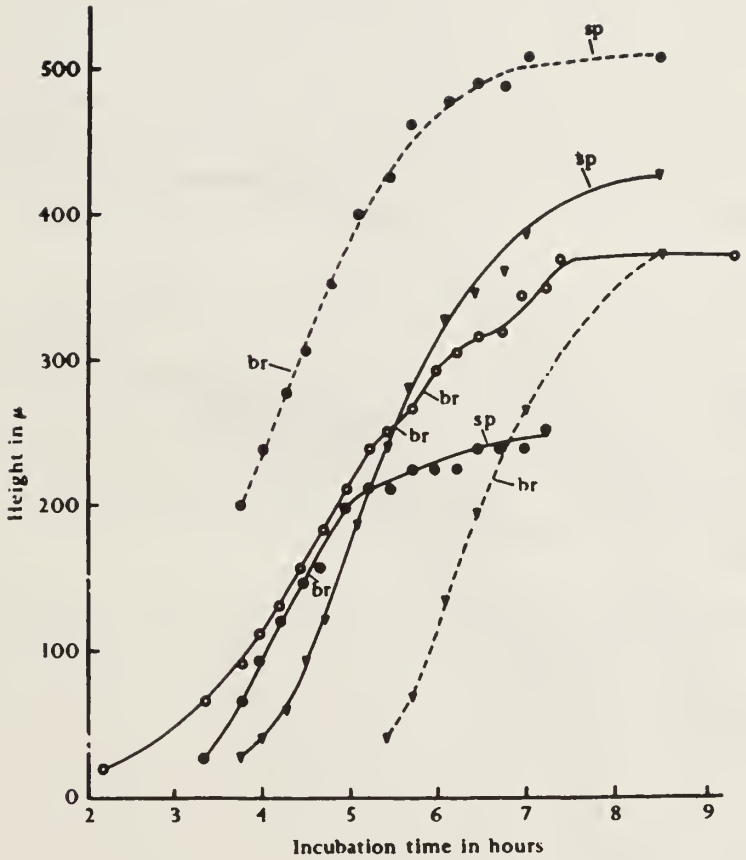


Fig. 20. Increase in length of five individual conidiophores growing in the humidity chamber. br: time at which branching commenced; sp: spore formation (Reprinted from E.M. Davison. 1968. Development of sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 623-631, by permission of the author and the publisher Academic Press Limited, London).

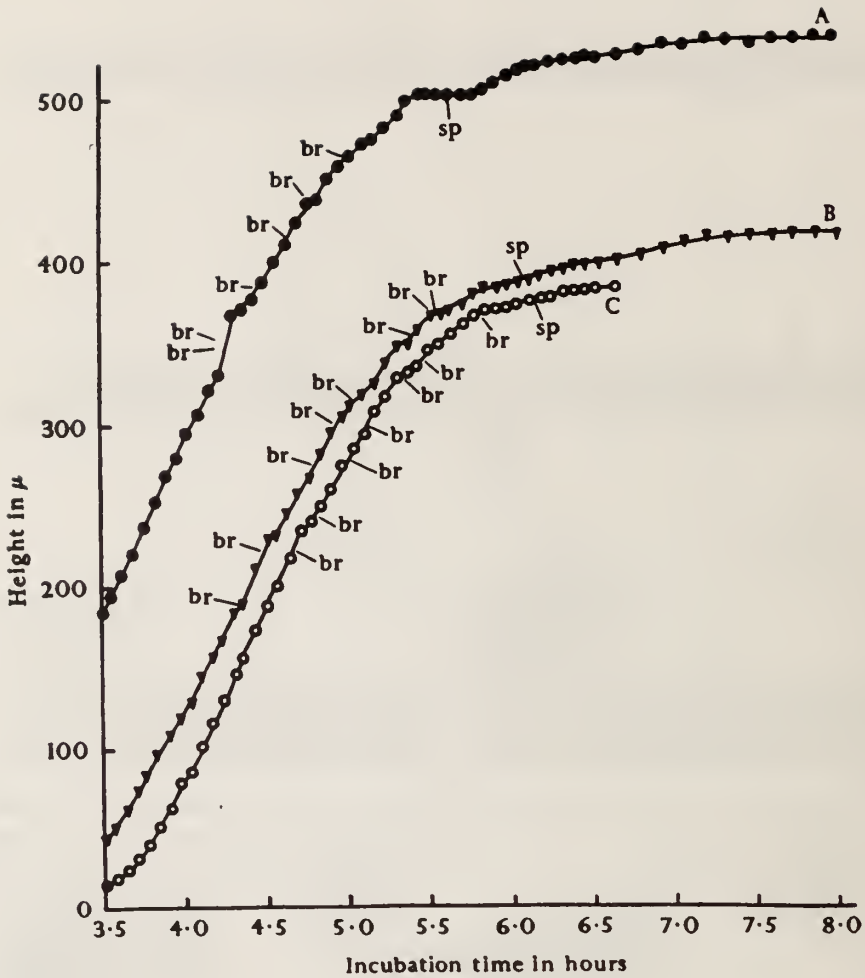


Fig. 21. Increase in length of conidiophore s A, B and C. br: formation of primary branch; sp: spore formation (Reprinted from E.M. Davison. 1968. Development of sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 623-631, by permission of the author and the publisher Academic Press Limited, London).

about two third of the way up the final length of the conidiophore stalk, late conidiophores are usually shorter, less profusely branched, and bear fewer spores than the conidiophores formed earlier. Although increase in volume may be approximately linear during the growth of unbranched conidiophores and branch production, there is a decrease in the rate of volume increase just before spore formation. This is followed by a massive increase in volume just after spore formation, when the total conidiophores volume may be more than quadrupled (Fig. 22) depending on the number of spores produced. The inflation of the branch apex is a gradual process which occurs without any interruption (Fig. 23) in branch elongation (Davison, 1968b).

vi) Conidial formation: Surface ultrastructure of conidia, germ tubes, appressoria and conidiophores of *P. parasitica* infecting Japanese radish has been observed by Shiraishi et al. (1974) through scanning electron microscopy (Figs. 24-27). Mature conidia are approximately 7 μm in width and 10 μm in length. Conidia are formed directly from the swelling tips of the conidiophores, and they have the same surface structure as the conidiophores. Old conidia have many wart-like structures, although the mature conidiophores have a smooth surface (Figs. 26, 27).

vii) Host response: The host protoplast of epidermal and mesophyll cells respond differently to infection by the downy mildew pathogen (Chou, 1970). The epidermal cells in most cases respond vigorously to infection resulting in a severe disruption of the protoplast. The central vacuoles contract and undergo fragmentation. The plasmalemma is broken down or detached from the wall and numerous vesicles are formed from it. The cytoplasm, which originally appeared as a thin peripheral coating of the wall, is either dislocated and aggregated into a vacuolated blob or completely dispersed to the extent that its identity cannot be discerned. Apparently intact mitochondria and chloroplasts appear to be set free from the groundplasm. Consequently, haustoria in epidermal cells are not surrounded by a clearly defined layer of host cytoplasm. Haustoria formation in a mesophyll cell is less disruptive. The host cytoplasm is invaginated by the invading haustorium while the tonoplast and plasmalemma remain intact.

viii) Cytology and genetics: The haploid chromosome number of *P. parasitica* is n = 18-20 and it is a tetraploid (Sansome and Sansome, 1974). Nuclei, mitochondria, lipid material, protein and RNA in the intercellular mycelia and haustoria of *P. parasitica* are uniformly distributed (Davison, 1968a). Insoluble carbohydrate material has been detected in the fungal cell wall. Callose sheaths are occasionally seen partially surrounding the haustoria. A distinct plasmalemma, porate nuclei, tubular endoplasmic reticulum, mitochondria with tubular cristae, golgi dictyosomes and lipid bodies are present within the protoplast (Ehrlich and Ehrlich, 1966). The distribution of organelles, storage products and other substances within the developing conidiophores of *P. parasitica* is very different from the distribution observed within the

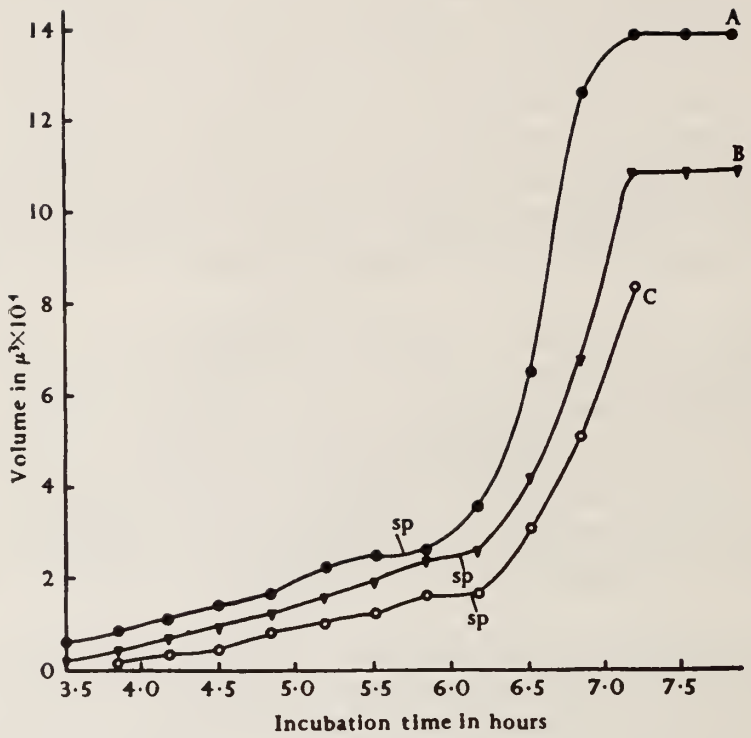


Fig. 22. Increase in volume of conidiophores A, B, and C. sp: spore formation (Reprinted from E.M. Davison. 1968. Development of sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 623-631, by permission of the author and the publisher Academic Press Limited, London).

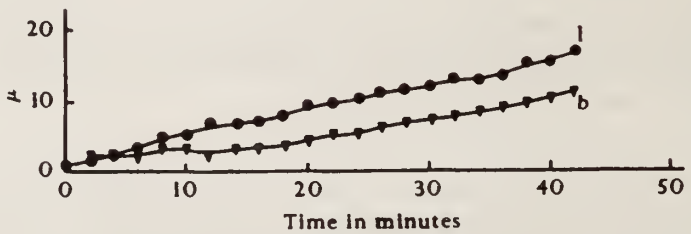


Fig. 23. Increase in branch length and apical diameter during spore formation. l: branch length; b: apical diameter (Reprinted from E.M. Davison. 1968. Development of sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 623-631, by permission of the author and the publisher Academic Press Limited, London).

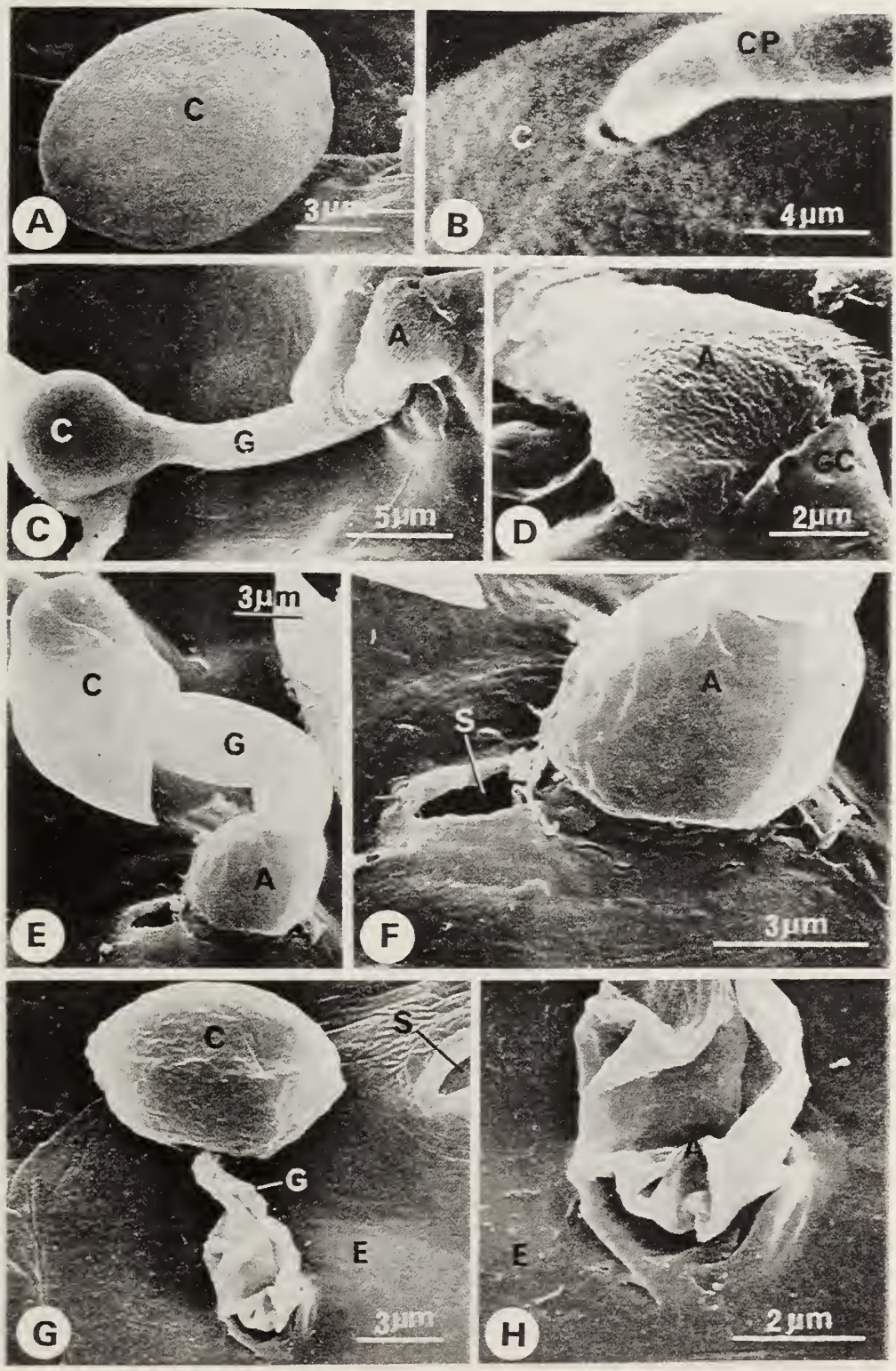


Fig. 24. (See legend page 50a)

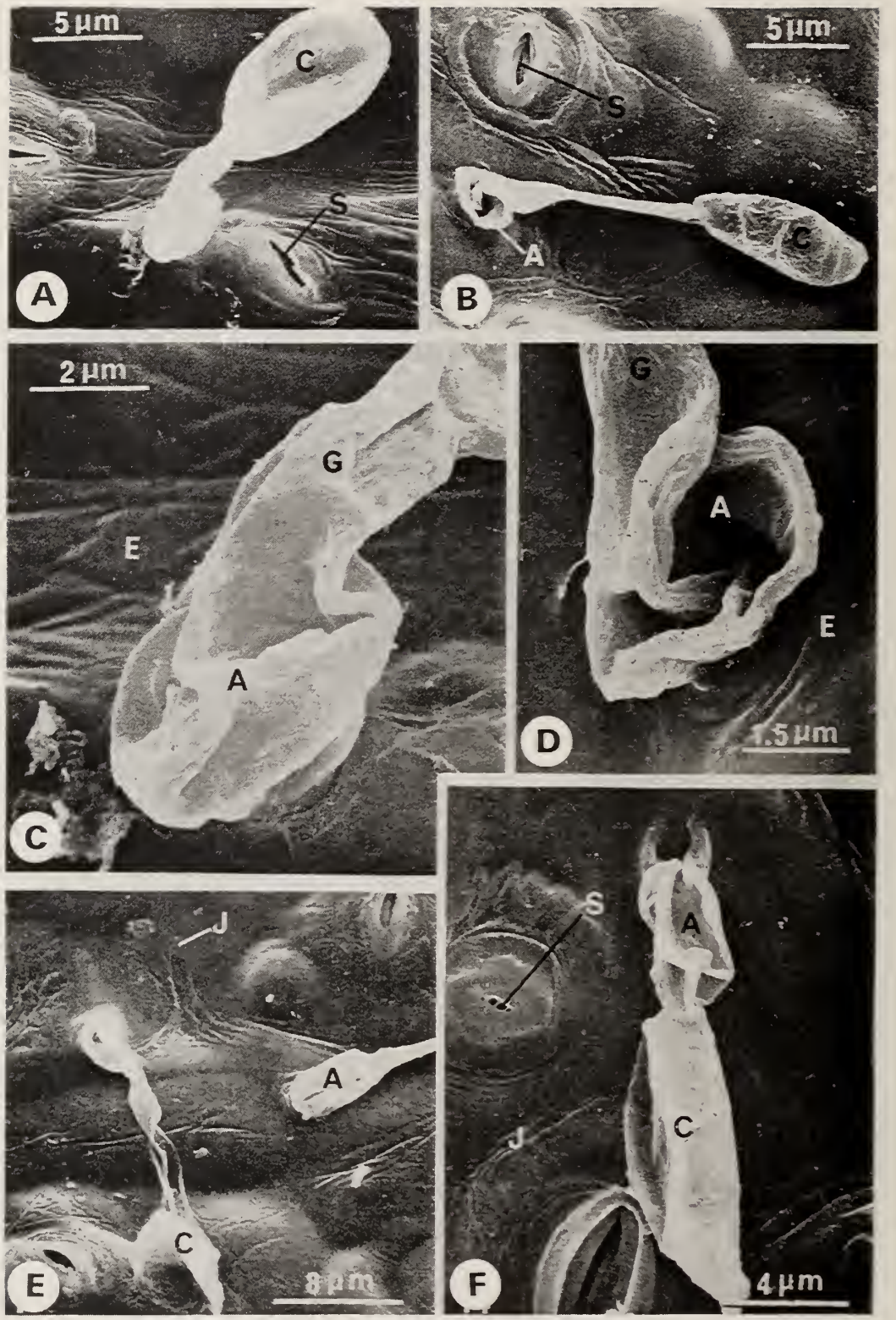


Fig. 25. (See legend page 50a)

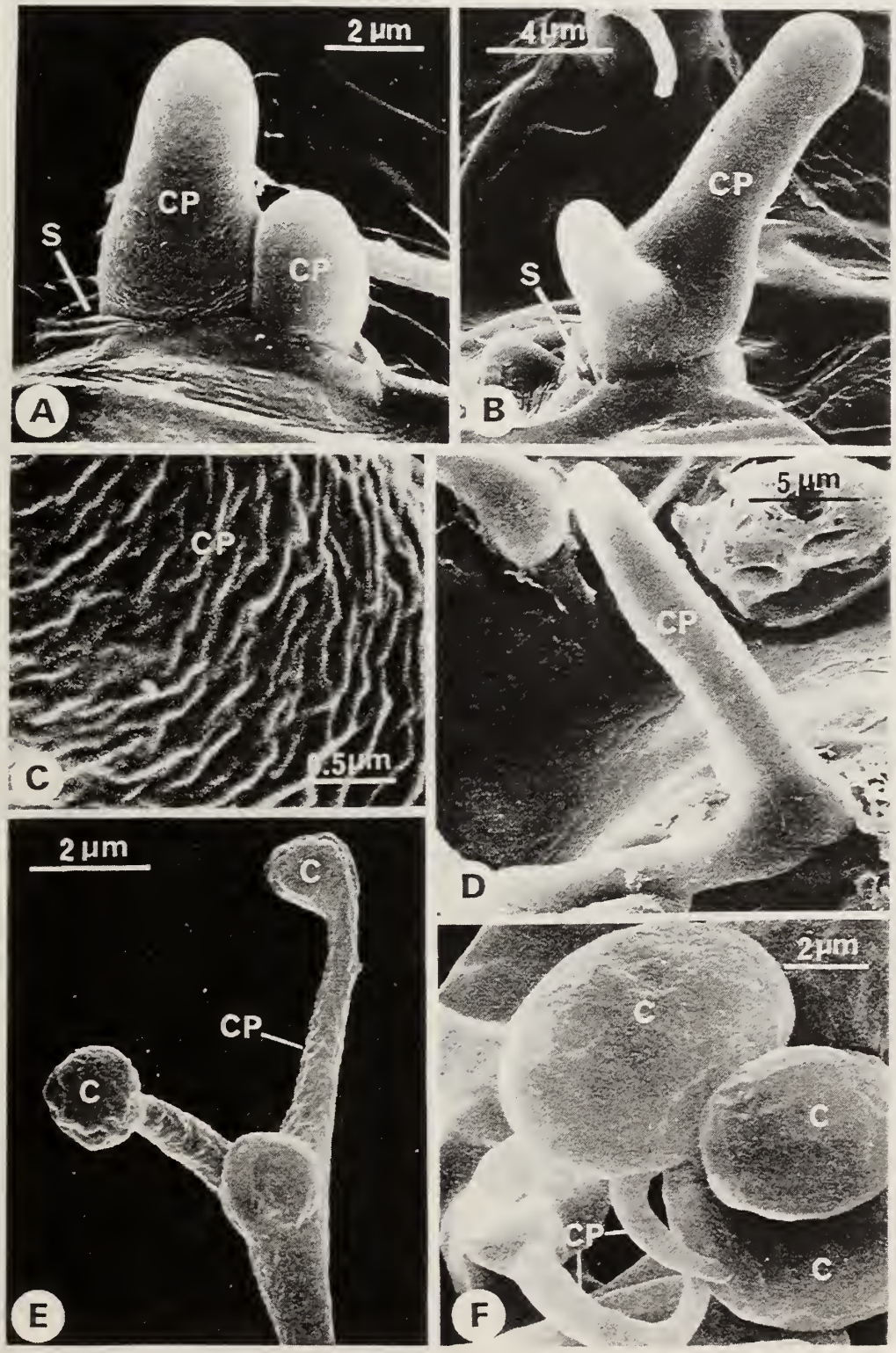


Fig. 26. (See legend page 50a)

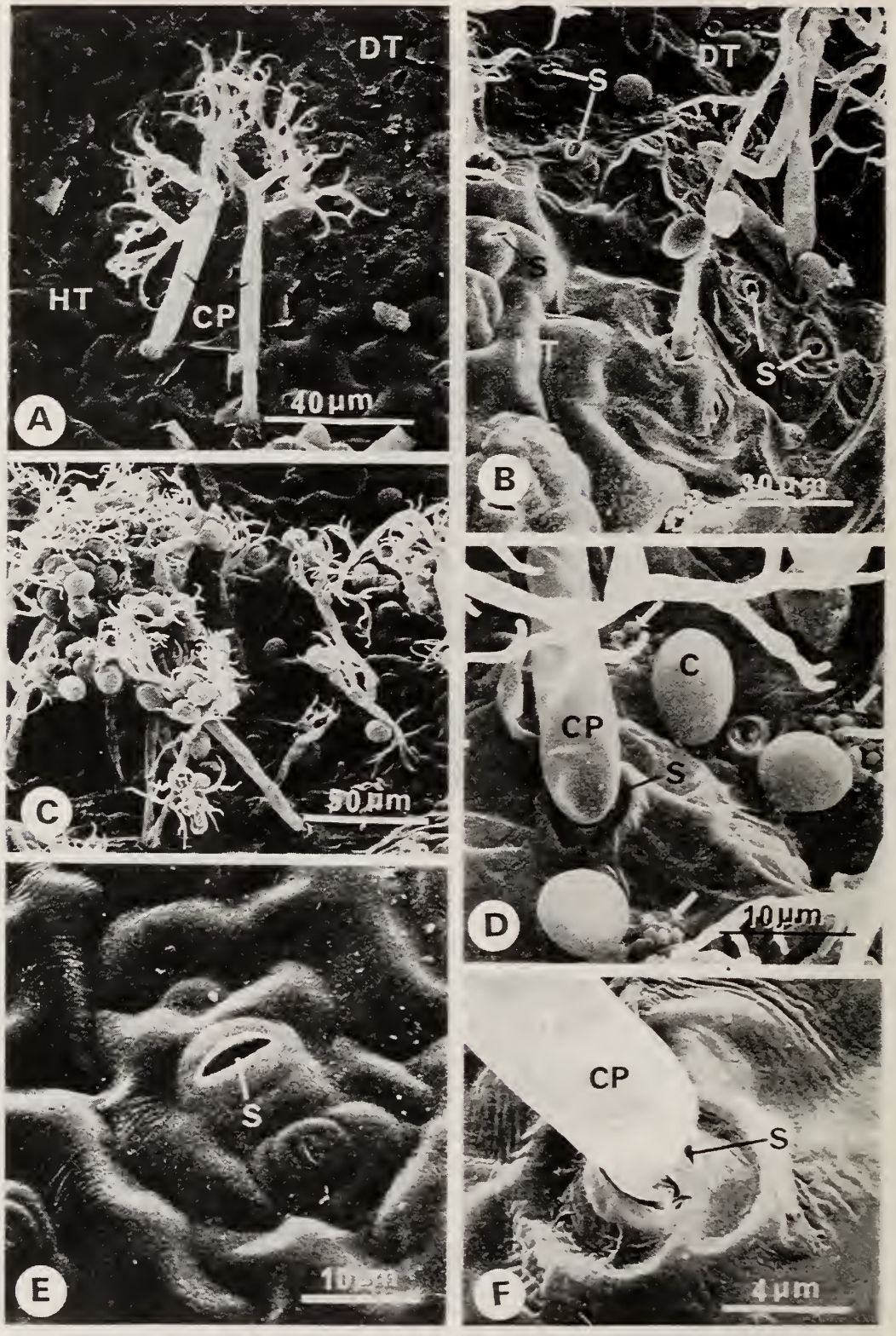


Fig. 27. (See legend page 50a)

Fig. 24 Electron micrograph of conidia, germ tubes and initial period of *Peronospora parasitica* invasion on Japanese radish leaves. (A) Mature conidium. The surface is rough, with wart-shaped structure; (B) Separation of a mature conidium from its conidiophore; (C) An appressorium above a stoma, and a penetration peg into the stomatal cavity; (D) Enlargement of C. Wrinkly structures on an appressorium in the initial period of formation; (E) An appressorium over a stoma 48-h after germination; (F) Enlargement of E. Slight degeneration of the epidermal cells where the appresorium is in contact with the stomatal guard cells; (G) Cuticular invasion. Germ tube growing from the side of a spore; (H) Enlargement of G. The appressorium is quite contracted (Reprinted from M. Shiraishi, K. Sakamoto, Y. Asada, T. Nagatani and H. Hidaka. 1975. A scanning electron microscopic observation on the surface of Japanese radish leaves infected by *Peronospora parasitica* (Fr.) Fr. Ann. Phytopath. Soc. Japan 41:24-32, by permission of the authors and the publisher Phytopathological Society of Japan.)

Fig. 25 Electron micrograph of initial period of *Peronospora parasitica* invasion on Japanese radish leaves. (A) Invasion through a junction between a stomatal guard cell and an auxillary cell; (B) Cuticular invasion of an auxillary cell. The germ tube is quite extended, but invasion does not depend on a stoma being present; (C) Enlargement of B. The viscous substance used by the appressorium to adhere to the epidermal cell wall is not very visible; (D) Enlargement of C. The germ tube and appressorium are clearly contracted, and circular traces of where the penetration peg has entered can be seen in the epidermal cell wall; (E) Cuticular invasion with a long germ tube; (F) Cuticular invasion through a short germ tube. Although the conidium is adjacent to a stoma, germination has occurred from the conidium wall on the side away from the stoma, and cuticular invasion is taking place (Reprinted from M. Shiraishi, K. Sakamoto, Y. Asada, T. Nagatani and H. Hidaka. 1975. A scanning electron microscopic observation on the surface of Japanese radish leaves infected by *Peronospora parasitica* (Fr.) Fr. Ann. Phytopath. Soc. Japan 41:24-32, by permission of the authors and the publisher Phytopathological Society of Japan.)

Fig. 26. Electron micrograph showing development of conidiophores and conidia of *Peronospora parasitica* on Japanese radish leaves (A) Conidiophores invariably grow out of stomata, sometimes two at a time; (B) A conidiophore branching during the initial stage of new growth; (C) Surface of a conidiophore during the initial stage of new growth, with a wavy structure; (D) An extended conidiophore with appearance of a crimp at the base; (E) Initial stage of conidium formation. The tips of the conidiophore swell, forming conidia. The conidiophores and conidia have similar surface structures; (F) Clusters of conidia that have matured and begun to take on a tuft-like shape (Reprinted from M. Shiraishi, K. Sakamoto, Y. Asada, T. Nagatani and H. Hidaka. 1975. A scanning electron microscopic observation on the surface of Japanese radish leaves infected by *Peronospora parasitica* (Fr.) Fr. Ann. Phytopath. Soc. Japan 41:24-32, by permission of the authors and the publisher Phytopathological Society of Japan.)

Fig. 27. Electron micrographs showing conidiophores and conidia of *Peronospora parasitica* on Japanese radish leaves (A) Conidiophores without conidia. The area at the top right is a relatively young diseased area, and exfoliation of epideral cell wax and cuticular material can be seen; (B) Diseased area with advanced signs of disease. Wrinkles have appeared in the epidermis of the diseased area, and open stomata can be seen; (C) Diseased area with many developed conidiophores; (D) Diseased area with advanced symptoms of disease. A crimp in the base of the conidiophore is visible. Yeast-shaped fungi area also present; (E) Stoma in a healthy area. It is formed of two stomatal guard cells and several auxillary cells; (F) The base of the conidiophore is crimped, perhaps due to mechanical force exerted by the stoma. Wrinkles on the surface of the host are clearly visible (Reprinted from M. Shiraishi, K. Sakamoto, Y. Asada, T. Nagatani and H. Hidaka. 1975. A scanning electron microscopic observation on the surface of Japanese radish leaves infected by *Peronospora parasitica* (Fr.) Fr. Ann. Phytopath. Soc. Japan 41:24-32, by permission of the authors and the publisher Phytopathological Society of Japan).

Abbreviations for Figures 24-27

A = Appressorium; C = Conidium; CP = conidiophore; DT = Diseased tissue; E = Epidermal cell wall; G = Germ tube; GC = Guard cell; HT = Healthy tissue; J = Junction line of the epidermal cell wall; S = Stoma.

intercellular mycelium (Davison, 1968c). In developing conidiophores, the nuclei, mitochondria, protein and lipid material are more or less uniformly distributed at first, but gradually shift into the conidia so that by maturity all these substances have relocated, leaving the conidiophores stalk and branches almost completely empty (Figs. 28-30). Glycogen has not been detected within conidiophores or conidia of *P. parasitica*. Trehalose and either glucose or mannose are identified in the conidiophores and conidia of *P. parasitica* but sugar alcohols are absent.

d. Physiologic specialization (Pathogenic variability)

Specificity in the downy mildew fungus on crucifers is very complex since it occurs on a wide range of wild hosts as well as agricultural and horticultural species. For most of these there has been little sustained effort to introduce resistance to the disease, and hence there has been less selection pressure exerted on the pathogen population than is the case with many other obligate parasites. Further impetus has been added by the exponential growth in research on the wild crucifer *Arabidopsis thaliana*, as a host for *P. parasitica*, and serving as a model system for genetic and molecular analysis (Uknes et al., 1992). Discontinuities in the host range of isolates from different host genera and species suggest that the fungus may exist as a series of pathotypes adapted to each host of origin, although some cross-infection may occur. There is also growing evidence that within host species, specificity may be determined by genotype specific interactions consistent with a gene for gene recognition system (Lucas et al., 1988, 1994; Nashaat et al., 1995). Specificity might therefore be expressed at several levels including family, genus, species and cultivar or accession. In view of the close cytogenetic relationship between the major *Brassica* species, coupled with the strongly outbreeding nature of several of these, some overlap in the host range of species-adapted isolates is perhaps predictable.

At the generic level, pathogenic specialization has been observed by several workers all around the world. Gardener (1920) and Kobel (1921) suggested that *P. parasitica* is highly specialized and seldom occurs in the same biological form on more than one crucifer. An isolate of *P. parasitica* obtained from turnip is able to infect seedlings of turnip but not rutabaga or radish (Gardner, 1920). In Holland disease on cabbages is classified in two groups, both representing distinct biological forms of the fungus. The first is characterised by short, ellipsoid conidia, and the second by larger, elongated conidia with protuberant apices. The average dimensions of the later group are 32.51 x 25.66 μ and that of the former 26.67 x 23.13 μ, the corresponding ratios of length to breadth being 1.26 and 1.11, respectively (Thung, 1926). However, Gaumann (1926) sub-divided *P. parasitica* (= *P. brassicae*) into three biological strains, namely: 1. f. sp. *brassicae*, the chief hosts of which are *B. oleracea, B. napus, B. rapa, B. nigra, B. juncea, B. tournefortii* and *B. fructiculosa,* but can also cause some infection on *Sinapis arvensis, S. alba, Raphanus raphanistrum, R. sativus* and *Eruca sativa;* 2. f. sp.

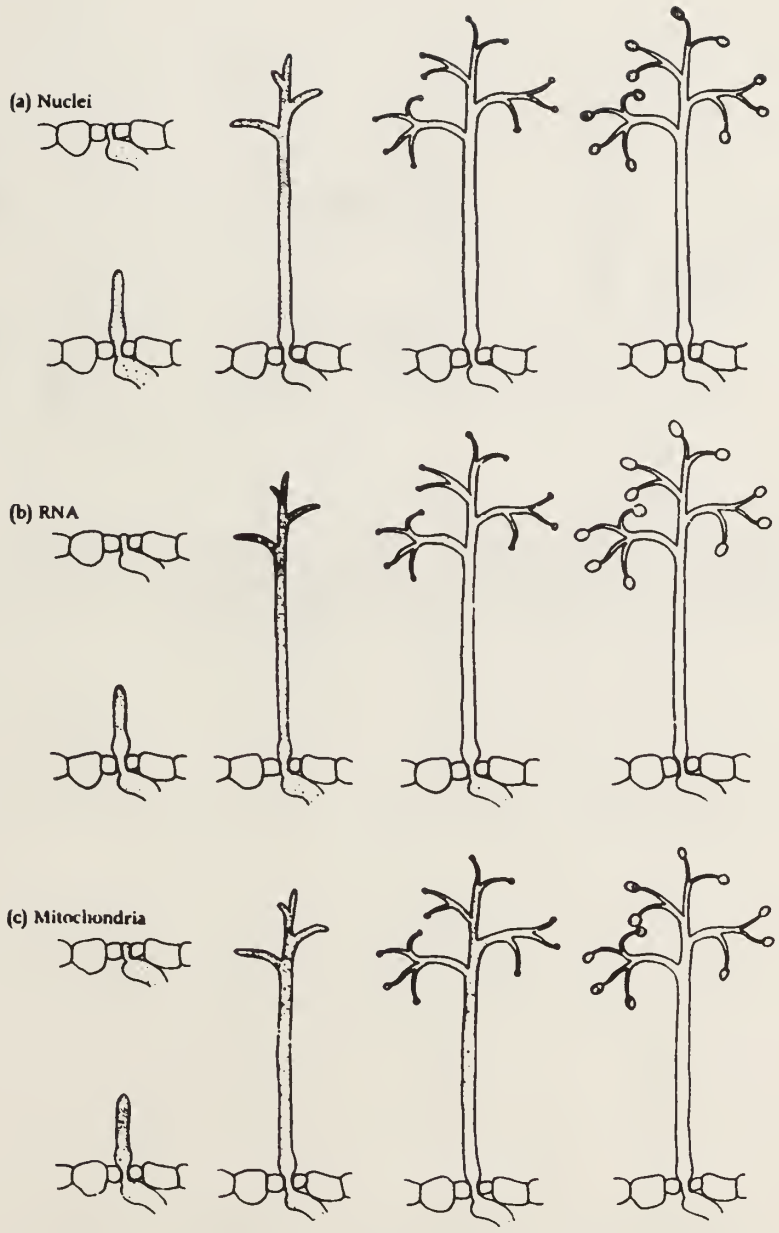


Fig. 28. The distribution of (a) nuclei; (b) RNA; and (c) mitochondria in the developing conidiophores of *Peronospora parasitica* (Reprinted from E.M. Davison. 1968. The distribution of substances in the sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 633-647, by permission of the author and the publisher Academic Press Limited, London).

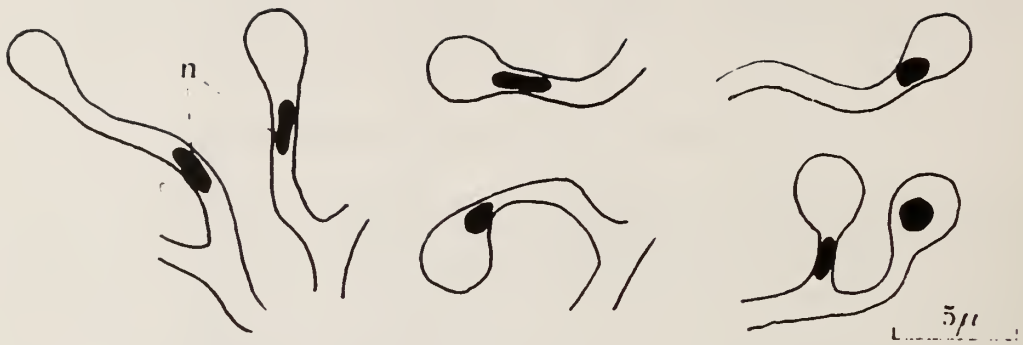


Fig. 29. Migration of nuclei (n) in to the anucleate spores of *Peronospora parasitica* (Reprinted from E.M. Davison. 1968. The distribution of substances in the sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 633-647, by permission of the author and the publisher Academic Press Limited, London).

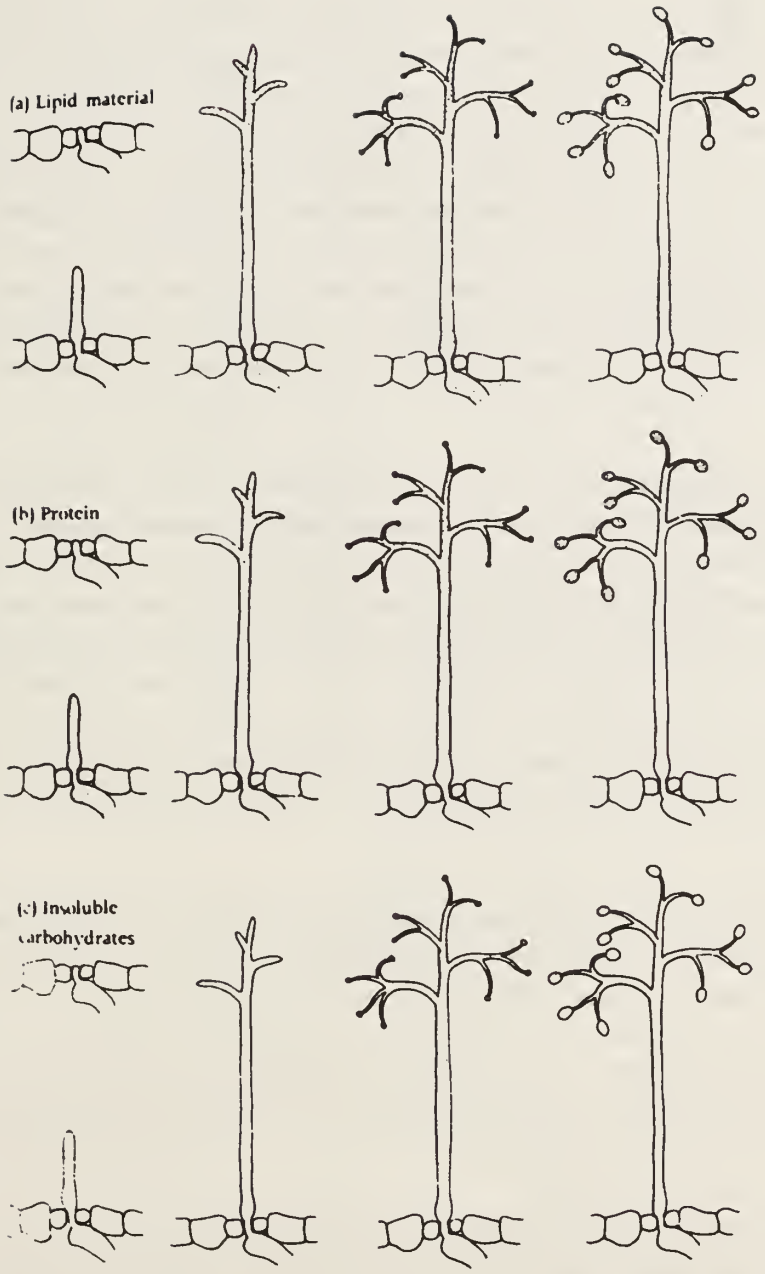


Fig. 30. The distribution of (a) lipid material; (b) protein; and (c) insoluble carbohydrates in the developing conidiophores of *Peronospora parasitica* (Reprinted from E.M. Davison. 1968. The distribution of substances in the sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 633-647, by permission of the author and the publisher Academic Press Limited, London).

sinapidis, the principal hosts of which are *S. arvensis* and *S. alba*, but is also able to produce sub-infections on all the above mentioned species of *Brassica* (except *B. rapa* and *B. juncea*) and *Raphanus*, with occasional conidiophore formation on *B. oleracea*; and 3. f. sp. *raphani,* the chief hosts of which are *R. raphanistrum* and *R. sativus*, but can also produce sub-infections on all the above mentioned species of *Brassica* (except *B. fructiculosa)*, as well as on *S. arvensis* and *S. alba,* with occasional conidiophore formation on *B. oleracea* and *B. napus*. The downy mildew on radish does not attack cabbage *(B. oleracea* var., *bullata* and *capitata)* and is slightly pathogenic on Chinese cabbage *(B. pekinensis, B. chinensis),* rape *(B. campestris)* and mustard *(B. juncea)* (Hiura and Kanegae, 1934). Conversely, the form derived from *B. pekinensis* does not infect radish but is allied to one on *B. chinensis* and rape. The forms derived from *B. pekinensis, B. chinensis* and rape are mutually pathogenic on one another (Hiura and Kanegae, 1934).

Wang (1944) classified the reaction of the hosts into four categories: 1. susceptible with normal symptoms; 2. resistant, showing large necrotic spots; 3. para-immune, showing slightly visible necrotic dots; and 4. immune, with no visible symptoms. Three pathotypes of *P. parasitica* were differentiated: *P. parasitica* Brassicae on *Brassica, P. parasitica Raphani* on *Raphanus* and *P. parasitica Capsellae* on *Capsella.* The three pathotypes were not mutually compatible with each other's host. Six forms of *P. parasitica Brassicae* were differentiated by their reaction to *B. chinensis, B. oleracea, B. juncea* and *B. napobrassicae.* Wang (1944) prepared a dichotomous key to physiological forms from China as follows:

A. *Capsella bursa-pastoris,* immune
B. *Raphanus sativus,* immune or para-immune variety Brassicae
C. *B. oleracea,* resistant or para-immune
D. *B. chinensis,* susceptible
E. *B. juncea* (Meitan Dav Yu Tsai), susceptible Ph.fm. 1.
EE. *B. juncea* (Meitan Dav Yu Tsai,) resistant
F. *B. napobrassica,* immune Ph. fm. 2.
FF. *B. napobrassica,* resistant Ph. fm. 3.
DD. *B. chinensis,* resistant
E. *B. juncea* (Dav Ching Tsai), susceptible Ph. fm. 4
EE. *B. juncea* (Dav Ching Tsai), resistant Ph. fm. 5
CC. *B. oleracea*, susceptible Ph. fm. 6
BB. *R. sativus*, susceptible variety *Raphani*
AA. *C. bursa-pastoris,* susceptible variety *capsellae*

Felton and Walker (1946) and Natti (1958) differentiated the races of *P. parasitica* found on *R. sativus*, and *B. oleracea* on the basis of their host specificity. Morris and Knox-Davies (1980) also indicated distinct races of *P. parasitica* on *B. oleracea* and *R.*

raphanistrum based on host specificity.

P. parasitica f. *brassicae* on cabbage, f. *rapae* on turnip, f. *rapiferae* on *B. rapa,* f. *rapifera,* f. *napi* on rape, f. *raphani* on radish, and f. *sinapidis* on *sinapis alba* have been distinguished as special forms of *P. parasitica* from Leningrad though all are similar morphologically (Dzhanuzakov, 1963).

Three vars. of *P. parasitica* have been differentiated in 35 samples of downy mildew from *B. pekinensis* and other crucifers, namely f. sp. *brassicae* on *Brassica,* f. sp. *raphani* on *Raphanus,* and f. sp. *capsellae* on *Capsella* (Chang et al. 1964). *P. parasitica* f. sp. *Brassicae* exists in at least 3 different subforms *(pekinensis, oleracea* and *juncea).* Isolates from *B. pekinensis, B. chinensis* and turnip were classified in the same group and can attack all three hosts but did not infect *Capsella bursa-pastoris,* radish, cabbage, Chinese mustard, and *B. juncea* var. *multiceps. B. juncea* var. *megarrhiza* expressed various reactions to these isolates. Isolates from Chinese mustard, *B. juncea* var. *megarrhiza*, and *B. juncea* var. *multiceps* were limited to these hosts, except that those from Chinese mustard did not infect some vars. of *B. pekinensis, B. chinensis* and turnip. Radish isolates are of two types, one infects only radish, the other vars. of *B. pekinensis,* cabbage and turnips as well as radish. Isolates from cabbage and *C. bursa-pastoris* are host specific.

In Norway, cross-inoculation experiments with downy mildew from cabbage, turnip rape and radish indicated the occurrence of different races on cabbage and radish (Semb, 1969).

According to Natti et al. (1967) the predominant physiologic race of *P. parasitica* pathogenic to broccoli and other types of *B. oleracea* grown commercially in New York were race 1 and race 2. The later race was pathogenic to plants resistant to race 1.

Dickinson and Greenhalgh (1977) observed a wide variation in the reaction of seedlings of different crucifers species to isolates of *Peronospora* derived from *Brassica* and *Raphanus* species (Table 2).

In India, *P. parasitica* isolates from different hosts vary in host range. Isolates from *Brassica, Raphanus, Eruca* and *Sisymbrium* are not cross infective (Bains and Jhooty, 1983). Recently Mehta and Saharan (1994) tested the host range of 9 isolates of *P. parasitica* collected from the leaves and stagheads of *6* host species on 17 host differentials (Tables 7, 8). Isolates from brassica oilseeds infected all species, except *B. alba*, whereas isolates from cauliflower leaves do not infect *B. carinata*, *B. alba*, *B. nigra*, *B. chinensis*, *B. pekinensis* and *B. napus* (Table 8). There was no significant differences among the conidial size of the isolates collected from leaves and stagheads, but significant differences were observed among these groups (Table 9). The isolates

Table 7. List of host differentials (Mehta and Saharan, 1994)

Common name	Species	Cultivar
Indian Mustard (Raya)	*Brassica juncea*	RH-30
Toria	*Brassica campestris* var. *toria*	TH-68
Yellow sarson	*Brassica campestris* var. yellow *sarson*	YSPB-24
Brown sarson	*Brassica campestris* var. brown *sarson*	BSH-1
Ethiopian mustard	*Brassica carinata*	HC-1
White mustard	*Brassica alba*	Local
Black mustard	*Brassica nigra*	Local
Chinese mustard	*Brassica chinensis*	Local
Chinese mustard	*Brassica pekinensis*	Local
Rapeseed	*Brassica napus*	GSL-1
Wild turnip	*Brassica tournefortii*	Local
Cabbage	*Brassica olercea* var. *capitata*	Pride of India
Cauliflower	*Brassica oleracea* var. *botrytis*	Snowball-16
Turnip	*Brassica rapa*	White Purple Top
Knol Khol	*Brassica caulorapa*	Early White Vienna
Taramira	*Eruca sativa*	Local
Radish	*Raphanus sativus*	HR-1

were classified into two distinct pathotypes, one from cauliflower and other from oilseeds brassica. There was no significant difference between the isolates in percentages of spore germination (Table 10).

Specific populations of *P. parasitica* differing in pathogenesis and host specificity were reported from Bulgaria (Masheva et al., 1996a, b). The populations formed at a lower temperature were more aggressive on cabbage heads.

In the United Kingdom, differential host resistance in relation to pathogenic variation of isolates derived from the same host species were identified in *B. rapa* (Moss et al., 1991; Silue et al., 1996), *B. napus* (Nashaat and Rawlinson, 1994), *B. juncea* (Nashaat and Awasthi, 1995) and *B. oleracea* (Silue et al., 1996). Isolates from different *Brassica* species found to be most virulent on their species of origin, were nevertheless able to grow to less extent on other *Brassica* species (Sheriff and Lucas, 1990). Nashaat and Awasthi (1995) identified five groups of *B. juncea* accessions with differential resistance to U.K. isolates R1 and P003 derived from oilseed rape (*B. napus* ssp. *oleifera*) and Indian isolates IP01 and IP02 derived from mustard (*B. juncea*) (Table 11). All *B. juncea* accessions were resistant to isolates from *B. napus,* but at the same time *B. napus* cv. Arian was resistant to isolates from *B. juncea*. Twenty-one differential responses to *P. parasitica* isolates from *B. oleracea* and two from *B. rapa* were identified. Of the seven isolates tested, four were from crops of cauliflower in France,

Table 8. Response of seventeen *Brassica* species to nine isolates of *Peronospora parasitica* (Mehta and Saharan, 1994)

Differential Hosts	Sources of *P. parasitica* isolates/reaction								
	Toria (T_1)	Yellow YS_1	Sarson YS_2	Brown BS_1	Sarson BS_2	Raya R_1	R_2	Cauliflower C_1	B. nigra (BN_2)
B. campestris var. toria	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. campestris var. Y. Sarson	+(6)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. campestris var. B. Sarson	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. juncea	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. carinata	+(6)	+(6)	+(4)	+(6)	+(4)	+(4)	+(4)	-	+(4)
B. alba	-	-	-	-	-	-	-	-	-
B. nigra	+(6)	+(6)	+(4)	+(4)	+(4)	+(4)	+(4)	-	+(4)
B. chinensis	+(6)	+(6)	+(4)	+(4)	+(4)	+(4)	+(4)	-	+(4)
B. pekinensis	+(6)	+(6)	+(4)	+(4)	+(4)	+(4)	+(4)	-	+(4)
B. napus	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	-	+(4)
B. tournefortii	+(6)	+(6)	+(4)	+(6)	+(4)	+(4)	+(4)	+(4)	+(4)
Eruca sativa	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
Raphanus sativus	+(4)	+(4)	+(6)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. oleracea var. capitata	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. oleracea var. botrytis	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. rapa	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)
B. caulorapa	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)

( ) = Incubation period in days; + = Infection; - = No infection
B = Brassica; 1 = Leaf inoculum; 2 = Inflorescence inoculum

two from oilseed rape in the U.K. and one was from mustard in India. All *Raphanus sativus* accessions were resistant to all seven isolates (Silue et al., 1996).

Differential resistance of rapid cycling and commercial genotypes of *B. rapa* were also reported from the U.K. (Moss et al., 1988). A range of differential host responses were characterized by four homologous isolates (Table 12). Pathogen isolates were also characterized in relation to their sexual compatibility type and response to phenylamide fungicides (Moss et al., 1988).

Table 9. Conidial size of *Peronospore parasitica* isolates derived from eleven*Brassica* species (Mehta and Saharan, 1994)

Source of Isolates	*P. parasitica* Isolates*	Conidial dimensions (u) Range Length	Range Width	Average Length	Average Width
B. campestris var. *toria*	T_1	19.50-29.25	14.62-27.30	25.93	19.30
B. campestris var. *Y. Sarson*	YS_1	21.45-29.25	19.50-24.37	25.35	21.74
B. campestris var. *Y. Sarson*	YS_2	19.50-29.25	19.50-24.37	26.81	23.39
B. campestris var. *B. Sarson*	BS_1	21.45-29.25	19.00-24.37	25.35	21.45
B. campestris var. *B. Sarson*	BS_2	21.93-29.25	19.50-26.81	26.56	23.64
B. juncea	R_1	20.47-29.25	19.50-26.32	25.64	21.84
B. juncea	R_2	19.50-29.25	19.50-26.81	27.05	22.90
B. oleracea var. *botrytis*	C_1	19.50-29.25	17.55-24.37	23.30	20.76
B. tournefortii	BT_2	19.50-24.37	16.57-19.50	23.59	20.96
Raphanus sativus	RS_2	19.50-24.37	18.52-21.45	22.03	19.69
B. nigra	BN_2	21.93-29.25	19.50-26.81	26.81	22.90
CD 0.05		-	-	2.43	1.78

* Source of inoculum: 1. leaves; 2. hypertrophied inflorescences

Eleven isolates of *P. parasitica* tested on rapid cycling populations of B. *rapa* (aa, CrGc-1-1), *B. nigra* (bb, CrGc-2-1), *B. oleracea* (cc, CrGc-3-1), *B. juncea* (aabb CrGc-4-1), *B. napus* (aacc CrGc-5-1), *B. carinata* (bbccCrGc-6-1) and *R. sativus* (rr CrGc-7-1) indicated specificity towards particular genotypes within each rapid cycling population (Hill et al., 1988).

Variation in the response of different host lines to *P. parasitica* has also been detected within wild crucifer species such as Shepherd's Purse *(Capsella bursa-pastoris)* and *Arabidopsis thaliana* (Lucas et al., 1994).

Moss et al. (1994) attempted to cross fungal isolates originating from different *Brassica* spp. by co-inoculating host lines previously identified as susceptible to these

Table 10. Percent conidial germination of *Peronospora parasitica* isolates at 18°C (Mehta and Saharan, 1994)

Incubation period (h)	cauliflower (C_1)	Toria (T_1)	Brown Sarson (BS_1)	Yellow Sarson (YS_1)	Raya (R_1)
0	-	-	-	-	-
0.5	0	0	0	0	0
1.0	0	0	0	0	10.57
1.5	12.20	13.73	15.72	11.87	32.46
2.0	36.50	33.25	45.87	39.31	52.68
3.0	66.30	63.25	69.12	61.06	69.12
4.0	84.25	83.86	80.99	81.92	88.85
5.0	86.02	84.91	82.61	84.16	88.85
6.0	86.51	87.36	82.90	85.42	89.19
	(68.72)	(70.11)	(65.80)	(67.85)	(73.20)

LSD 0.05 = NS

isolates. A proportion of oospore progeny recovered from these crosses appeared to be hybrids and had reduced virulence on both hosts of origin. The differential resistance to *P. parasitica* identified in *Brassica* spp. can be used for future studies of the genetics of the host-pathogen interaction and for breeding for disease resistance.

Tham et al. (1994) used RAPD analysis to compare 16 isolates of *P. parasitica* from two different *Brassica* species, *B. napus* and *B. oleracea*. Two out of twenty random primers screened gave reproducible band patterns capable of discriminating between the different host-adopted isolates (Fig.31).

e. Heterothallism and homothallism: Induction of the sexual process in several downy mildew species requires the presence of two strains of opposite mating type. Such heterothallic behaviour has been reported for *P. parasitica* (DeBruyn, 1937; McMeekin, 1960; Kluczewski and Lucas, 1983; Sherriff and Lucas, 1989b; Sequeira and Monteiro, 1996). Homothallic forms of *P. parasitica* have also been observed (De Bruyn, 1937; Sherriff and Lucas, 1989b; Sequeira and Monteiro, 1996). The two forms of sexual reproduction are very important for the maintenance and evolution of the fungal strains and epidemiology of the disease. On *B. oleracea* and *B. campestris* heterothallic isolates of two mating types designated as P_1 and P_2 have been identified. Isolates from

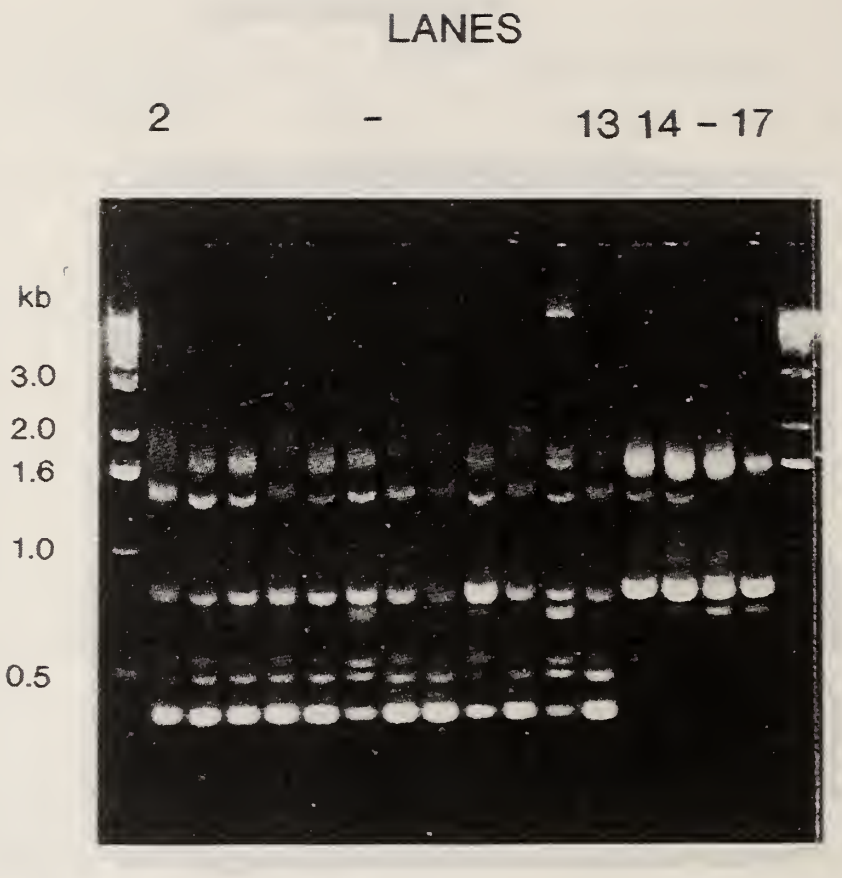


Fig. 31. Random amplified polymorphic DNA (RAPD) from 16 isolates of crucifer downy mildew *(Peronospora parasitica)*. Lanes 2-13 are isolates from oilseed rape *Brassica napus* and lanes 14-17 are isolates from cauliflower *B. oleracea* (Reprinted from F.Y. Tham, J.A. Lucas and Z.A. Wilson. 1994. DNA fingerprinting of *Peronospora parasitica*, a biotrophic fungal pathogen of crucifers. Theoretical and Applied Genetics 88:490-496, by permission of the authors and the publisher Springer-Verlag New York Inc., U.S.A.).

Table 11. Sources of seed for accessions of *Brassica juncea*, arranged in five groups according to the response of their seedlings at the cotyledon stage to *Peronospora parastica* and one accession of *B. napus* (Reprinted from N.I. Nashaat and R.P. Awasthi. 1995. Evidence for differential resistance to *Peronospora parasitica* (downy mildew) in accessions of *Brassica juncea* (mustard) at the cotyledon stage. J. Phytopathology 143:157-159, by permission of the authors and the publisher Blackwell Wissenschafts-Verlag GmbH, Germany)

B. juncea[a]	Seed source[b]	*B. juncea*	Seed source[b]
GROUP A		Group C	
RES-BJ01 (Kranti)	(India)	Ecotype/BGRC 34263	FAL
RES-BJ02 (Krishna)	(India)	BGRC 34283	FAL
RES-BJ03 (Varuna)	(India)	BGRC 34291	FAL
RES-BJ04 (BGRC 34253)	(FAL)	BGRC 34781	FAL
		BGRC 34789	FAL
		BGRC 46069	FAL
GROUP B			
Chang Yang Huang Jie	HAU	BGRC 46071	FAL
BGRC 34294	FAL	PPBJ-1	India
		Skorosjelka-2/	
GROUP C		BGRC 34275	FAL
Aurea/BGRC 28602	FAL	Stephniacka/	
Blaze/BGRC 30288	FAL	BGRC 34274	FAL
Burgonde/BGRC 30289	FAL	Stoke/BGRC 51764	FAL
Commercial Brown	Ag Cda	Yi Men Feng Wei Zi	HAU
Cutlass	Ag Cda	Zaria/BGRC 16254	FAL
Ecotype BGRC 34255	FAL		
GROUP D		**GROUP E**	
Hatano/BGRC 22527	FAL	BGRC 34282	FAL
BGRC 34239	FAL		
Landrace/BGRC 46323	FAL	*B. napus*	
Larja/BGRC 34273	FAL	Ariana	Semundo
Line/BGRC 34295	FAL		

[a] RES-BJ01 to RES-BJ05, lines selected from seedling population of accessions in parethesis.

[b] Agriculture and Agri-Food Canada, Saskatoon Research Centre, Canada. FAL, Institut für Pflanzenbau und Pflanzenzüchtung, Braunschweig, Germany. HAU, Huazhong Agricultural University, Wuhan, P.R. China. Semundo, Semundo Ltd., Cambridge, UK.

oilseed rape *B. napus* have been found to be uniformly homothallic and remained self-fertile even after months of laboratory subculture (Sherriff and Lucas, 1989b). In a cytogenetic study of heterothallic and homothallic isolates of *P. parasitica* at metaphase 1 of meiosis, a ring of four chromosomes is found (Sherriff and Lucas, 1989a). This ring is interpreted as a reciprocal translocation complex between chromosomes carrying the mating type alleles. In homothallic isolates a fifth chromosome is associated with the ring of four. The self fertility of these isolates may therefore be due to the presence of a third mating type allele on the fifth chromosome, a condition known as secondary homothallism. The determination of sexual compatibility type (SCT) of an unknown isolate can be achieved by mixing conidia in a 1:1 ratio with isolates of known SCT and inoculating to a common compatible host. In heterothallic isolates, oospores may form in combination with isolates of opposite SCT. Isoenzyme markers are particularly important in discriminating between self and true hybrid progeny (Moss et al., 1988).

f. Perpetuation

Oospores formed in malformed and senesced host tissues constitute an important means of survival of *P. parasitica* over periods of unfavourable conditions (Gauman, 1926; Kolte, 1985). It is also known to survive through mycelium and conidia (Jang and Safeeula, 1990b; Krober, 1970; McMeekin, 1969; Vishunavat and Kolte, 1993).

i) Mycelium: The presence of *P. parasitica* mycelium in the seed coat of Chinese cabbage has been recorded by Chang et al. (1963). According to Jang and Safeeulla (1990c) the presence of mycelium in the pericarp and embryo of radish seeds varies from 0.1 to 12.5% (Table 13, 14). The coenocytic branched mycelium is clearly visible in the intercellular space of the pericarp. In the embryonal tissues, the mycelium is comparatively thin. The percentage of seeds with viable mycelium is directly correlated with the percentage of embryo infection.

ii) Conidia: Conidia of *P. parasitica* on cabbage survive longer under cool, dry conditions (Krober, 1981). Relative humidity is more important than temperature. In the field conidia can survive on detached leaves of Kohlrabi for 10 days during warm days. When buried in dry soil conidia can survive for 110 days. The survival period is greatly reduced to a maximum of 22 days if the soil is moist. Marked reduction in survival has also been observed after storage in both dry and moist soils during the

Table 12. Differential virulence of *P. parasitica* isolates from *B. campestris* on six hosts lines (Reprinted from N.A. Moss, I.R. Crute, J.A. Lucas and P.L. Gordon. 1988. Requirements for analysis of host-species specificity in *Peronospora parasitica* (downy mildew). Cruciferae NewsLetter 13:114-116, by permission of the authors and the publisher)

Host Lines	Reaction of *P. parasitica* isolates			
	P007	P008	P013	P014
CA88014*[a]	+	+	+	+
JADE PAGODA	+	-	+	+
CA87063*	-	-	+	+
SNOWBALL	-	-	+	-
CA87068*	-	-	-	+
CA87065*	-	-	-	-

+ = susceptible, - = Resistant, a = universally susceptible, * = rapid cycling lines

summer. The conidial viability is longest, up to 130 days, when the spores are stored in air-dried soil at a constant temperature of 5°C (Krober, 1970). Conidia kept at -25°C and relatively dry on leaf disks (air dried at 20°C) maintain a relatively high rate of germination after 1 year or longer.

iii) Oospores: Ooospores formation is abundant in the infected tissues of all crucifers and they form primary source of survival for the pathogen (Le Beau, 1945; McMeekin, 1960; Chang et al., 1963; Kolte, 1985). In radish and rapeseed-mustard there is abundant production of oospores in infected leaf tissues, on the seed surface and pericarp and embryo of seeds (Jang and Safeeulla, 1990c; Vishunavat and Kolte, 1993). However, in rapeseed and mustard, seed transmission is low and may be nonsystemic, ranging from 0.4% to 0.9% in the seedlings grown from infected seeds (Vishunavat and Kolte, 1993). In radish seed transmission to the extent of 14% was observed by Jang and Safeeulla (1990d).

iv) Axenic culture: *P. parasitica* hyphae grow on water agar from the infected tissues and form haustorium like structures (Ohguchi and Asada, 1989). The growth is greater on the modified Knop medium with many haustorium-like structures and conidiophores being formed on this medium. If cod-liver oil or minerals are added to

Table 13. Percentage seed infection by *P. parasitica* in *R. sativus* (Reprinted from P. Jang and K.M. Safeeulla. 1990c. Seed-borne nature of *Peronospora parasitica* in *Raphanus sativus*. Indian Acad. Sci. (Plant Sci.) 100:255-258, by permission of the authors and the publisher Indian Academy of Sciences)

Cultivar	Place of collection	Seed showing infection (%)		
		Pericarp	Endosperm	Embryo
Japanese white	Mysore Seed Multiplication Farm	12.8	0	12.5
Arka nishant	Indian Council of Agricultural Research Station, Bangalore	0.5	0	0.5
Pusa desi	Bangalore Seed Health Testing Station	0.2	0	0.3
Pusa reshmi	Bangalore Seed Health Testing Station	0	0	0.1

this medium then branched hyphae are formed. The fungus does not grow on Japanese radish root homogenate medium but grows well on the dialized homogenate medium. The decoction of residuum of the root homogenate and the sap in the intercellular spaces of the root tissues also stimulates the growth of the fungus. In the decoction medium the growth of the hypha is vigorous and the formation of conidiophores is stimulated. In the sap medium, the formation of a haustorium like structure is promoted (Ohguchi and Asada, 1989).

g. Conidial discharge: The maximum conidial discharge of *P. parasitica* from Kohlrabi leaves is between 5-6 a.m. (258 conidia/cm^2) (Lin and Liang, 1974). The conidial discharge decreases greatly from 12 noon to 8 p.m. If infected leaves are covered with plastic during the night, then the production and discharge of conidia decreases drastically and the disease index is half that of uncovered seedlings. The discharge of conidia from diseased leaves of Chinese cabbage shows a periodic cycle each day (Fig. 32A) (Lin, 1981). Conidial release increases steadily after 2 a.m. each day, and reaches a peak around 6-8 a.m. Conidial discharge decreases rapidly after 8 a.m. Few conidia can be detected from noon to 10 p.m. The discharge of conidia is favoured by temperatures below 18°C and RH above 75% (Fig. 32B). If the conidia are ready to

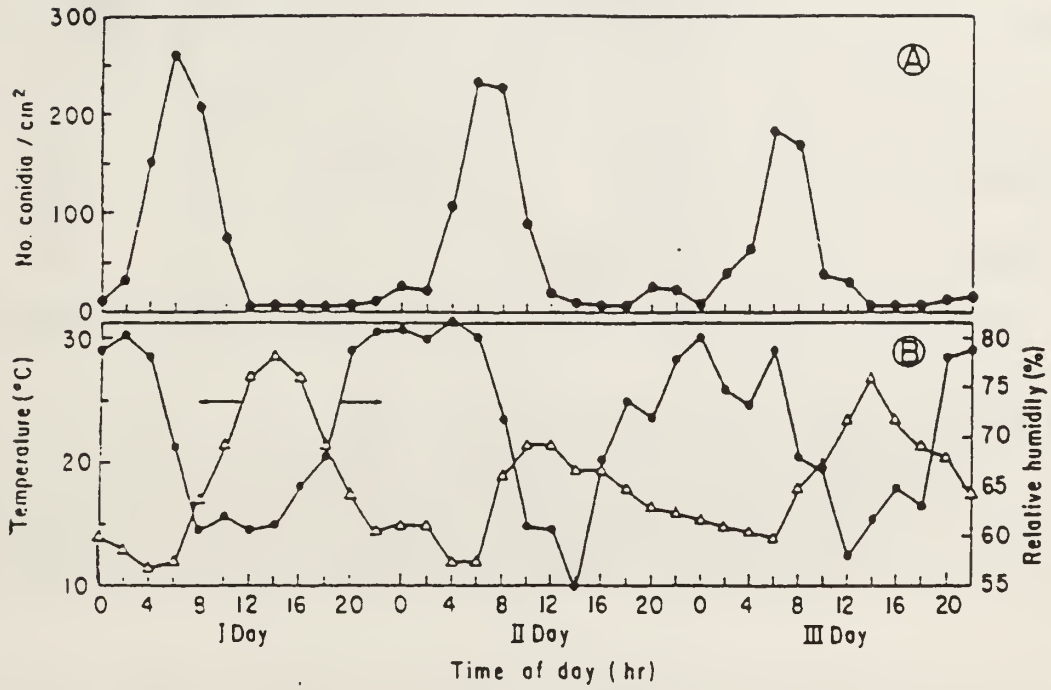


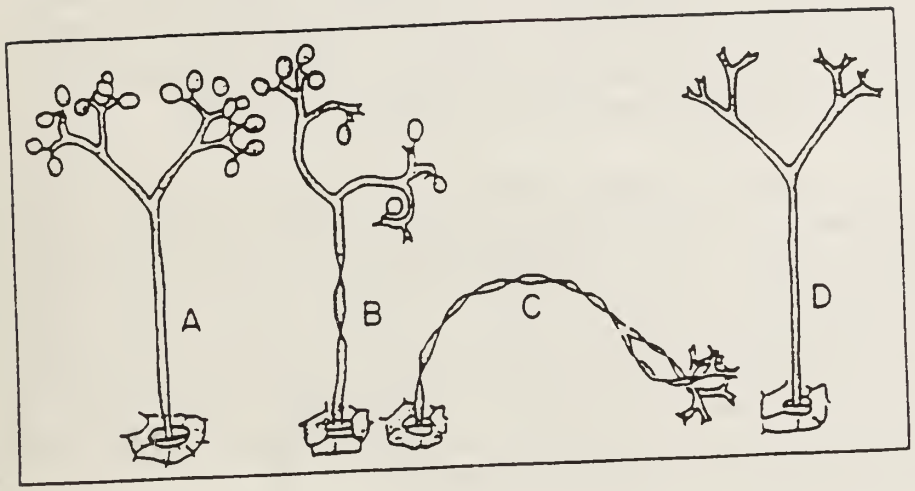
Fig. 32. (A) Pattern of *Peronospora parasitica* conidia discharge from infested Chinese cabbage plants; and (B) temperature and humidity on three fine days in November 1978 (Reprinted from C.Y. Lin. 1981. Studies on downy mildew of Chinese cabbage caused by *Peronospora parasitica*. Proc. First Intern. Symposium, Asian Vegetable Research Development Centre, Shanhua, Taiwan: pp. 105-112, by permission of the author and the publisher Asian Development Research Development Centre, Taiwan).

Table 14. Percentage of seedling infection by *P. parasitica* and seed transmission in *R. sativus* (Reprinted from P. Jang and K.M. Safeeulla. 1990c. Seed-borne nature of *Peronospora parasitica* in *Raphanus sativus*. Indian Acad. Sci. (Plant Sci.) 100:255-258, by permission of the authors and the publisher Indian Academy of Sciences)

Cultivar	Seedling infection (%)	Seed Infection (%)	
		Pericarp	Embryo
Japanese white	14.0	13.5	12.8
Arka nishant	1.5	0.5	0.4
Pusa desi	1.0	0.1	0.2
Pusa reshmi	1.0	0.0	0.1

be released from (Fig. 33) conidiophores but the RH suddenly decreases, then the branched conidiophores become dry and the twirling movement of the drying conidiophores may flick the spores in to the air and discharge abundant conidia from the diseased leaf. According to Shao et al. (1990) conidia release during favourable temperature and RH conditions is three times higher in the morning than in the afternoon.

Dispersal of conidia of *P. parasitica* on *Lepidium virginicum* begins with incipient desiccation and conclude with hygroscopic distortion of the aerial fructifications (Pinckard, 1942). Several complete twists occur in the portion of tall conidiophores extending up to the first branch, with a lesser number between each successively shorter branch. With the progress of drying, a twisting and binding motion is imparted to the sterigma-like structure on which the conidia are borne. If the process of desication stops, the twisting motion also stops. However, if humidity increases, the rotation reverses itself. Under conditions of delicate moisture balance, the breath of an observer is sufficient to induce the above mentioned movements. The outcome of the movement is the release of mature conidia. By slowly decreasing the vapour pressure, a point is reached when abscission occur, and the conidia are forcibly released with the stimulus for the requisite energy being derived from differential stresses set up within the sterigmata. The mechanical action of wind and rain, during periods of atmospheric saturation, does not appear to contribute significantly to dispersal of conidia.



The mechanism of *Peronospora parasitica* conidia discharge. (A) condiophore in damp air with attached conidia; (B) and (C) changes in conidiophore on exposure to dry air; and (D) recovery on return to damp condition (Reprinted from C.Y. Lin. 1981. Studies on downy mildew of Chinese cabbage caused by *Peronospora parasitica*. Proc. First Intern. Symposium, Asian Vegetable Research Development Centre, Shanhua, Taiwan: pp. 105-112, by permission of the author and the publisher Asian Development Research Development Centre, Taiwan).

h. Conidial germination

For germination of conidia collected from Chinese cabbage, 8-20°C is favourable with an optimum range of 12 - 16°C (Fig. 34) (Lin, 1981). Germ tubes usually grow normally and extensively at these temperatures. The germination rate of conidia is low and the germ tubes show limited and malformed growth at temperatures below 8°C and above 20°C. Conidia fail to germinate at extreme temperatures even after a long period of incubation. Conidial germination usually increases after treatment with hot water of up to 42°C (Fig. 35). Germination of conidia from Chinese cabbage (*B. pekinensis*) was optimum at 15 - 20 °C and was stimulated by light (Shao et al., 1990).

P. parasitica sporulates on intact cabbage seedlings when incubated at 13°C or 18°C in the presence of free water, or to atmospheric water potentials (ψ) of 0 or -30 $\pm$ 10 bars (Table 15) (Hartman et al., 1983). The pathogen fails to sporulate at these temperatures when the atmospheric ψ is -60, -90 or -120 bars. More conidia are produced at 13°C (1466 - 2265 conidia/45 mm^2 cotyledons) than at 18°C (821 - 1042 conidia/45mm^2 cotyledon). Conidia germinate in the presence of free water but do not germinate when exposed to atmospheric ψ of 0, -30, -60, or -90 bars for 24 h. The level of atmospheric ψ and the presence or absence of free water during sporulation exerts preconditioning effects on the ability of conidia to germinate.

Conidia collected from *B. campestris* (Toria, Brown sarson, Yellow sarson) and *B. oleracea* (cauliflower) leaves germinate after 1.5h at 18°C, whereas conidia derived from *B. juncea* germinate after 1h (Mehta and Saharan, 1994). Germination increases as the incubation period is increased. For instance, more than 80% conidia germinate after 4h (Table 10).

i. Oospore germination

The germination of oospores of *P. parasitica* infecting radish is dependent on temperature, light, pH of the medium, and age of oospores (Jang and Safeeulla, 1990a). The optimum temperature for germination is 23°C. Drying and chilling of oospores has no marked effect on germination. At a pH of 7.5 germination is 42% but at a pH of 4.5 only 1% oospores germinate. Oospore germination also increases with age (Jang and Safeeulla, 1990a).

4. INFECTION AND PATHOGENESIS

Infection may be either general or local. In the former case, all or most of the leaves and inflorescence (which may be hypertrophied as a result of pre-infection with *A. candida*) may bear conidiophores. Although some parts (especially the stem) may show no external injury, microscopic examination shows that the mycelia are in the tissues (Butler, 1918). Generalized infection is restricted to young tissues and this is why seedlings show completely infected leaves.

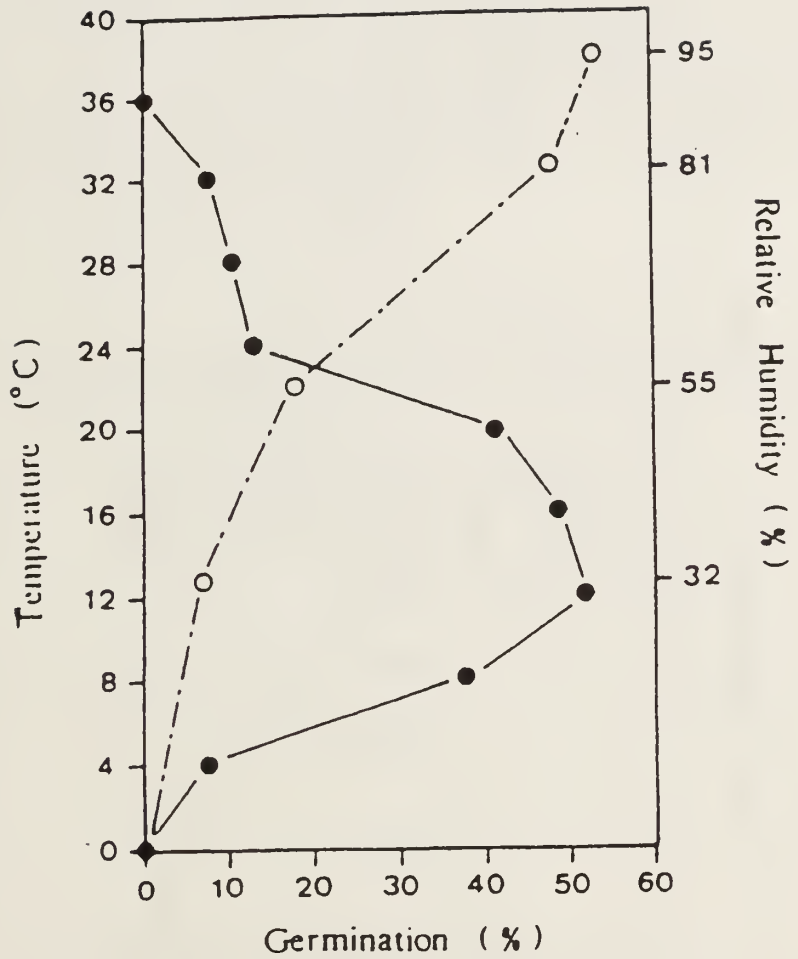


Fig. 34. The effect of temperature and relative humidity on the germination of conidia of *Peronospora parasitica* (Reprinted from C.Y. Lin. 1981. Studies on downy mildew of Chinese cabbage caused by *Peronospora parasitica*. Proc. First Intern. Symposium, Asian Vegetable Research Development Centre, Shanhua, Taiwan: pp. 105-112, by permission of the author and the publisher Asian Development Research Development Centre, Taiwan).

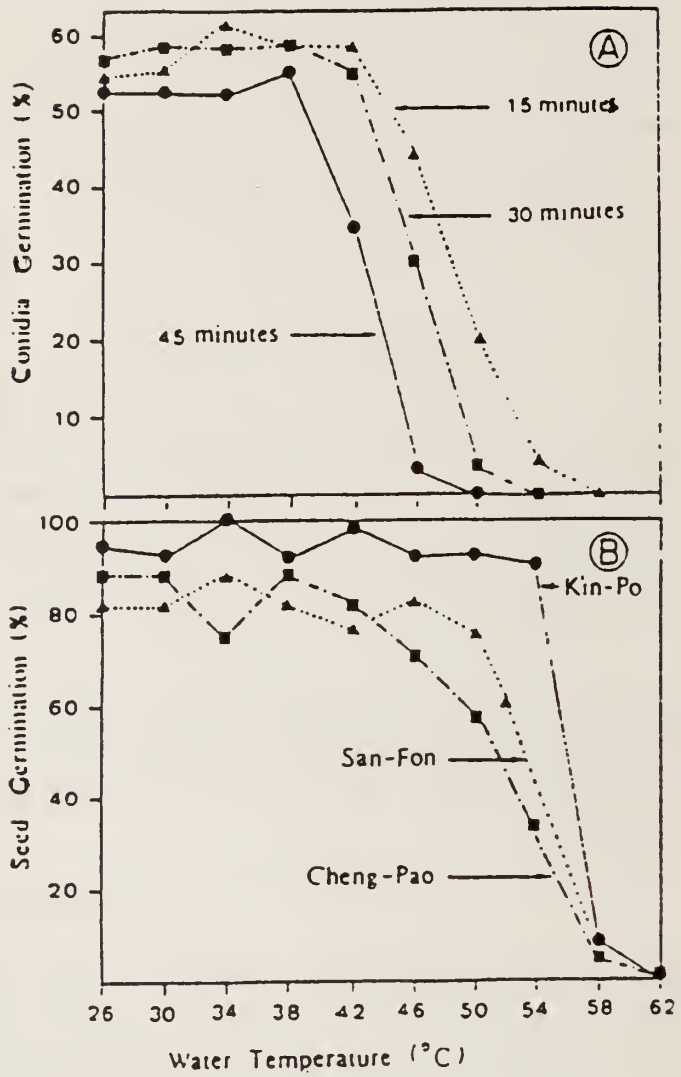


Fig. 35. Effect of hot water treatment on the germination of (A) conidia of *Peronospora parasitica*, and (B) seeds of three Chinese cabbage cultivars. Conidia were held at each temperature for 15, 30 and 45 minutes whereas seeds were held for 30 minutes only (Reprinted from C.Y. Lin. 1981. Studies on downy mildew of Chinese cabbage caused by *Peronospora parasitica*. Proc. First Intern. Symposium, Asian Vegetable Research Development Centre, Shanhua, Taiwan: pp. 105-112, by permission of the author and the publisher Asian Development Research Development Centre, Taiwan).

Table 15. An analysis of sporulation of *P. parasitica* on cabbage cotyledons at two temperatures and in free water or at atmospheric water potentials of 0 or -30 bars (Reprinted from H. Hartman, J.C. Sutton and R. Procter. 1983. Effects of atmospheric water potentials, free water and temperature on production and germination of sporangia in *Peronospora parasitica*. Can. J. Plant Pathol. 5:70-74, by permission of the authors and the publisher the Canadian Phytopathological Society)

	Numbers of sporangia/45 mm2 cotyledon* and integers** assigned for contrasts of these numbers in the following treatments†:						
Contrast number	13°C FW	13°C ψ=0	13°C ψ=-30	18°C FW	18°C ψ=0	18°C ψ=-30	F test‡
	1978§	1466	2265	1042	821	855	
1	-1	-1	-1	1	1	1	P=0.01
2	0	-1	0	1	0	0	NS
3	0	0	0	1	-1	0	NS
4	0	-1	1	0	0	0	P=0.01
5	0	0	0	0	-1	1	NS
6	-1	1	0	0	0	0	P=0.05
7	-1	0	1	0	0	0	NS

* Numbers of sporangia were contrasted using nonorthogonal coefficients and the differences were assessed by an F test. F tests are approximate for nonorthogonal contrasts.

** Treatments assigned positive integers were contrasted with those assigned negative integers.

† Treatments are identified according to temperatures and free water (FW) or atmospheric water potentials (ψ in - bars) at germination.

§ This value is the summation of mean numbers of sporangia/mm2 for each of 45 pairs of cotyledons.

‡ Significance level or nonsignificance (NS) for results of the F test.

Localized infection also occurs in young tissues, especially those still in active division. In the hypertrophy caused by *Albugo* (Awasthi et al., 1997), the cells of the epidermis and cortex are dividing and may readily give entrance to *Peronospora*. Young inflorescence may wholly or partly be infected while normal tissues of older stems and leaves below the initial site of infection may remain free.

When environmental conditions are suitable, conidia of *P. parasitica* on the surface of a susceptible host form germ tubes from which appressoria develop (Preece et al., 1967). In cauliflower, appressoria are found at the junction of the anticlinal walls of the epidermal cells. The contents of the conidium pass into the appressorium from which an infection hypha develops (Chou, 1970). Penetration is usually direct and only occasionally through a stoma(Shiraishi et al., 1975). It breaks a hole, 4-5 μ in dia., through the cuticle and after entering the host the hypha expands to a diameter of 7-8 μ. The fungus grows initially in the region of the middle lamella between the anticlinal walls of the epidermal cells. Penetration between adjacent epidermal cells rather than via stomata had been earlier reported by Chu (1935). The infection hypha continues its growth between the cells of the host tissues branching in all directions and varying in diameter and form according to the size and shape of the intercellular spaces (Chou, 1970).

A single conidium of *P. parasitica* is sufficient to infect kohlrabi (Krober, 1969) and radish (Achar, 1992a). The disease intensity and rate of infection increases as the number of spores in the inoculum increases. Commensurately, more conidia are required to produce a comparable responses in older plants which are less susceptible to infection than young plants (Krober, 1969). Disease intensity increases with increasing inoculum concentrations up to 30,000 conidia/ml of water but further increases in inoculum have no significant effect on the host (Achar, 1992a).

The rate of spore germination and host penetration are markedly affected by temperature. At 15°C, conidia germinate in 4-6h, appressoria form in 12h, and penetration occurs in 18-24h (Chu, 1935). Felton and Walker (1946), however, reported that on cabbage, germination of conidia and the subsequent penetration of the host takes place most rapidly at 8-12°C and 16°C, respectively. Jonsson (1966) found that development of the disease on winter rape is also favoured by temperatures of 8-16°C. In contrast Chou (1970) noted that at 20-25°C, infection occurs within 6h of the conidium deposition on the surface of the host cotyledon.

Haustoria develop from the infection hyphae in the epidermis as well as in those of the inner tissues (Chu, 1935). The typical symptoms of infection by *P. parasitica* begin to appear two days after inoculation at 15°C, and a day or two later the formation of conidiophores and conidia is initiated. The haustoria in turnip and radish roots are at first spherical to pyriforms, becoming cylindrical or clavate, often di- or trichotomously branched; the maximum dimensions of an unbranched haustorium in

this situation being 18 x 25 μm compared with only 11 x 8 μm in the leaves. They are usually spherical and bi- to trilobate and 57 x 14 μm in the stem of *B. chenensis*, where they are cylindrical or clavate and sometimes dichotomous. Some haustoria are surrounded by a sheath of variable extent from a collar round the neck to a third or half the length of the organ itself. The few full grown haustoria found completely enveloped in vigourous roots inoculated with the fungus are probably incapable of functioning (Fig. 36).

The systemic invasion of the hypocotyls and cotyledons of cabbage seedlings may take place from the soil contaminated with oospores (LeBeau, 1945). Further spread of the pathogen is by dissemination of conidia released from conidiophores formed on the cotyledons and hypocotyls (Chang et al., 1963).

The pathogen can also enter directly through the inflorescence axis (Jang and Safeeulla, 1990d). The infection through the stigma and ovary wall results in embryonal infection.

Pathogenesis in a susceptible combination is accompanied by large increases in electrolyte leakage, and increased activity of the enzymes, *B*-glucosidase, ribonuclease and peroxidase (Kluczewski and Lucas, 1982). The large increase in *B*-glucosidase originates from the pathogen and the enhanced ribonuclease activity is due to a new post infectional form of the enzyme. Infected *B. juncea* produce cellulase, indo-PMG and endo-PG (Singh et al., 1980).

5. DISEASE CYCLE

Downy mildew of crucifers is essentially a disease of foliar and other aerial plant tissues. The fungus survives as oospores in *A. candida* - induced malformed inflorescence and senesced host tissues, as conidia on leaves and inflorescence, and as latent systemic mycelium in seeds or infected plant debris. Infections are favoured by temperatures between 10 and 15°C and by high atmospheric humidity following rain or heavy dew. The conidia produce germ tubes which penetrate anticlinal cell walls often on the lower surface of the leaves. The penetration is usually direct but occasionally also occurs through a stoma (Shiraishi et al., 1975). Primary infection from soil-borne oospores has been obtained (LeBeau, 1945; Chang et al., 1963). Transmission by infected seed is possible but its importance has not been well documented. Further spread of the pathogen is by dissemination of conidia released from conidiophores formed on the cotyledons or hypocotyls. The true leaves are usually infected through wind-borne conidia, resulting in spread of the disease through secondary infection. The pathogen dispersal over short distances in water droplets can also occur. Although, there is no exact information on the relationship between leaf and floral infection under

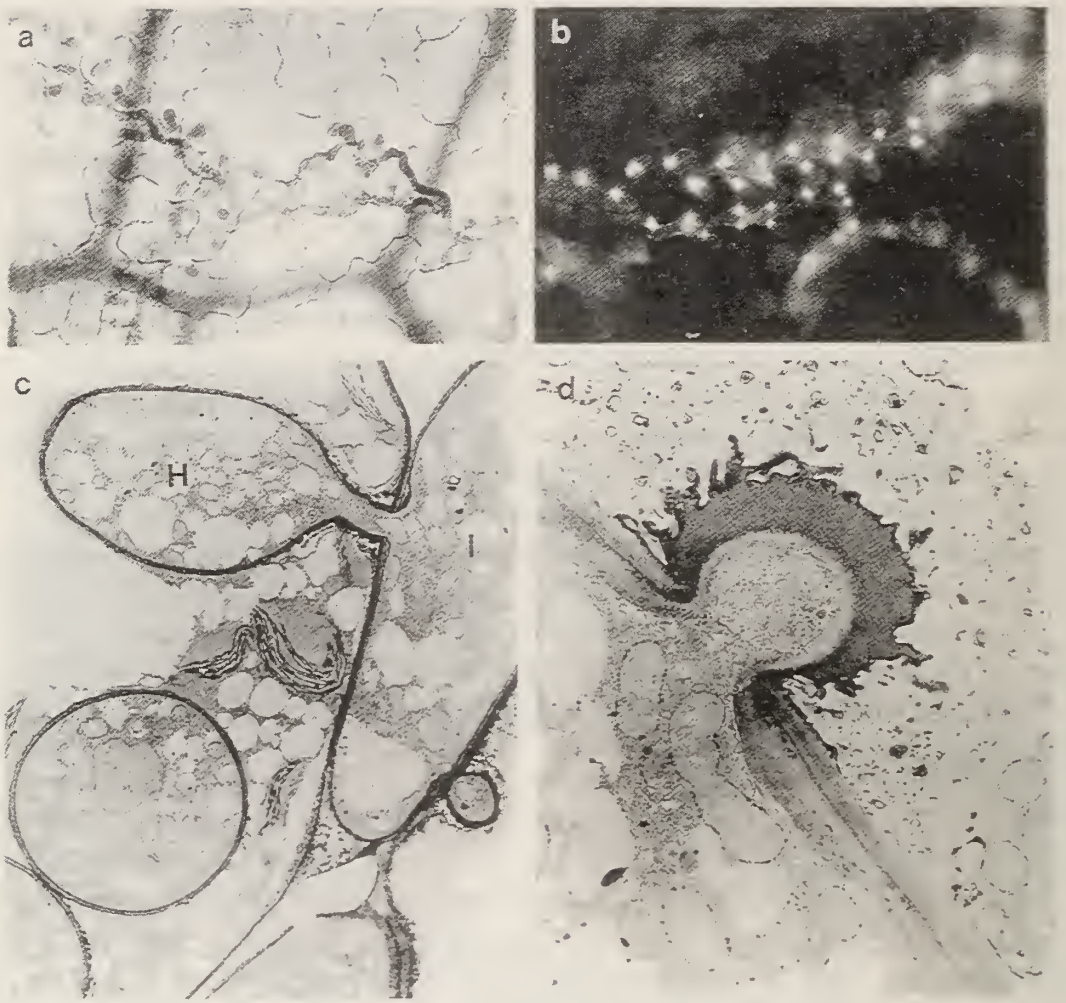


Fig. 36. Growth of crucifer downy mildew, *Peronospora parasitica*, in cotyledon tissues of Brassica. (A) intercellular hyphae forming club-shaped intracellular haustoria in host cells, stained with trypan blue (x 250); (B) fluorescence micrograph of similar preparation, stained with aniline blue, showing bright collars, presumed to callose-like material of host origin, at sites of haustorial penetration (x 280); (C) electron micrograph of intercellular hypha (I) and haustorium (H) in host cell (HC). A second haustorium can be seen in the same cell (x4200); and (D) cell wall encasement surrounding developing haustorium at site of attempted penetration. Such host cell responses are commonly seen during development of the pathogen in partially resistant hosts (x 10,500). (Reprinted from J.A. Lucas, J.B.R. Hayter and I.R. Crute. 1995. The downy mildews: host specificity and pathogenesis, IN: Pathogenesis and host specificity in plant diseases, Volume 2, Eucaryotes, Chapter 13:217-238, by permission of the authors and the publisher Elsevier Science ltd., The Boulevard, Langford Lane, Kidlington OX5 1GB, U.K.).

natural conditions, most inflorescence infections, as is *Albugo candida*, probably result from secondary spread of the pathogen rather than systemic infection. The diagrammatic life cycle of the disease developed by Lucas et al (1995) is given in Fig. 37.

6. EPIDEMIOLOGY

In epidemics of downy mildews, the pathogen population starts from a low level of initial inoculum which then increases exponentially through successive cycles on the host during the growing season. Therefore downy mildew of crucifers is a compound interest disease. The seasonal increase of the pathogen population has been investigated much more thoroughly than that of the initial inoculum. Information has been generated on the multiplication phase of the disease which relates to the sequence of events in the life of the pathogen on its host, which are infection, colonization and sporulation.

a. Disease development in relation to temperature, humidity, rainfall and leaf wetness

The relationship of host-pathogen-environment interaction in case of downy mildew of crucifers is a complex phenomenon which determines the rate of disease development (Fig. 38). Among the major environmental factors which markedly influence the development of downy mildew are air temperature and relative humidity. The rates of spore germination and host penetration is affected by temperature variations. Chu (1935) found that at 15°C conidia germinate in 4-6h, appressoria form in 12h, and penetration occurs in 18-24h. According to Eddins (1943) the downy mildew of cabbage is most destructive when the temperature ranges between 10° and 15°C, and when the plants remain wet until mid-morning for 4 consecutive days. However, Felton and Walker (1946) reported that on cabbage, germination of the conidia (Fig. 39) and subsequent penetration of the host takes place most rapidly at 8-12°C and 16°C, respectively. Formation of haustoria and growth of the fungus in the host tissues is most rapid at 20-24°C (Fig. 40). Symptoms develop quickly at 24°C, but sporulation and reinfection is limited at 24°C and 28°C. The lower temperature of 16°C results in slower growth of both the host and the pathogen, less damage, more prolific sporulation, more reinfection, and consequently, more profuse disease development. The severity of the disease at 10-15°C seems to be the effect of temperature upon production of inoculum, spore germination and infection (Figs. 41, 42). However, according to Nashaat (1997) a temperature of 15°C seems to be the most favourable for epidemic development as this favours slower growth of both host and pathogen resulting in less drastic damage and hence more profuse disease development. By contrast, Chou (1970) noted 20-25°C, and Nakov (1972) found 15-20°C as the most favourable temperature

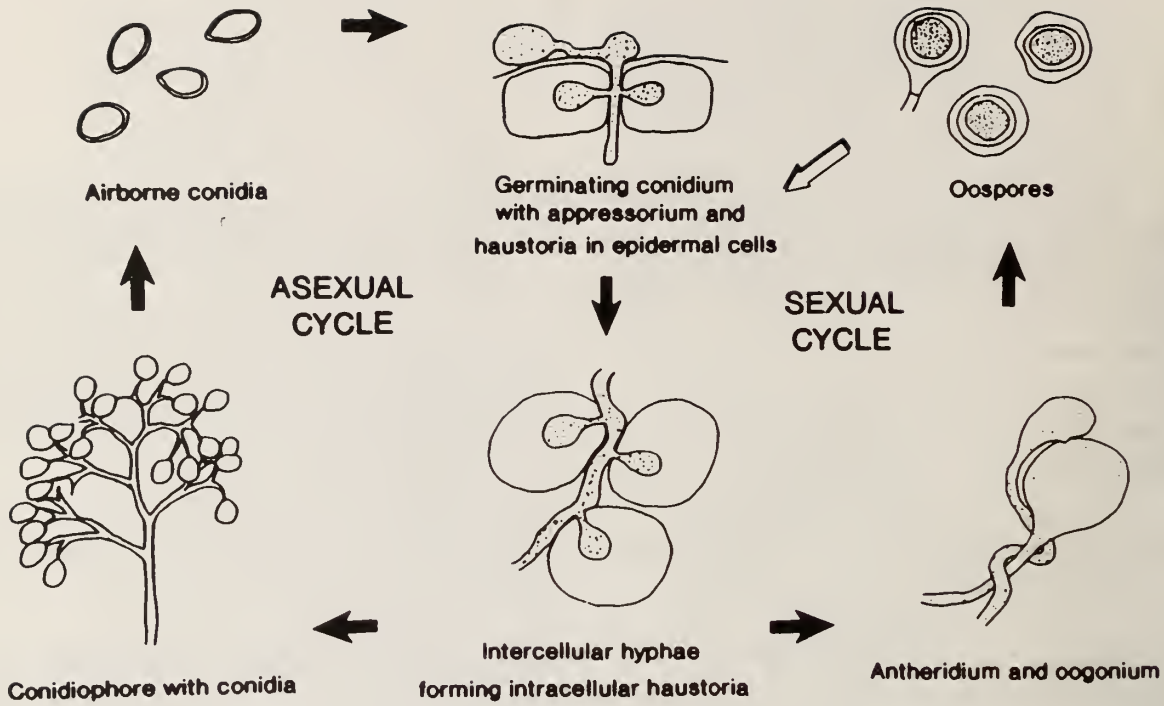


Fig. 37. Diagramatic life cycle of *Peronospora parasitica* causing downy mildew of crucifers (Reprinted from J.A. Lucas, J.B.R. Hayter and I.R. Crute. 1995. The downy mildews: host specificity and pathogenesis, IN: Pathogenesis and host specificity in plant diseases, Volume 2, Eucaryotes, Chapter 13:217-238, by permission of the authors and the publisher Elsevier Science ltd., The Boulevard, Langford Lane, Kidlington OX5 1GB, U.K.).

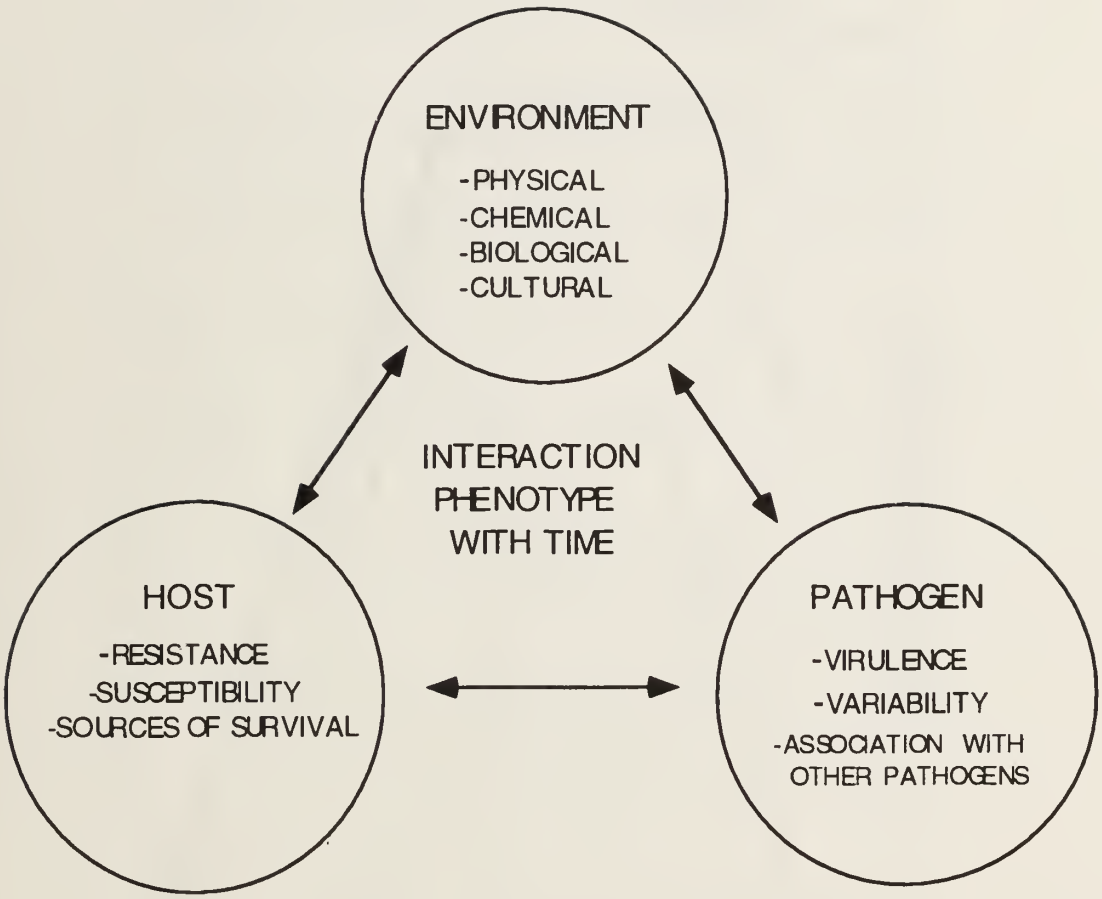


Fig. 38. The relationship of host, pathogen and environment in the interaction phenotype of downy mildew of crucifers.

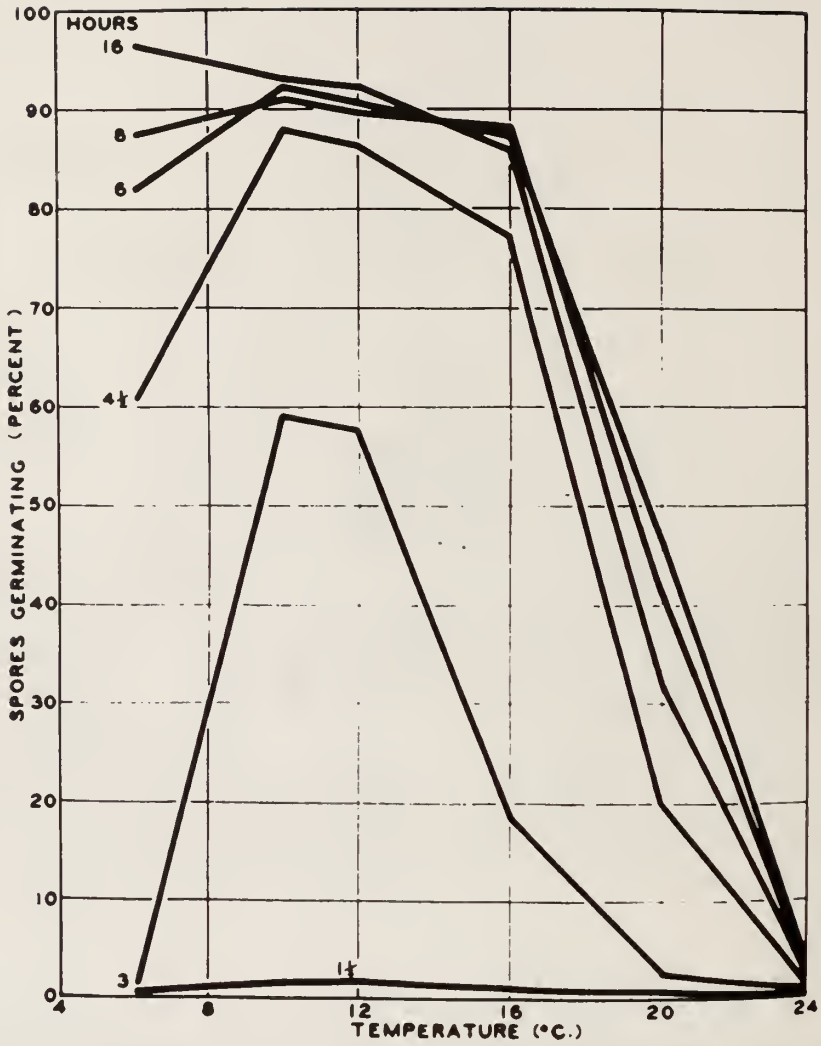


Fig. 39. Effect of time and temperature on germination of conidia of *Peronospora parasitica* (Reprinted from M.W. Felton and J.C. Walker. 1946. "Environal factors affecting downy mildew of cabbage." J. Agric. Res. 72: 69-81, by permission of the authors and the publisher superintendent of Documents, United States Government Printing Office, Washington, D.C.).

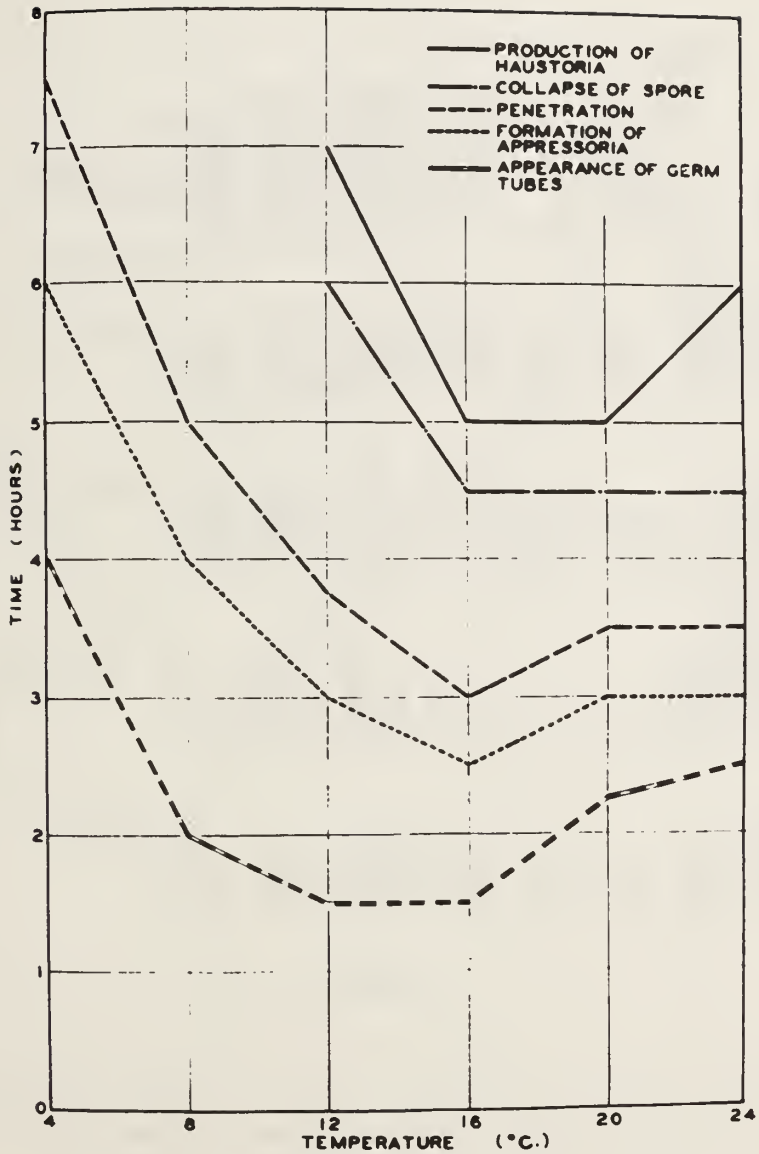


Fig. 40. Effect of temperature upon penetration and development of haustoria of *Peronospora parasitica* (Reprinted from M.W. Felton and J.C. Walker. 1946. "Environal factors affecting downy mildew of cabbage." J. Agric. Res. 72: 69-81, by permission of the authors and the publisher superintendent of Documents, United States Government Printing Office, Washington, D.C.).

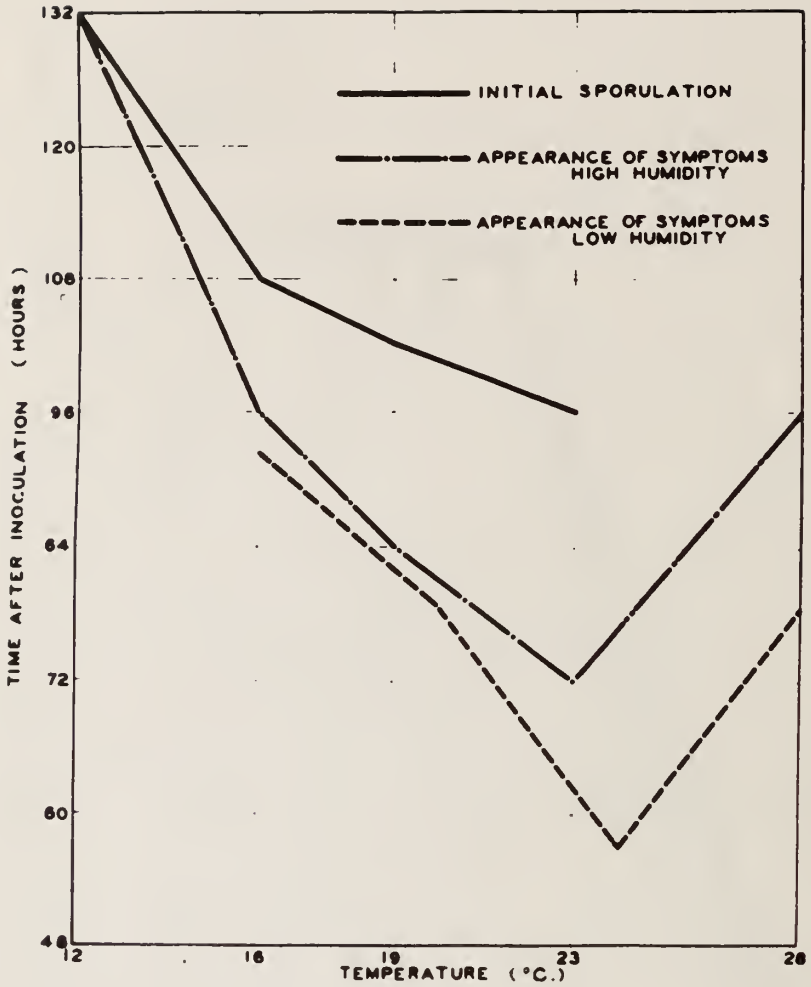


Fig. 41. Effect of five different temperatures on the initial sporulation of *Peronospora parasitica* at high humidity and upon initial appearance of symptoms at low and at high humidity (Reprinted from M.W. Felton and J.C. Walker. 1946. "Environal factors affecting downy mildew of cabbage." J. Agric. Res. 72: 69-81, by permission of the authors and the publisher superintendent of Documents, United States Government Printing Office, Washington, D.C.).

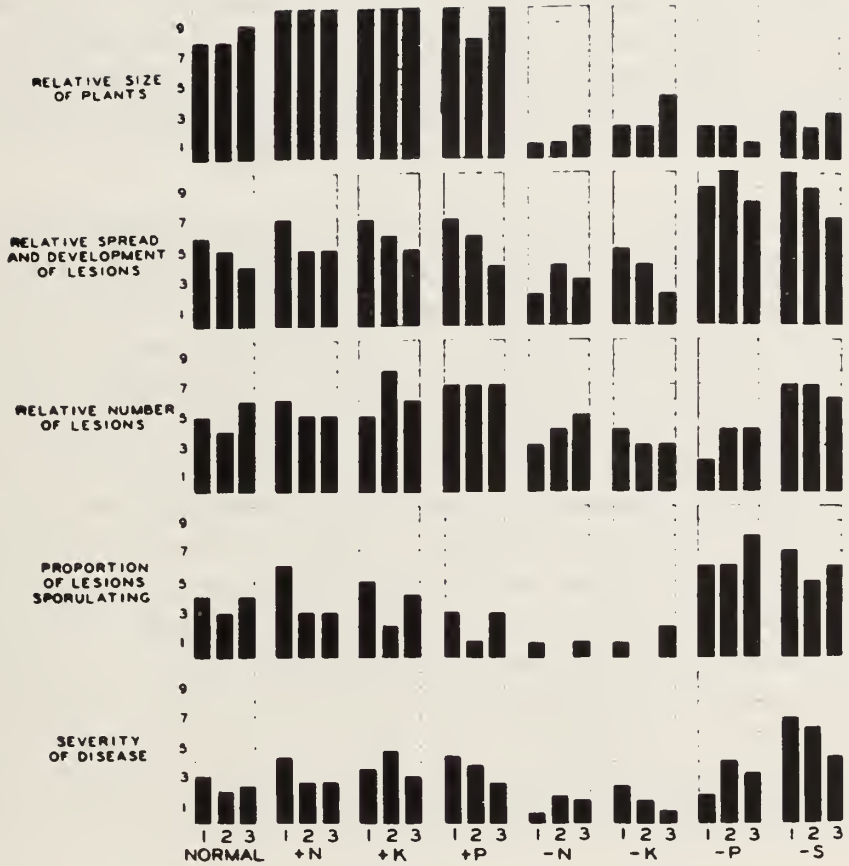


Fig. 42. Graphic summary of infection by and development of *Peronospora parasitica* on cabbage plants grown in sand culture supplied with various nutrient solutions (Reprinted from M.W. Felton and J.C. Walker. 1946. "Environal factors affecting downy mildew of cabbage." J. Agric. Res. 72: 69-81, by permission of the authors and the publisher superintendent of Documents, United States Government Printing Office, Washington, D.C.).

for infection. In temperate coastal regions of Madison, Wisconsin, USA where Chinese cabbage is grown from late summer through the winter and spring, downy mildew thrives during periods of frequent rains and high humidity. There is an 8-12h requirement of 100% RH for the production and dissemination of its air-borne conidia. Once inside the Chinese cabbage, hyphae spread through the leaves, petioles and stems, first feeding on the cells without apparent injury then suddenly causing yellowing, collapse and death of the tissues. Conidiophores and conidia are produced primarily on the lower side of the leaves (Williams and Leung, 1981).

On brassica oilseeds, *P. parasitica* is favoured by temperatures of 8-16°C, moist air, and weak light (Jonsson, 1966; D'Ercole, 1975). According to Bains and Jhooty (1979), a 17°C and 51 mm rainfall results in low infection of mustard in contrast to high infection at 14°C and 152 mm rainfall during the crop season. In a subsequent study, 15 - 20°C were the best temperatures for infection and development of downy mildew. At this temperature regime infection occurs within 24h of inoculation (Table 16, Fig. 43). The infection frequency is reduced at 25°C with no infection observed at 30°C (Table 16, Fig. 43). The maximum area under disease progress curve occurs at 20°C (AUDPC-60.54%, Fig. 43). Leaf wetness duration of 4-6h at 20°C, and for 6-8h at 15°C is essential for severe infection and disease development on mustard (Tables 17, 18, Figs. 44, 45). The infection frequency and disease development increases significantly with the increase in duration of leaf wetness (Mehta et al., 1995). According to Kolte et al. (1986), sunshine has a significant negative correlation, whereas total rainfall has a significant positive correlation with *A. candida* - induced staghead development on rapeseed-mustard (Table 19, Fig. 46). A reduced period of sunlight (2-6 h/d) and rainfall of up to 161 mm during the flowering period favours severe occurrence of the stagheads.

In Ukraine and Russia, downy mildew of white cabbage is more severe with abundant rain (75 - 100 mm/10 yr) and a 14-15 h of day light (Vladimirskaya et al., 1975).

b. Disease development in relation to planting time

In India, infection of mustard foliage starts by the end of October (cotyledon stage) and progresses up to November (Tables 20, 21). The crop planted after mid-November may not contract downy mildew. However, downy mildew growth as a mixed infection with white rust on floral parts can be seen up to March (Saharan, 1984; Kolte et al., 1986; Mehta, 1993).

c. Disease development in relation to host nutrition

Peronospora parasitica is severe on cauliflower plants which suffer from potash deficiency, while plants with a sufficient quantity of potash are only slightly attacked

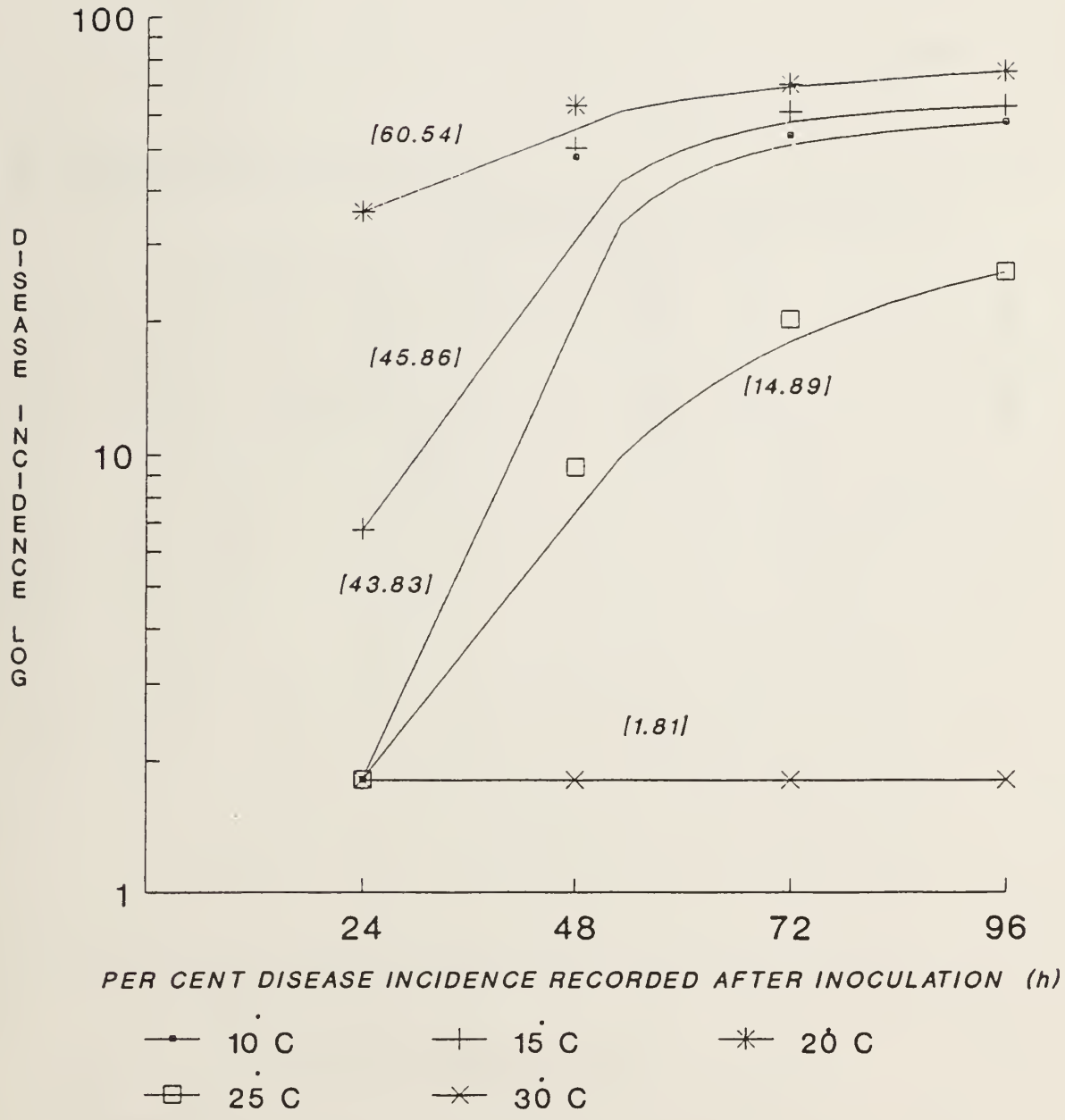


Fig. 43. Progression of downy mildew *(Peronospora parasitica)* of mustard *(Brassica juncea)* in relation to temperature (AUDPC) (Reprinted from N. Mehta, G.S. Saharan and O.P. Sharma. 1995. Influence of temperature and free moisture on the infection and development of downy mildew of mustard. Plant Dis. Res. 10:114-121, by permission of the authors and publisher).

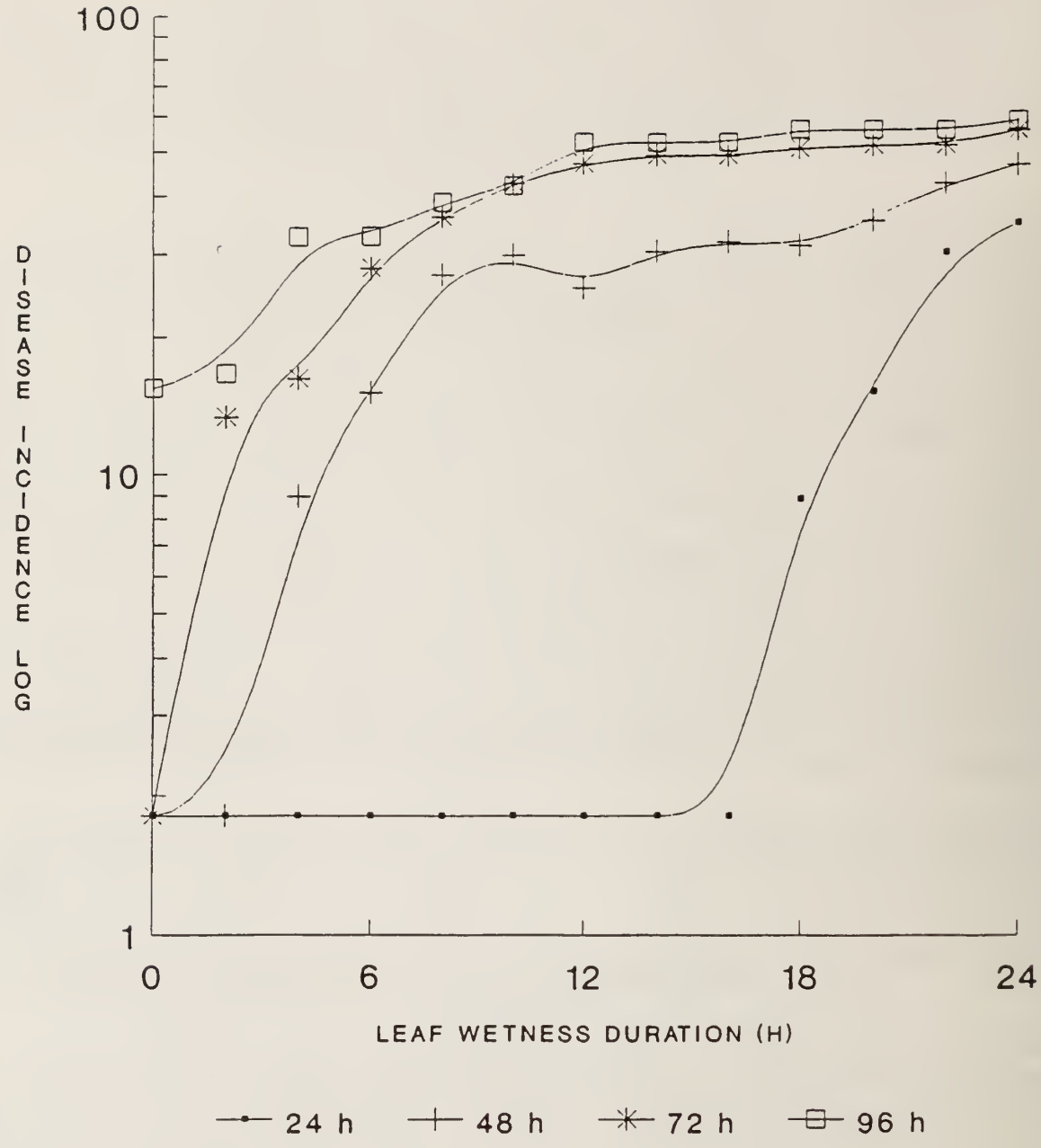


Fig. 44. Effect of leaf wetness duration on the development of downy mildew *(Peronospora parasitica)* infection on mustard *(Brassica juncea)* cultivar RH-30 at 20°C (Reprinted from N. Mehta, G.S. Saharan and O.P. Sharma. 1995. Influence of temperature and free moisture on the infection and development of downy mildew of mustard. Plant Dis. Res. 10:114-121, by permission of the authors and publisher).

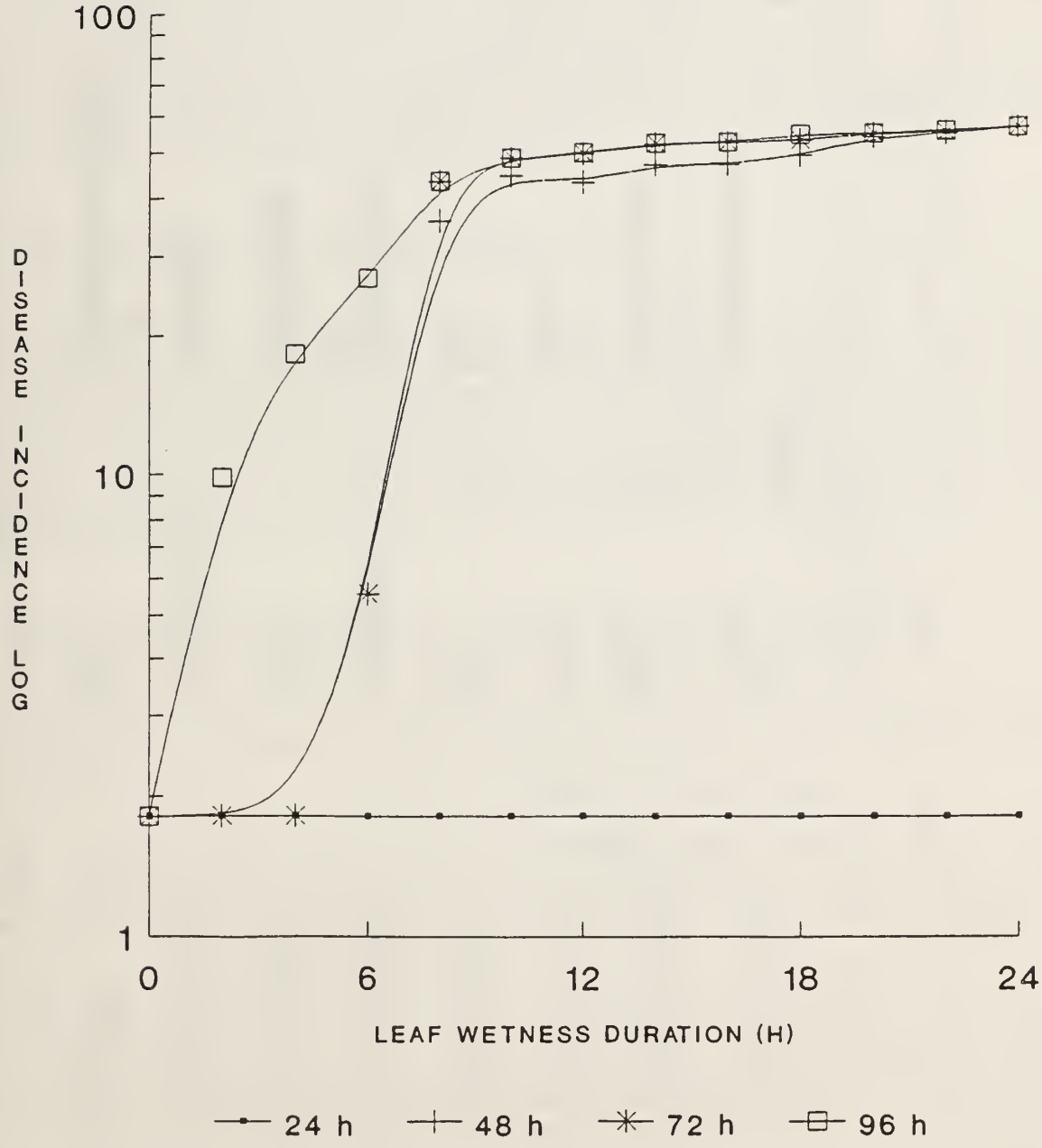


Fig. 45. Effect of leaf wetness duration on the development of downy mildew *(Peronospora parasitica)* on mustard *(Brassica juncea)* seedlings of cultivar RH-30 at 15°C (Reprinted from N. Mehta, G.S. Saharan and O.P. Sharma. 1995. Influence of temperature and free moisture on the infection and development of downy mildew of mustard. Plant Dis. Res. 10:114-121, by permission of the authors and publisher).

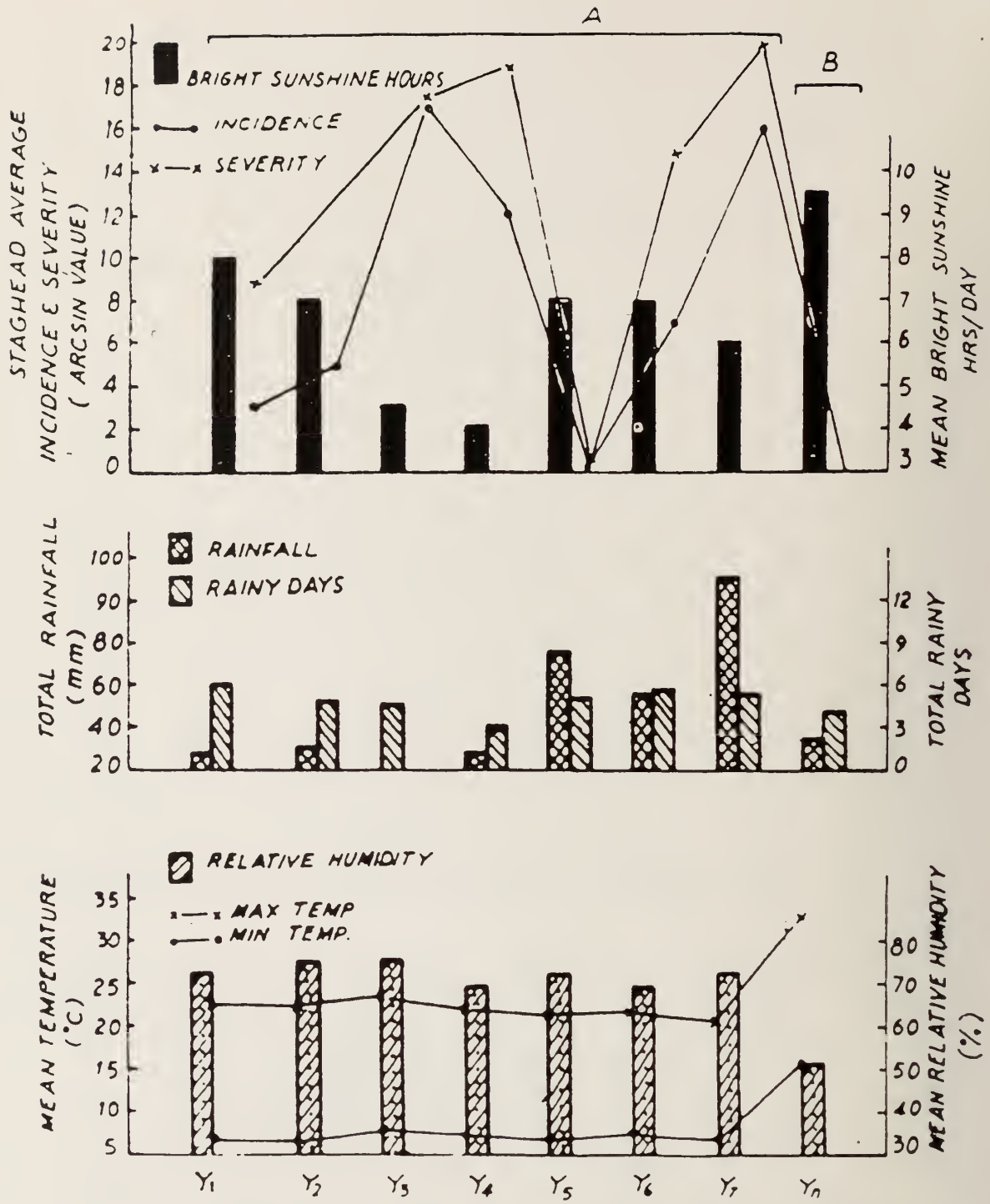


Fig. 46. Weather factors associated with occurrence (A) and no occurrence (B) periods of staghead phase of white rust *(Albugo candida)* and downy mildew *(Peronospora parasitica)* on mustard *(Brassica juncea)* in crop seasons Y_1 (1976-77), Y_2 (1977-78), Y_3 (1978-79), Y_4 (1979-80), Y_5 (1980-81), Y_6 (1981-82) and Y_7 (1982-83). Symbol Y_n represents the number of crop seasons covering the period from 1977-78 through 1982-83 under no occurrence periods of stagheads (B) (Reprinted from S.J. Kolte, R.P. Awasthi and Vishwanath. 1986. "Effect of planting dates and associated weather factors on staghead phase of white rust and downy mildew of rapeseed and mustard." Indian J. Mycol. Plant Pathol. 16: 94-102, by permission of the authors and the society of mycology and Plant Pathology, Udaipur, India).

(Quanjer, 1928). Cabbage plants grown in soil fertilized with less potash and more phosphorus are more prone to downy mildew than cabbages grown in unfertilized soil (Townsend, 1935). However, according to Butler and Jones (1949) there is no consistent effect of fertilizers on the development of downy mildew of brassicas. Felton and Walker (1946) found no direct relationship between mildew incidence and any excess or deficiency of nitrogen, phosphorus or potash. On radishes tubers, conidiophores and conidia appear to be relatively large which is probably due to the availability of ample nutrient supply in the tubers (Hammarlund, 1931).

d. Disease interaction with insecticidal sprays

The incidence of downy mildew in plots of broccoli sprayed with emulsifiable insecticide formulations containing a solvent and a wetting agent is significantly greater than in plots sprayed with an insecticide formulation containing no solvent or wetting agent or in unsprayed plots (Natti et al., 1956). It is possible that emulsifiable insecticide formulations remove the bloom from the leaves and dissolve the wax from the cuticle of the leaves creating conditions favourable for the germination of *P. parasitica* spores (Natti et al., 1956).

7. MIXED INFECTION AND ASSOCIATION WITH WHITE RUST

The association of downy mildew and white rust infection on oilseed brassicas, vegetable brassicas, wallflowers and stocks have long been observed (Butler, 1918; Wiese, 1927).

On horse-radish leaves and petioles, *A. candida* (white rust) and *P. parasitica* (downy mildew) are frequently associated with each other causing brown rot commencing at the head of the rootstock and extending downwards (Boning, 1936).

According to Bains and Jhooty (1985), *A. candida* often appears first in combined infections. It is followed by infection with *P. parasitica* which develops in and around *A. candida* colonies (Fig. 3). *A. candida* predisposes the host tissues towards susceptibility to this pathogen. The development of hypertrophied tissues of the staghead phase are attributed to infection with *A. candida*. The intensity of mixed infection by *A. candida* and *P. parasitica* on *B. juncea* inflorescence has been reported to be from 0.5 to 29.0 percent under Punjab (India) conditions (Bains and Jhooty, 1979, 1985). There is preferential parasitism of *P. parasitica* on galls of *B. campestris* caused by *A. candida* (Chaurasia et al., 1982). The hypertrophied malformed floral organs of mustard infected with *A. candida* are usually heavily covered with white sporulating fungal growth of *P. parasitica* consisting of conidia and conidiophores (Saharan and

Table 16. Effect of temperature on infection by *Peronospora parasitica* and disease development on mustard seedlings (cv. RH-30) (Mehta, Saharan and Sharma, 1995)

Temp (°C)	Per cent Disease Incidence after inoculation (h)								Mean		AUDPC*
	24		48		72		96				
10	0.00	(1.81)	54.09	(47.52)	64.19	(53.52)	70.53	(54.49)	47.20	(40.08)	43.83
15	2.91	(6.71)	58.47	(50.30)	73.88	(60.37)	75.55	(62.44)	52.70	(44.88)	45.86
20	34.31	(35.59)	78.33	(62.49)	87.56	(69.98)	90.27	(75.00)	72.61	(60.76)	60.54
25	0.00	(1.81)	5.08	(9.32)	14.25	(20.90)	19.72	(25.82)	9.76	(14.26)	14.89
30	0.00	(1.81)	0.00	(1.81)	0.00	(1.81)	0.00	(1.81)	0.00	(1.81)	1.81
Mean	9.40	(9.54)	48.99	(34.23)	59.97	(41.51)	64.01	(44.51)		-	-
Correlation coefficient (r)	0.08		0.78		0.82		0.80				
LSD (0.05)	Temperature (T) 3.55				Observations (0) 3.55		Temp. x Observation (T X 0) :7.11				

Figures in the parentheses are angular transformed values after adding 0.1.

* Area under disease progress curve

Verma, 1992; Saharan, 1992a). Incidence and severity of mixed infections by *A. candida* and *P. parasitica* on *B. juncea* inflorescence is higher on detopped than on normal plants (Bains, 1989). Severity of mixed infections on leaves is not related to infections on inflorescence. It seems that greater susceptibility of new inflorescence and their availability over extended periods of time is associated with this phenomenon (Bains and Sokhi, 1986). *Peronospora parasitica* also causes severe infections and high levels of sporulation on plants of mustard systematically infected with mustard mosaic virus (Bains and Jhooty, 1978).

In artificially inoculated leaves of mustard, the stimulatory effect of *A. candida* infection is more intense when *P. parasitica* is inoculated 7 days after *A. candida* (Chaudhury and Verma, 1987). When *Peronospora* and/or *Albugo* are inoculated alone or in different combinations, the downy mildew infection takes 7 days, while white rust appears within 5-6 days of inoculations. When both the pathogens are inoculated simultaneously in a 50:50 spore concentration then there is delay in the expression of infections by *Peronospora* for 2-3 days (Mehta et al., 1995).

Table 17. Effect of leaf wetness duration on infection by *Peronospora parasitica* and disease development on mustard seedlings (cv. RH-30) at 20°C (Mehta, Saharan and Sharma, 1995)

Leaf Wetnes Duration (h)	Percent Disease Incidence after inoculation (h) 24		48		72		96		Mean	
0	0.0	(1.81)	0.00	(1.81)	0.0	(1.81)	7.08	(15.41)	1.77	(5.21)
2	0.0	(1.81)	0.00	(1.81)	5.18	(13.30)	8.51	(16.57)	3.42	(8.37)
4	0.0	(1.81)	3.05	(8.94)	7.77	(16.14)	29.04	(32.48)	9.96	(14.84)
6	0.0	(1.81)	6.80	(15.05)	22.12	(27.87)	29.35	(32.63)	14.56	(19.34)
8	0.0	(1.81)	21.52	(27.00)	35.83	(35.99)	35.30	(38.69)	24.16	(25.79)
10	0.0	(1.81)	24.58	(29.70)	45.37	(42.39)	44.44	(41.85)	28.59	(28.93)
12	0.0	(1.81)	18.57	(25.30)	53.03	(46.84)	62.26	(52.24)	33.46	(34.05)
14	0.0	(1.81)	25.27	(30.18)	56.46	(48.78)	61.94	(52.02)	35.91	(33.19)
16	0.0	(1.81)	27.91	(31.79)	56.52	(48.84)	62.50	(52.31)	36.73	(33.68)
18	5.0	(8.83)	26.80	(31.22)	59.86	(50.78)	68.33	(55.88)	39.99	(36.68)
20	9.16	(15.15)	34.38	(35.48)	61.57	(51.80)	68.61	(56.06)	43.43	(39.62)
22	26.25	(30.39)	46.11	(42.82)	61.66	(57.98)	69.16	(56.04)	50.79	(45.30)
24	32.96	(35.01)	53.19	(46.90)	67.80	(55.96)	72.77	(58.83)	56.68	(49.17)
Mean	5.64	(8.12)	22.16	(25.23)	41.01	(37.88)	47.94	(43.13)	29.18	(28.59)

LSD (0.05) Observation (0) = (2.54) Leaf Wetness duration (W) = (4.59) 0*W = (9.19)

Correlation coefficient (r)	0.72	0.95	0.95	0.94

Figures in parentheses are angular transformed values after adding 0.1.

Histopathological studies carried out by Mehta et al. (1995) indicated that conidia of *Peronospora* and sporangia of *Albugo* inoculated on mustard leaves germinate 24h after inoculation. Two days after inoculation infection is normally confined to the host epidermis. The pathogens penetrate up to 1/3 of the mesophyll cells by the third day after inoculations. Six days after inoculation, the pathogens progress deeper into the tissues. When *P. parasitica* is inoculated prior or after *A. candida*, mycelium can be seen in the intercellular spaces with globose to knob like haustoria in the mesophyll cells. When *A. candida* is inoculated alone or in combination with *P. parasitica*, the pathogen emerges from the lower epidermis and forms pustules. However, on its own *Peronospora* causes necrosis in the mesophyll cells. When both the pathogens are inoculated together, the infection is confined to the upper layer of the mesophyll with limited colonization of the cells and few haustoria or mycelium in the intercellular spaces. Nine days after inoculation, characteristic disease symptoms are visible. The white rust pustules show hyaline sporangiophore bearing globose to oval shaped

Table 18. Effect of leaf wetness duration on infection by *Peronospora parasitica* and disease development on mustard seedlings (cv. RH-30) at 15°C (Mehta, Saharan and Sharma, 1995)

Leaf Wetness Duration (h)	Percent Disease Incidence after inoculation (h) 24		48		72		96		Mean	
0	0.0	(1.81)	0.00	(1.81)	0.00	(1.81)	0.00	(1.81)	0.00	(1.81)
2	0.0	(1.81)	0.00	(1.81)	0.00	(1.81)	3.75	(9.84)	0.93	(3.81)
4	0.0	(1.81)	0.00	(1.81)	0.00	(1.81)	10.00	(18.21)	2.50	(5.91)
6	0.0	(1.81)	1.66	(5.55)	1.66	(5.55)	20.83	(26.90)	8.52	(9.95)
8	0.0	(1.81)	34.02	(35.73)	47.10	(43.41)	47.10	(43.41)	32.05	(31.09)
10	0.0	(1.81)	49.32	(44.69)	56.41	(48.74)	56.41	(48.74)	40.53	(35.99)
12	0.0	(1.81)	46.84	(43.26)	58.05	(49.74)	58.47	(49.99)	40.84	(36.20)
14	0.0	(1.81)	53.82	(47.18)	62.73	(52.36)	62.78	(52.48)	44.83	(38.00)
16	0.0	(1.81)	54.02	(47.39)	63.31	(52.81)	63.31	(52.81)	45.16	(38.70)
18	0.0	(1.81)	57.93	(49.55)	64.60	(53.49)	66.97	(54.94)	47.37	(39.49)
20	0.0	(1.81)	65.83	(54.31)	67.50	(55.33)	67.50	(55.33)	50.20	(41.69)
22	0.0	(1.81)	67.50	(55.33)	68.09	(55.69)	68.88	(56.17)	51.11	(42.25)
24	0.0	(1.81)	70.07	(57.06)	70.07	(57.06)	70.07	(57.06)	52.55	(46.51)
Mean	0.0	(1.81)	38.53	(34.28)	43.04	(38.53)	45.85	(42.26)	31.85	(29.22)

LSD (0.05)	Observation (0) = (1.38)	Leaf Wetness duration (W) = (2.49)	0*W = (4.99)
Correlation coefficient (r)	0.00	0.91	0.91	0.93

Figures in parentheses are angular transformed values after adding 0.1.

sporangia in chains. The *Peronospora* mycelium is intercellular with lobe shaped haustoria in the distorted tissue of leaves. When both pathogens are inoculated together, infection is extended to mesophyll cells and there is development of pustule below the epidermis. Twelve days after inoculation, complete colonization of the host tissues by the pathogen is evident from the development of necrotic zone by *P. parasitica* and bursting of pustules releasing sporangia in case of *A. candida*. In the inflorescence, the mycelium passes through the epidermis, hypodermis, cortex and finally reaches to the pith region. The mycelium is in abundance in the cortex and produces conidiophores bearing conidia above the epidermis layer. For *A. candida,* numerous sporangiophores bearing sporangia are observed below the epidermis layer in the form of pustules and knob like haustoria in the tissues. In the colonized tissues both pathogens cannot be distinguished based on somatic morphology.

Table 19. Prediction equations for the progress of downy mildew and white rust of rapeseed-mustard using different combinations of weather factors (Reprinted from S.J. Kolte, R.P. Awasthi and Vishwanath. 1986. Effect of planting dates and associated weather factors on staghead phase of white rust and downy mildew of rapeseed and Indian J. Mycol. Plant Pathol. 16:94-102, by permission of the authors and the publisher the Society of Mycology and Plant Pathology, Udaipur, India)

Equations	b_0	x_1	x_2	x_3	x_4	x_5	x_6	R^{2*}
Staghead incidence (Y_1) (%)	+16.925	+0.019	-0.132	-0.086	+0.158	+0.030	-1.469	0.6849
Staghead severity (Y_2) (%)	+86.169	-1.241	-0.129	-0.503	+0.054	+0.472	-2.125	0.6283

x_1 = mean maximum temperature
x_2 = mean minimum temperature
x_3 = mean relative humidity
x_4 = total rainfall (mm)
x_5 = total rainy days
x_6 = mean bright sunshine period (h/day)
* Significant at 5% level

8. BIOCHEMISTRY OF THE HOST PATHOGEN INTERACTION

Biochemical studies of the growth and survival of a pathogen and the changes it induces in its host can ultimately lead to a better understanding of the disease development, its epidemiology and control. Ideal prerequisites for meaningful studies of the biochemistry of the host-parasite interaction are: (a) A clear understanding of the genetic control of virulence and avirulence in the parasite and of susceptibility and resistance in the host, (b) Precise histological and cytological descriptions of spore germination, infection, and the establishment and development of the host-pathogen interaction, and (c) The availability of methods for maintaining the pathogen alone and in combinations with its host, under controlled conditions. Unfortunately, these criteria have not yet been satisfactorily met for downy mildew of crucifers.

a. Metabolic changes

Many marked shifts were observed in the metabolic processes of plant tissues following infection by biotrophic parasites. These included changes in respiration, photosynthesis, nucleic acid and protein synthesis, and phenol metabolism. There could

Table 20. Effect of planting time on the severity of downy mildew and white rust of mustard (Saharan, 1984)

Planting time	Percent disease intensity		
	Hisar	Kanpur	Pantnagar
06.10.1978	10.0	-	-
21.10.1978	8.6	-	-
28.10.1978	18.6	-	-
06.11.1978	55.4	-	-
18.11.1978	68.5	-	-
02.12.1978	72.8	-	
01.10.1979	-	24.16	-
10.10.1979	4.6	28.30	-
20.10.1979	10.0	34.34	-
30.10.1979	22.5	36.18	-
09.11.1979	46.8	40.91	-
20.11.1979	57.5	46.15	-
03.10.1981	-	-	15.04
23.10.1981	-	-	19.85
13.11.1981	-	-	32.85

also be changes in the translocation and accumulation of nutrients and in the levels of endogenous growth substances.

The respiration rate was raised sharply soon after *P. parasitica* infection of cabbage cotyledons and reached a maximum, almost twice that of uninfected controls, at the time of the initiation of sporulation (Fig. 47). The chlorophyll content of infected and noninfected cotyledons did not differ significantly at any time (Fig. 48). The increased respiration rate of the infected tissues did not reflect any significant changes to the pentose phosphate pathway in this infection since no acyclic polyhydric alcohols were detected in soluble extracts of either infected leaves or fungal conidida (Thornton and Cooke, 1974) (Figs. 49, 50).

Pathogenesis in *Brassica* - *Peronospora* combinations was observed to be accompanied by large increases in electrolyte leakage (Fig. 54-57) and increased activity of β.-glucosidase (Fig. 55), ribonuclease (Fig. 56) and peroxidase (Fig. 57) (Kluczewski and Lucas, 1982). The large increase in β.-glucosidase were of pathogen origin while enhanced ribonuclease activity was due to a new post-infectional form of the enzyme.

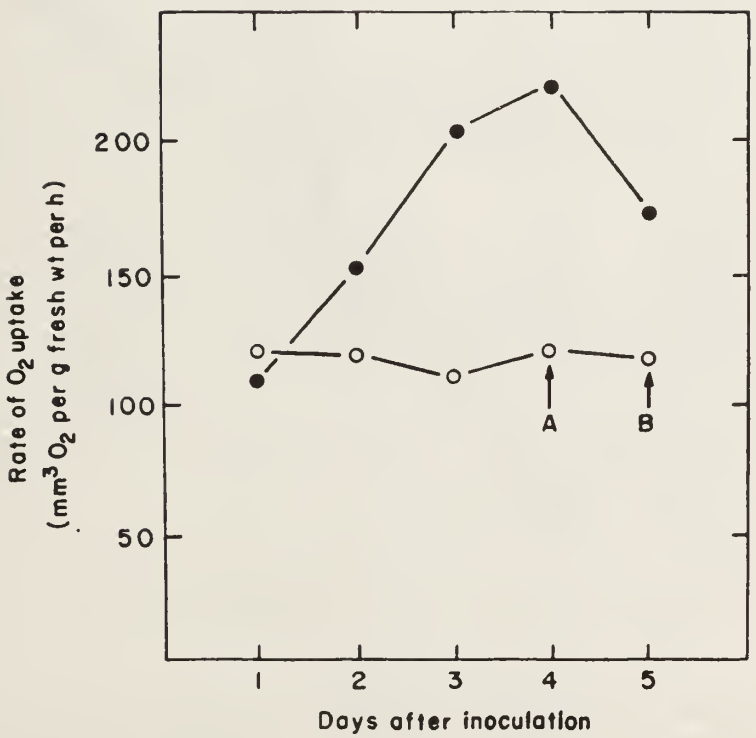


Fig. 47. Rates of O_2 uptake of infected and uninfected cotyledons at various times after inoculation: (•-•), infected; (o-o), uninfected; A, visible signs of sportulation (Reprinted from J.D. Thornton and R.C. Cooke. 1974. Changes in respiration, chlorophyll content and soluble carbohydrates of detached cabbage cotyledons following infection with *Peronospora parasitica* (Pers. ex Fr.), by permission of the authors and the publisher Academic Press Limited, London).

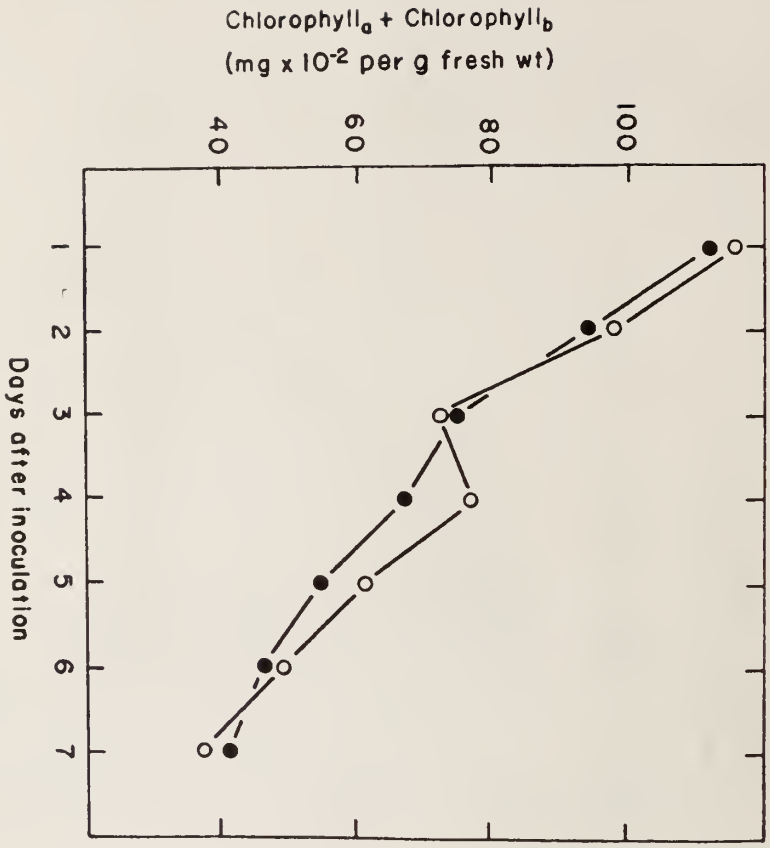


Fig. 48. Chlorophyll$_a$ plus chlorophyll$_b$ content of infected and uninfected cotyledons at various times after inoculation: (•-•), infected; (o-o), uninfected (Reprinted from J.D. Thornton and R.C. Cooke. 1974. Changes in respiration, chlorophyll content and soluble carbohydrates of detached cabbage cotyledons following infection with *Peronospora parasitica* (Pers. ex Fr.), by permission of the authors and the publisher Academic Press Limited, London).

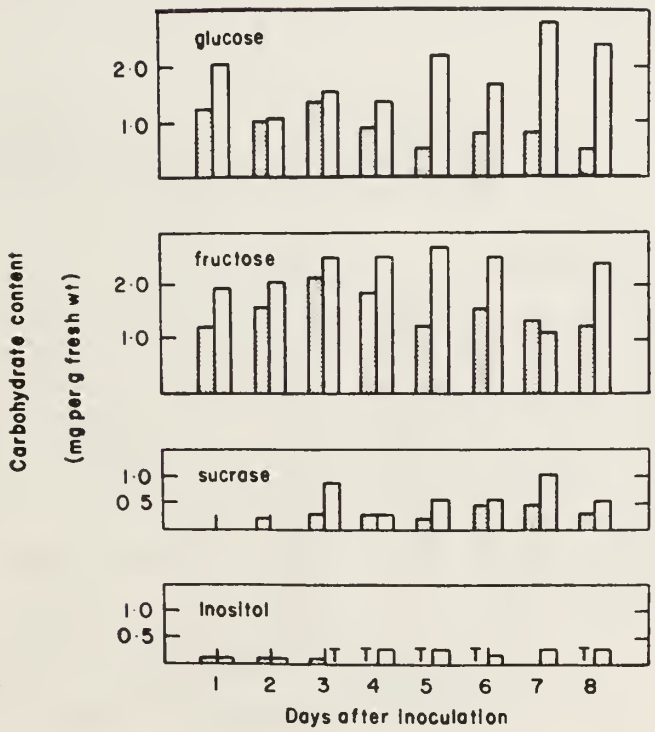


Fig. 49. Carbohydrate content of the alcohol soluble fraction of infected and uninfected cotyledons at various times after inoculation with *Peronospora parasitica*: ■ = infected; □ = uninfected; T = trace (indicating that the peak height of the TMS derivative was indeterminable at an attenuation of 20 x 10^3 (Reprinted from J.D. Thornton and R.C. Cooke. 1974. Changes in respiration, chlorophyll content and soluble carbohydrates of detached cabbage cotyledons following infection with *Peronospora parasitica* (Pers. ex Fr.), by permission of the authors and the publisher Academic Press Limited, London).

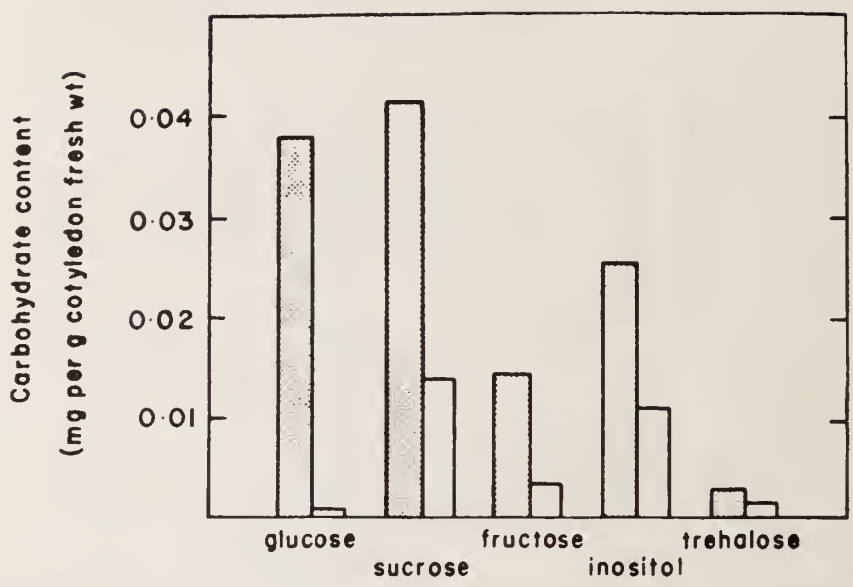


Fig. 50. Principal carbohydrates of the alcohol soluble fraction of sporangia from infected cotyledons and control washings, 7 days after inoculation with *Peronospora parasitica*: ■ = infected; □ = uninfected (Reprinted from J.D. Thornton and R.C. Cooke. 1974. Changes in respiration, chlorophyll content and soluble carbohydrates of detached cabbage cotyledons following infection with *Peronospora parasitica* (Pers. ex Fr.), by permission of the authors and the publisher Academic Press Limited, London).

Table 21. Influence of planting dates on staghead incidence and severity of white rust and downy mildew of rapeseed and mustard in three *rabi* crop seasons starting from 1977-78 to 1979-80 (Reprinted from S.J. Kolte, R.P. Awasthi and Vishwanath. 1986. Effect of planting dates and associated weather factors on staghead phase of white rust and downy mildew of rapeseed and mustard. Indian J. Mycol. Plant Pathol. 16:94-102, by permission of the authors and the publisher the Society of Mycology and Plant Pathology, Udaipur, India)

	1977-78						1978-79						1979-80					
	Yellow Mustard		Sarson		Toria		Yellow Mustard		Sarson		Toria		Yellow Mustard		Sarson		Toria	
	I	S	I	S	I	S	I	S	I	S	I	S	I	S	I	S	I	S
Oct. 1-6	2.7	11.0	0.0	0.0	0.0	0.0	20.6	15.6	24.3	23.1	10.2	20.3	8.9	20.3	4.8	15.7	0.7	4.4
Oct. 11-14	6.7	15.6	1.2	8.4	0.0	0.0	14.6	20.4	23.7	24.0	7.7	21.8	14.2	17.7	8.3	32.4	1.8	6.4
Oct. 20-22	10.2	23.4	4.1	24.8	0.0	0.0	10.6	11.3	17.2	14.9	18.2	16.8	7.8	14.6	3.6	17.7	3.6	16.9
Oct. 31-Nov.1	10.2	20.9	8.2	35.2	10.2	23.9	7.8	9.7	11.6	17.2	24.9	13.5	5.3	11.5	11.2	43.4	9.2	22.4
Nov. 1-11	9.3	22.4	9.7	25.8	10.6	25.3	9.6	11.1	11.2	16.5	9.4	14.4	3.1	5.2	6.7	16.3	182347	Nov.
20-22	14.2	22.8	38.2	25.7	22.2	24.0	9.1	10.9	13.7	19.0	8.9	15.3	4.3	8.5	1.4	8.8	15.9	23.2

I = Incidence (% plants affected). S = Severity (% recemes affected/plant)

CD at 5%

		1977-78	1978-79	1979-80
For planting dates	I	5.6	2.8	3.6
	S	5.5	1.7	9.8
	I	NS	1.8	2.6
	S	3.4	1.3	4.2

In vivo infected leaves of *B. juncea* produced cellulase, endo - PMG and endo - PG (Singh et al., 1980).

b. Role of natural biochemical compounds

There are a number of natural biochemical compounds present in host tissues which may influence the defence mechanism of crucifers against downy mildew infection. The role of phenolic compounds, glucosinolates and flavour volatile compounds in providing resistance to crucifers against downy mildew infection has been explained in section "Biochemical basis of resistance" of chapter 9E.

9. RESISTANCE

Genetic resistance is the most important attribute of the host defense against *P. parasitica*. Host resistance provides an economical, environmentally benign, and widely accepted method of managing downy mildew of crucifers.

a. Mechanism of host resistance

The first defense barrier in crucifers is the cuticle which is often covered with a waxy layer, a hairy surface and a few stomata with narrow apertures. The mechanisms of resistance to *P. parasitica* in Chinese rape, cabbage and radish was studied by Wang (1949) through observations on pathogen entry, mycelial and haustorial development and sporulation. All plants regardless to whether they are susceptible or resistant, were initially penetrated directly through the epidermal cells or by entering the stomata. After penetration, the mycelia grew through the intercellular spaces of the leaf mesophyll and haustoria penetrate the cells of susceptible hosts. In the resistant and immune hosts, development of mycelia and formation of haustoria were curtailed with the death of the surrounding host cells (Fig. 58). The pathogen sporulated abundantly on susceptible hosts, but necrotic reaction was associated with the infection of the resistant hosts. On the immune hosts, few minute necrotic spots/or occasionally no visible symptoms were observed. Weak light and high moisture conditions may alter the resistant or immune reaction of the host.

The growth of two isolates of *P. parasitica* obtained from cauliflower and oilseed rape (*B. napus*) was assessed in their respective hosts of origin and also in the alternative combination by Kluczewski and Lucas (1982). Both isolates were capable of infecting either host, but there were marked contrasts in the time course and extent of mycelial development, the amounts of associated host cell necrosis, and eventual intensity of sporulation (Figs. 51-53). Oilseed rape which is partially resistant to the isolate from cauliflower, exhibits extensive necrosis of mesophyll cells in conjunction with reduced mycelial development and delayed and reduced sporulation by the pathogen (Figs. 51-53). The isolate from oilseed rape is virulent on both host species. Pathogenesis in the susceptible combination is accompanied by a large increase in electrolyte leakage, and increased activity of the enzymes β-Glucosidase, ribonuclease and peroxidase.

The growth of hyphae in the susceptible cultivar of Japanese radish was reported to be faster than that in the resistant cultivar (Ohguchi and Asada, 1991). Five days after inoculation, haustoria were formed in the cells of the 63rd cell layer from the inoculated layer in the susceptible cultivar and in the cells of 12th cell layer in the resistant cultivar. The haustoria formed in both cultivars were similar in size and shape. On the surface of haustoria spherical or semi-spherical granules, 1.7 - 3.7 μm

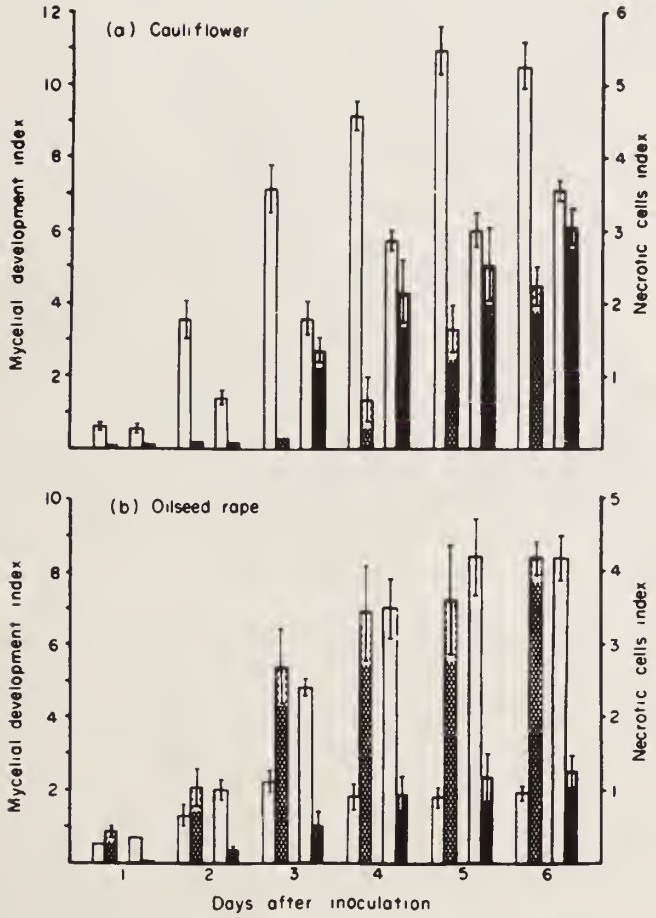


Fig. 51. Relationship between mycelial development and host-cell necrosis estimated as granulation and browning of cells in (a) cauliflower; and (b) oilseed rape inoculated with *Peronospora parasitica* isolates from cauliflower (C1) and oilseed rape (R1). □ C1 mycelial growth index; ■ C1 necrotic cell index; □ R1 necrotic cell index. Bars indicate ± standard deviation (Reprinted from S.M. Kluczewski and J.A. Lucas. 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant Brassica species. Plant Pathology 31:373-389, by permission of the authors and the publisher Blackwell Science ltd., Osney Mead, Oxford, U.K.).

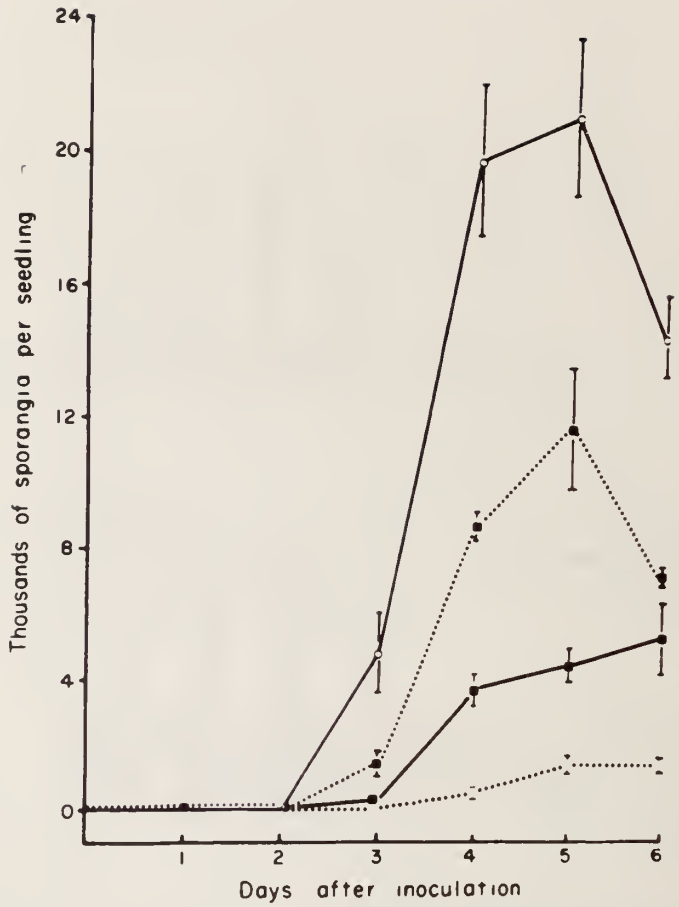


Fig. 52. Time course of sporulation of *Peronospora parasitica* isolate from cauliflower (o), and oilseed rape (■) on cauliflower (-) and oilseed rape (...). Bars indicate ± standard deviation (Reprinted from S.M. Kluczewski and J.A. Lucas. 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant Brassica species. Plant Pathology 31:373-389, by permission of the authors and the publisher Blackwell Science ltd., Osney Mead, Oxford, U.K.).

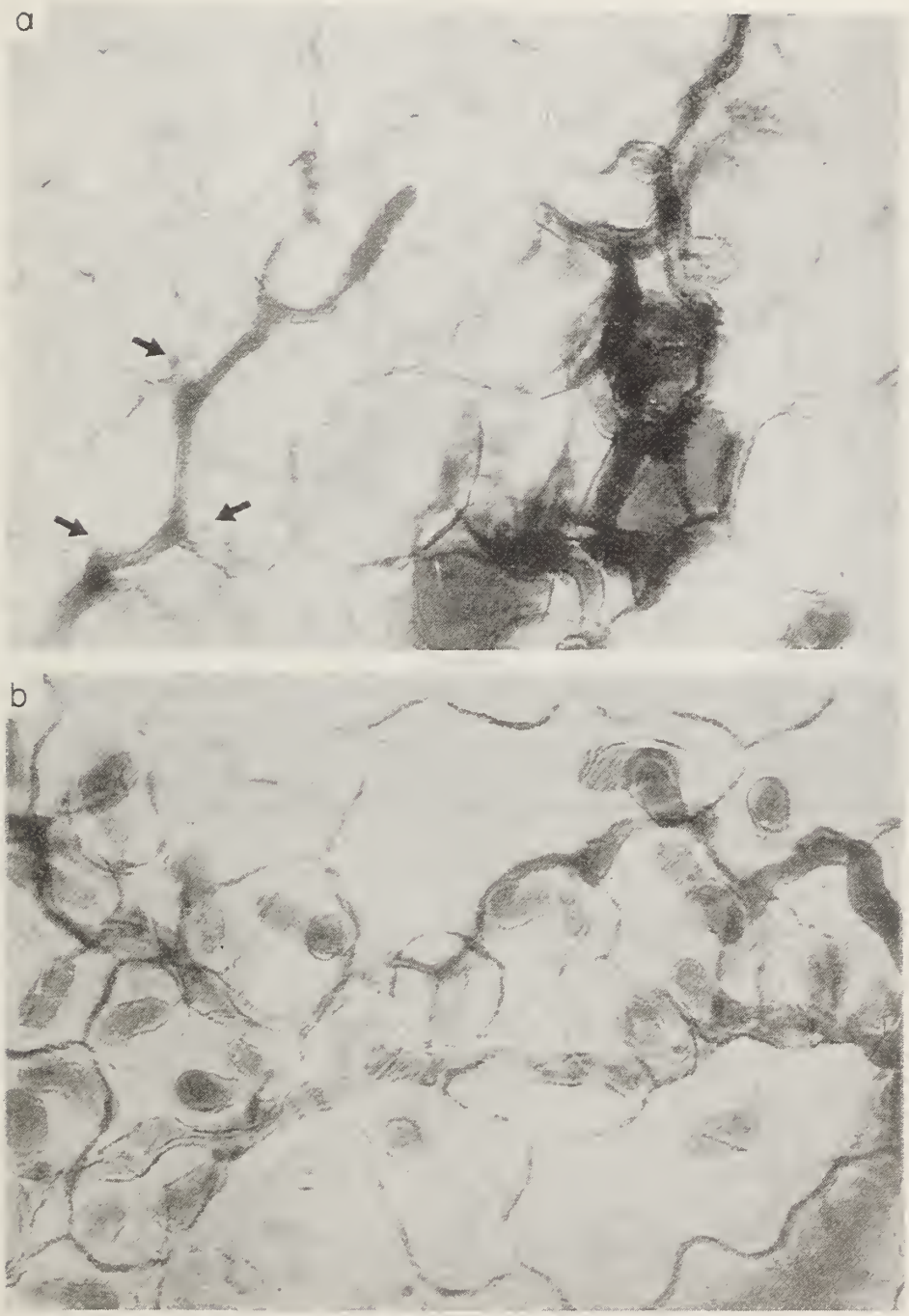


Fig. 53. Cotyledon tissue 4 days after inoculation with *Peronospora parasitica* cauliflower isolate stained with trypan blue and cleared in chloral hydrate. (A) intercellular hyphae in oilseed rape cultivar Primor showing left, developing haustoria (arrows) in host mesophyll cells close behind the hypha apex, and right, necrosis of penetrated host cells in older regions of a hypha. x 400; (b) Intercellular hyphae in cauliflower cultivar VSAG forming abundant intracellular haustoria. Note absence of host-cell necrosis. x 400 (Reprinted from S.M. Kluczewski and J.A. Lucas. 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant Brassica species. Plant Pathology 31:373-389, by permission of the authors and the publisher Blackwell Science ltd., Osney Mead, Oxford, U.K.).

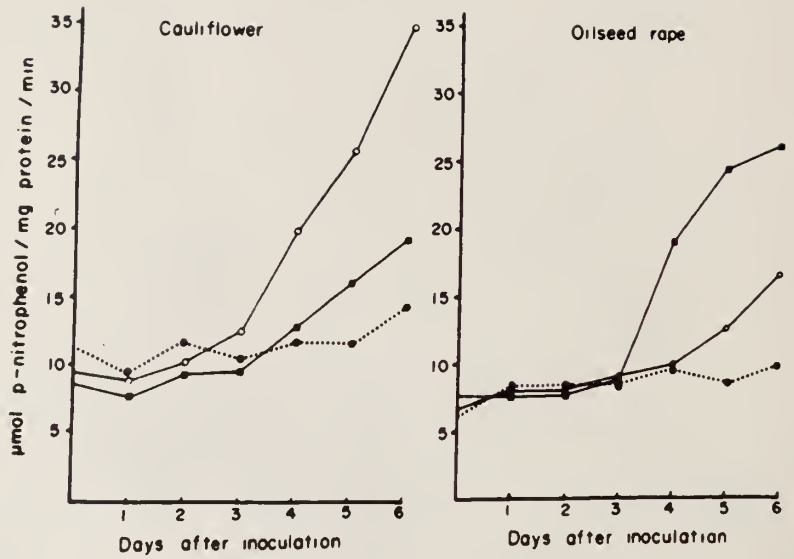


Fig. 54. Conductivity changes of deionized glass-distilled water containing samples of uninfected cotyledons (...) and cotyledons infected (-) by *Peronospora parasitica* isolate from cauliflower (o) and oilseed rape (■). Each point represents the mean of four replicates (Reprinted from S.M. Kluczewski and J.A. Lucas. 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant Brassica species. Plant Pathology 31:373-389, by permission of the authors and the publisher Blackwell Science ltd., Osney Mead, Oxford, U.K.).

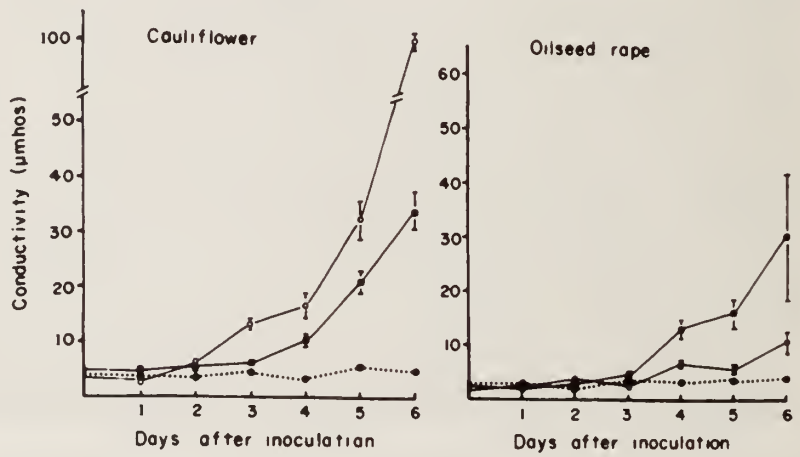


Fig. 55. β-glucosidase activity in extracts of control cotyledons (...) and cotyledons infected (-) by either cauliflower (o) or oilseed rape (-) isolate of *Peronospora parasitica* (Reprinted from S.M. Kluczewski and J.A. Lucas. 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant Brassica species. Plant Pathology 31:373-389, by permission of the authors and the publisher Blackwell Science ltd., Osney Mead, Oxford, U.K.).

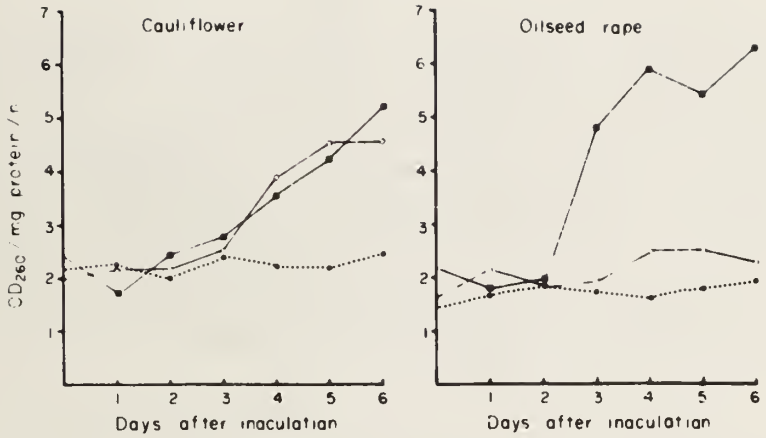


Fig. 56. Acid ribonuclease activity in extracts of control cotyledons (...) and cotyledons infected (-) by *Peronospora parasitica* isolate from cauliflower (o) and oilseed rape (-)(Reprinted from S.M. Kluczewski and J.A. Lucas. 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant Brassica species. Plant Pathology 31:373-389, by permission of the authors and the publisher Blackwell Science ltd., Osney Mead, Oxford, U.K.).

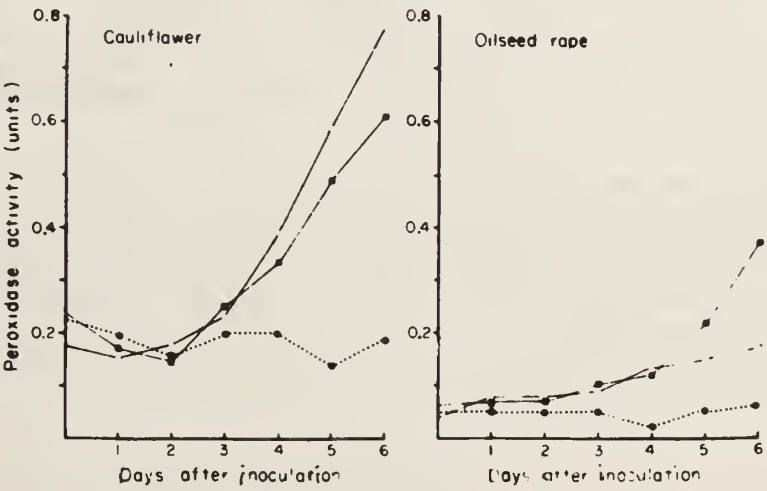


Fig. 57. Peroxidase activity in extracts of control cotyledons (...) and cotyledons infected by either cauliflower (o) or oilseed rape (■) isolate of *Peronospora parasitica* (Reprinted from S.M. Kluczewski and J.A. Lucas. 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant Brassica species. Plant Pathology 31:373-389, by permission of the authors and the publisher Blackwell Science ltd., Osney Mead, Oxford, U.K.).

Fig. 58. *Peronospora parasitica brassicae* rae 2. Entry of germ tube of the conidium, through (A) an epidermal cell and (B) a stoma. Mycelium in tissue of (C) the susceptible Chinese rape host, and (D) the immune radish host. Legend: Sp = conidium; Ap = appressorium; IH = infection hypha; My = mycelium; Ha = haustorium; Sh = sheath; Ep = epidermis; St = stoma; Sp = spongy mesophyll tissue; and DC = dead host cells (Reprinted from T.M. Wang. 1949. Studies on the mechanism of resistance of cruciferous plants to *Peronospora Parasitica.* Phytopathology 39: 541-547, by permission of the publisher American Phytopathological Society).

in diameter, were often observed in the susceptible cultivars, while rarely observed in the resistant cultivar.

b. Host-pathogen recognition system

The *Arabidopsis - P. parasitica* system was recently adopted as a model system for studying the recognition process for gene-for-gene interactions (Davis and Hammerschmidt, 1993). The determination of specificity and mechanism of recognition is a highly complex phenomenon. The comprehension of this phenomenon depends on better knowledge of molecular biology and genetics of the host-pathogen interaction. Cytological and biochemical studies are being attempted to identify the stages at which the incompatibility recognition events occur. Proposed steps between recognition, signal transduction and activated defence during the hypersensitive response in crucifers to *P. parasitica* are outlined in Table 22 (Lebeda and Schwinn, 1994).

c. Systemic acquired resistance

Systemic acquired resistance (SAR) has been demonstrated in *Arabidopsis* plants treated with chemical inducers such as 2, 6-dichloroisonicotinic acid (Uknes et al., 1992). Resistance is expressed within a few days of exposure to the inducer and inhibits subsequent infection by both bacterial and fungal pathogens, including *P. parasitica*. The degree of protection varies depending upon the concentration of inducer chemical used, but at higher dose rate sporulation of *P. parasitica* is completely inhibited. Microscopic examination of induced plants inoculated with the fungus reveals that hyphal growth is restricted to the initial penetration site, associated with a necrotic reaction in host cells. In plants treated with lower doses of the chemical, some hyphal development occurs, but haustoria are reduced in size and many are encased in material of host origin. Host cells penetrated by haustoria often become necrotic. Similar cytological events occur in uninduced hosts inoculated with incompatible isolates of *P. parasitica* (Kluczewski and Lucas, 1982). Induction of SAR appears to enhance the efficiency of host defence responses and thereby disrupts the development of a biotrophic relationship in a normally compatible host. The biochemical mechanism of SAR is not yet understood, but models envisage a translocated single molecule that induces changes in tissues removed from the initial inoculation site. Development of SAR is associated with induction of PR proteins (Uknes et al., 1992).

d. Genetics of host-pathogen relationship

Resistance derived from the Broccoli Introduction No. PI 189028 to *P. parasitica* race 1 is found to be governed by one dominant gene. The distribution of resistant

Table 22. Expected sequence of events leading to hypersensitive reaction expression in crucifers to *P. parasitca* infection (Reprinted from A. Lebeda and F.J. Schwinn. 1994. The downy mildews - an overview of recent research progress. J. Plant diseases and protection 101:225-254, by permission of the authors and the publisher Eugen Ulmer Verlag GmbH, Germany).

Differentiation in response to plant signal
Production of cultivar - specific elicitors
Primary recognition
Activated responses
De novo protein synthesis in penetrated cell
Irreversible membrane damage and release of phenolics
Release of endogenous elicitors
Accumulation of wall bound phenolics
Release of secondary signals
Secondary recognition
Transcriptions of mRNAs controlling biosynthesis of lignin
Precursors in surrounding
Deposition of lignins in and around the infection site.

plants in populations segregating for both downy mildew resistance and waxless foliage indicates that resistance is independent of foliage wax. Resistance to race 1 and race 2 obtained from the cabbage introduction PI 245015 is found to be inherited independently. Resistance is governed by one dominant gene for each race (Natti et al., 1967). However, in a later study, Hoser-Krause et al. (1991) found that in broccoli, (*B. oleracea* var *botrytis*) resistance to a Polish isolate of *P. parasitica* at the 4-5 leaf stage is determined by a single recessive gene different from genes determining resistance at the cotyledon stage. Subsequently they (Hoser-Krauze et al. 1995) found that in broccoli resistance to downy mildew is governed by 3 or 4 dominant complimentary genes. Both seedling and mature plant resistance has been reported in *B. oleracea* with the later being quantitative (Dickson and Petzoldt, 1996).

In Chinese cabbage resistance to downy mildew at the cotyledon stage expressed as a reduction in the sporulation capacity of *P. parasitia* was found to be under dominant monogenic control (Niu et al., 1983). However, Yuen (1991) while analysing Chinese cabbage lines with a reduced rate of mildew development found additive effects with involvement of several resistant genes.

The resistance to *P. parasitica* in radish cultivars Tokinaski (All Season) and Okura was found to be controlled by two dominant and independent genes (Bonnet and Blancard, 1987).

Cytoplasmic male sterile (*B. campestris*) breeding lines with resistance to downy mildew have been identified by Leung and Williams (1983). Downy mildew resistance was expressed in cotyledons as a reduction in the sporulation capacity of *P. parasitica*. In high and partially resistant hosts, spore production ranged from 7 to 30 spores/g host tissue as compared to 260 spores/g host tissue in the fully susceptible hosts. In the chinensis lines, 80% of the plants showed high to partial resistance to *P. parasitica* whereas in the other lines resistance ranged between 10% to 50%.

Differential host resistance to homologous isolates of *P. parasitica* has been identified in *B. rapa (B. campestris), B. napus, B. juncea* and *B. oleracea*. In *B. napus*, resistance in the oilseed rape cultivar Cresor is controlled by a single dominant allele (Lucas et al., 1988). In *B. oleracea,* differential resistance has been located in a land race cauliflower "Palermo Green". A model based on two or possible three major genes has been proposed by Moss et al. (1988) to account for the reaction patterns of individual plants to select fungal isolates within a host population (Table 23). In *B. campestris* both rapid cycling and commercial genotypes have been identified for differential resistance (Table 12). Four homologous isolates have identified a range of differential responses.

According to Nashaat et al. (1995a, b, 1996) resistance of the RES-01-1-4 and RES-26 lines of *B. napus* to isolate P003 of *P. parasitica* is conditioned by a single dominant resistant gene, whereas resistance of RES-02 is conditioned by two independent dominant resistance genes. Later, Nashaat and Awasthi (1995) selected differential putative homozygous resistant lines from seedling populations of accessions that exhibited a heterogeneous reaction to the isolates from *B. juncea* (Tables 24, 25).

In inoculation tests on *Arabidopsis thaliana* with seven pathogen isolates and eleven host accessions, a range of interaction phenotypes were observed including localized necrosis (flecking), more extensive cell collapse (pitting), delayed sporulation, or complete susceptibility (Holub et al., 1994). Segregation for these phenotypes among F_2 progeny from a half dialle cross between nine *A. thaliana* accessions, and ten host loci (termed RPP, recognition of *P. parasitica* loci) has been observed. Four of these loci (RPP1, RPP2, RPP4 and RPP7) were mapped (Tor et al., 1993), along with a further locus RPP5 (Parker et al., 1993). Three loci were apparently clustered together on chromosome four. There was also evidence for the existence of different alleles at a single locus, although corresponding crosses between pathogen isolates differing in specificity are required to confirm whether different alleles at closely linked loci might explain such results. A genetic model postulating the existence of complementary

Table 23. Inheritance of resistance in cauliflower to *P. parasitica* using Palermo green model (Reprinted from N.A. Moss, I.R. Crute, J.A. Lucas and P.L. Gordon. 1988. Requirements for analysis of host-species specificity in *Peronospora parasitica* (downy mildew). Cruciferae NewsLetter 13:114-116, by permission of the author and the publisher)

P. parasitica Isolate	Virulence		Resistance phenotype				Approx. % resistant seedlings observed
			-	R1	-	R1	
	A1	A2	-	-	R2	R2	
P005	1	2	+	-	-	-	95
P015	1	-	+	-	+	-	80
P018	-	2	+	+	-	-	80
P006	-	-	+	+	+	+	0
% Phenotype in seed stock			5	15	15	65	

A = avirulence gene, R = Resistance gene, + = susceptibility, - = resistance

recognition loci in the fungus designated ATR- *A. thaliana* recognition has been proposed (Holub et al., 1994). These results provide support for the gene-for-gene model of specificity in crucifers downy mildew. The interaction phenotypes between different host lines and pathogen isolates reveal a degree of complexity in the system, with partial dominance, epistasis and gene dosage effects.

e. Biochemical basis of resistance

The presence and absence of natural biochemical compounds like glucosinolates and other phenolic compounds play a significant role in providing resistance to the host plant. There is a correlation between high levels of flavour volatiles (e.g. allylisothiocyanate) released by tissue damage, and the limitation of fungal growth in both wild and cultivated *Brassica* lines. In cultivated brassicas, breeding has resulted in reduced levels of flavour volatiles with a consequent reduction in their general resistance to *P. parasitica*. The resistance to *P. parasitica* in the cabbage cultivar January King may be attributed to the high concentration of allyl-isothiocyanate(Greenhalgh and Mitchell, 1976).

Table 24. Response of groups A, B, C, D and E of *Brassica juncea* accessions and of one accession of *B. napus* at the cotyledon stage to infection with four isolates of *Peronospora parasitica* (Nashaat and Awasthi, 1995)

	Host category*	Disease Index: Isolates of *P. parasitica* IP01/IP02	P003	R1
B. juncea	A (4)	1	1	1
	B (2)	2-3	1	1
	C (19)	6-8	1	1
	D (5)	6-8	2-3	1
	E (1)	6-7	5-6	1
B. napus	Ariana	1	7-8	7-8

*) Number of accessions of each group in parenthesis.

The incidence and severity of downy mildew is positively correlated with glucosinolate concentration in seeds of oilseed rape (Rawlinson et al., 1989). In oilseed rape, downy mildew severity is lower on cultivars with high concentration of glucosinolate (>100 μmol g^{-1}) and greater on those with a lower concentrations (<15 μmol g^{-1}). In all cultivars grown in the UK incidence is lower in mid-February when glucosinolate products in the leaves reaches a maximum level (Anonymous, 1985).

A large number of *B. napus* species with different glucosinolate and erucic acid contents have been screened for resistance to four isolates of *P. parasitica* at the cotyledon stage (Nashaat and Rawlinson, 1994). Two groups of accessions with different resistance factors were identified. The first group was different from that of the cultivar Cresar which has an isolate specific gene for resistance to *P. parasitica*, and the second group was identical to that of Cresar. There was moderate to full susceptibility at the cotyledon stage but no clear differential response to any of the isolates. Those with high glucosinolate and high erucic acid content were significantly less susceptible than those with high glucosinolate and low erucic acid, or, low glucosinolate and low erucic acid content.

In preliminary experiments on the effect of treatment with abiotic elicitors on disease reaction in oilseed rape seedlings, salicylic acid and methyl jasmonate reduced

Table 25. Examples of a successful selection for putative homozygous resistance response to *Peronospora parasitica* from a heterogeneous starting population of *Brassica juncea* at the cotyledon stage (Nashaat and Awasthi, 1995)

*B. juncea**	Isolates of *P. parasitica* Disease Index (Standard deviation, n = 24) IP01	IP02	P003	R1
Kranti	3.0 (3.0)	4.1 (3.6)	1.0 (0.0)	1.0 (0.0)
RES-BJ01	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
Krishna	5.4 (2.5)	5.7 (2.5)	1.0 (0.0)	1.0 (0.0)
RES-BJ02	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
Varuna	6.1 (1.8)	7.1 (2.2)	1.0 (0.0)	1.0 (0.0)
RES-BJ03	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)

*) RES-BJ01, RES-BJ02 and RES-BJ03, lines selected from seedling populations of Kranti, Krishna and Varuna, respectively.

the severity of infection when cotyledons were subsequently inoculated with *P. parasitica* (Doughty et al., 1995).

f. Lignification of host cells

Lignin formation was observed in cell walls of parenchyma of Japanese radish root infected with downy mildew fungus. The observed lignin was mainly composed of guaincylpropane units and apparently differed from syringyl lignin which was present in vessels of the healthy tissues. Different pathways for lignin biosynthesis in the healthy and the diseased tissues were proposed (Tables 26, 27, Fig. 59-61) by Asada and Matsumoto (1972). Specific isoperoxidases synthesized de novo in diseased tissues were presumed to play an important role in the formation of the guaincyl lignin (Ohguchi et al., 1974; Ohguchi and Asada, 1975). Lignin was also formed in cell walls of parenchyma of Japanese radish root which was infiltrated with 700 x g supernatant of homogenate of downy mildew infected tissues. It began to form about 12h after infiltration of the homogenate (Matsumoto et al., 1978). The effective component in the homogenate for the induction of lignification is dialyzable, which is highly water soluble and seems to resemble monilicolin A (Asada et al., 1975). The lignification

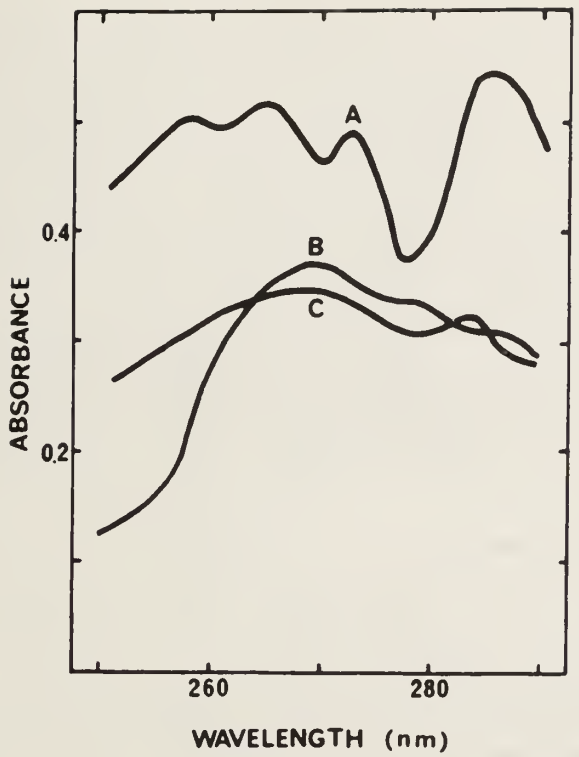


Fig. 59. The UV absorption spectra of the diseased parenchyma cell wall (A), the vessel wall (B), and the healthy parenchyma cell wall (C) of the Japanese radish root (Reprinted from Y. Asada and I. Matsumoto. 1972. The nature of lignin obtained from downy mildew-infected Japanese radish root. Phytopathol. Z. 73: 208-214, by permission of the authors and the publisher Blackwell Wissenschafts-Verlag GmbH, Berlin, Germany).

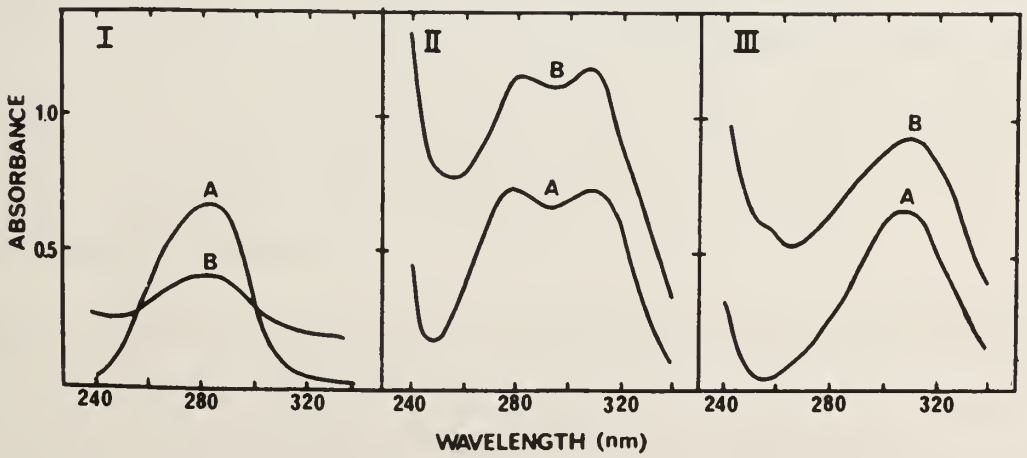


Fig. 60. UV absorption spectra of the authentic compounds (A) and the degradation products (B) obtained from the extraction of paper chromatograms. I = P - hydroxybenzaldehyde; II = vanillin; III = syringaldehyde (Reprinted from Y. Asada and I. Matsumoto. 1972. The nature of lignin obtained from downy mildew-infected Japanese radish root. Phytopathol. Z. 73: 208-214, by permission of the authors and the publisher Blackwell Wissenschafts-Verlag GmbH, Berlin, Germany).

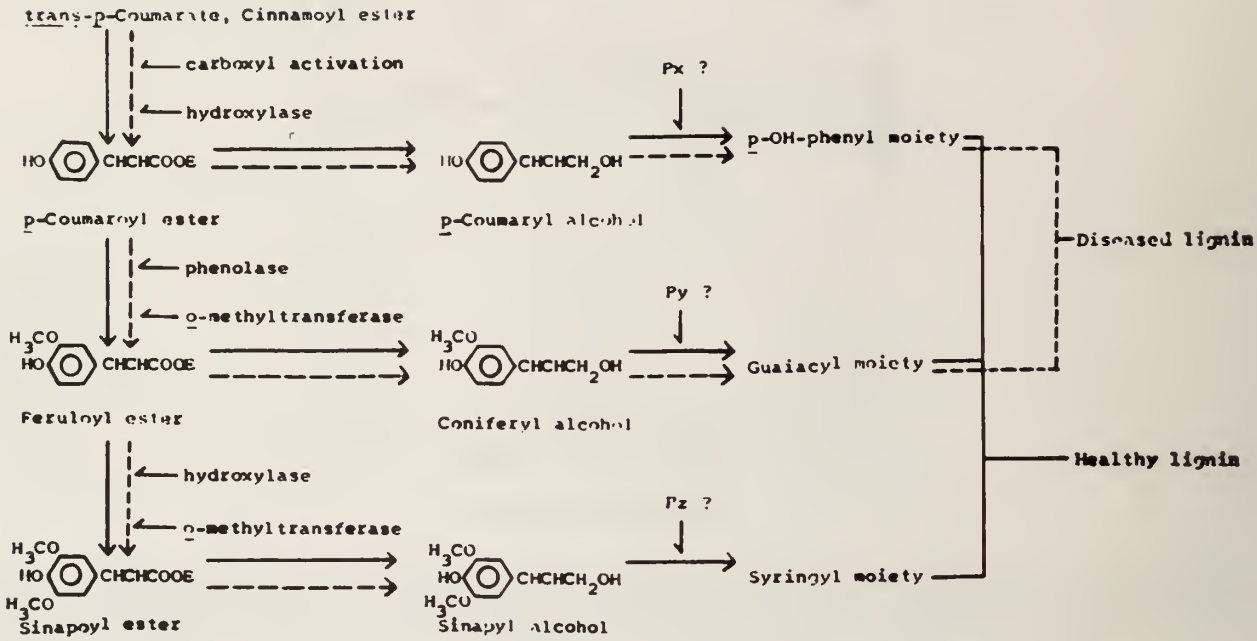


Fig. 61. Suggested pathway of lignin biosynthesis in healthy (full lines) and diseased (broken lines) plants. Px, Py, Pz: Peroxidase isoenzymes x,y,z (Reprinted from Y. Asada and I. Matsumoto. 1972. The nature of lignin obtained from downy mildew-infected Japanese radish root. Phytopathol. Z. 73: 208-214, by permission of the authors and the publisher Blackwell Wissenschafts-Verlag GmbH, Berlin, Germany).

inducing factor (LIF) plays a significant role in the induction of systemic resistance. Resistance to *P. parasitica* was induced in roots of susceptible radish cultivars when they were preliminarily inoculated with the pathogens or wounded. An increase in L-phenylalanine ammonia lyase (PAL) activity and lignification of cell walls occurred in these tissues after challenge inoculation with downy mildew. Histochemical observations indicated that the cell walls were lignified in the tissues beyond the site of fungal attack (Matsumoto and Asada, 1984). High peroxidase activity was located around the lignified cell walls (Asada and Matsumoto, 1969; Ohguchi et al., 1974). The higher amount of lignin accumulation was present in the middle portion of the cell wall (Asada and Matsumoto, 1971). Following infection of radish by *P. parasitica* the deposition of lignin in host cell walls may have a role to play in non-specific limitations of the growth of biotrophic fungi. Ohguchi and Asada (1975) have defined the pathways and enzymes involved in lignin biosynthesis in radish following infection by *P. parasitica*.

g. Sources of resistance

Differential host resistance to isolates of *P. parasitica* has been identified in *B. campestris*, *B. juncea*, *B. napus*, *B. oleracea* and *R. sativas* (Bonnet and Blancard, 1987; Lucas et al., 1988; Nashaat and Rawlinson, 1994; Nashaat and Awasthi, 1995; Nashaat et al., 1997; Silue et al., 1996). Sources of resistance to *Peronospora parasitica* in different host species of crucifers identified from different countries of the world are given in Table 28.

10. BREEDING FOR DISEASE RESISTANCE

Plant breeding offers one method for controlling diseases and has obvious advantages if successful. As with other traits, the breeder's task is firstly to find sources of disease resistance and effective ways to screen for resistant genotypes. The trait has then to be transferred into a useful cultivar or hybrid (Buzza, 1995). Transfer of resistance among crucifers and from other species is possible by using conventional and biotechnological techniques:

a. Germplasm evaluation for sources of resistance at national and international levels.
b. Selection for disease resistance through (i) pure line selection, (ii) mass selection, (iii) modified recurrent mass selection, and iv) recurrent selection.
c. Breeding for disease resistance by increasing the level of resistance through i) multiple crosses, ii) recurrent selection, iii) dialle crossing, and d) selective mating system
d. Transfer of resistance by i) intraspecific pedigree, backcross and modified

Table 26. Amounts of degradation products by alkaline nitrobenzene oxidation of the isolated lignin (Reprinted from Y. Asada and I. Matsumoto. 1972. The nature of lignin obtained from downy mildew-infected Japanese radish root. Phytopathol. Z. 73:208-214, by permission of the authors and the publisher Blackwell Wissenschafts-Verlag GmbH, Berlin, Germany)

Lignin	Product	Amount (%)	Ratio
Healthy	p-Hydroxybenzaldehyde (H)	0.39	V/H 6.05
	Vanillin (V)	2.36	S/H 3.90
	Syringaldehyde (S)	1.52	S/V 0.64
	Total	4.27	
Diseased	p-Hydroxybenzaldehyde (H)	0.99	V/H 2.75
	Vanillin (V)	2.72	S/H 0.0
	Syringaldehyde (S)	0.00	S/V 0.0
	Total	3.71	

recurrent mass selection methods, and ii) interspecific genome substitutions, chromosome substitutions and gene introgression.

e. Transfer of resistance through mutation breeding.

f. Use of biotechnological and genetic engineering techniques such as i) genome manipulation, ii) manipulation of cytoplasmic genomes, iii) use of transformation and foreign gene expression techniques, and iv) embryo rescue techniques for wide hybridization.

11. DEVELOPMENT OF RESISTANCE TO FUNGICIDES

The existence of metalaxyl resistant strains of *P. parasitica* has been reported (Crute, 1984; Crute and Gordon, 1986; Brophy and Laing, 1992). Metalaxyl resistant isolates were also shown to be cross-resistant to furalaxyl and ofurace, two related phenylamide fungicides. A differential degree of insensitivity to two related phenylamide fungicides has been demonstrated (Table 29) (Moss et al., 1988). Metalaxyl was more active against a sensitive isolate (P005) than cyprofuran but the

Table 27. Elemental compositions and empirical formulae of the isolated lignins and the related compounds (Reprinted from Y. Asada and I. Matsumoto. 1972. The nature of lignin obtained from downy mildew-infected Japanese radish root. Phytopathol. Z. 73:208-214, by permission of the authors and the publisher Blackwell Wissenschafts-Verlag GmbH, Berlin, Germany)

Lignin	C (%)	H (%)	OCH_3 (%)	Formula
Healthy root	64.38	7.27	18.93	$C_9H_{10.3}O_{2.20}(OCH_3)_{1.16}$
Diseased root	63.47	5.74	12.58	$C_9H_{8.33}O_{2.80}(OCH_3)_{0.75}$
Birch*)	58.82	6.49	21.51	$C_9H_{9.03}O_{2.77}(OCH_3)_{1.58}$
Spruce*)	63.48	6.35	14.84	$C_9H_{8.83}O_{2.37}(OCH_3)_{0.96}$
DHP**)	64.00	6.00	16.90	$C_9H_{8.21}O_{2.50}(OCH_3)_{1.02}$
Coniferyl alc.	66.67	6.67	17.22	$C_9H_9O_2(OCH_3)_1$

*) From BJÖRKMAN and PERSON (1957).

**) Dehydrogenation polymerization product, from NOZU (1967).

The formulae were obtained from the following equations.

$C_9H_xO_y(OCH_3)_z$ where

$$x = \frac{108.09\,[H\,(\%) - \frac{1.008 \times 3}{31.035} \times OCH_3\,(\%)]}{1.008D} \qquad z = \frac{108.9 \times OCH_3\,(\%)}{31.035D}$$

$$y = \frac{108.09\,[O\,(\%) - \frac{16}{31.035} \times OCH_3\,(\%)]}{16D} \qquad D = C\,(\%) - \frac{12.01}{31.035 \times OCH_3\,(\%)}$$

converse was true with an insensitive isolate (P006). The inheritance of fungicide insensitivity to *P. parasitica* may reveal the true picture of genes controlling this phenomenon.

12. LABORATORY AND FIELD TECHNIQUES AND BIOASSAYS

a. Culturing of *Peronospora parasitica*

Table 28. Sources of resistance to *Peronospora parasitica*

Host species/genotypes	References
Brassica alba (white mustard)	
All Indian accessions	Saharan, 1992a, b
B. carinata (Ethiopian mustard)	
All Indian accessions	Saharan, 1992a, b
HC1	Saharan, 1996
B. campestris	
candle	Saharan, 1992a, b
B. campestris var *toria* (Toria)	
1B - 586	Kolte & Tewari, 1980
B. campestris var *yellow sarson* (Yellow Sarson)	
YST-6	Kolte & Tewari, 1980
B. campestris var *brown sarson* (Brown Sarson)	
BS-15	Kolte & Tewari, 1980
B. juncea (Indian mustard)	
PI 340207, PI 340218, PI 347618	Ebrahimi et al., 1976
Domo, RC 781, EC 126743, Zem, YRT 3, 45, 72	Saharan, 1992a, b
PR 8805, RN 248, EC 129126-1, PC 3	Saharan, 1996
RESBJ-01, RESBJ-02, RESBJ-03	Nashaat and Awasthi, 1995
B. oleracea var. *botrytis* (cauliflower)	
Igloo, Snowball y, Dok Elgon, RS-355	Kontaxis et al., 1979
PI 181860, PI 188562, PI 189028 (MR), PI 204765, PI 204768, PI 204772, PI 204773, PI 204775, PI 204779, PI 241612, PI 264656, PI 291567, PI 373906, PI 462225 (MR)	Thomas and Jourdain, 1990
KPS-1	Sharma et al., 1991
PI 231210, PI 189028	Hoser-Krause et al., 1991
B. oleracea var *capitata* (cabbage)	
January King	Greenhalgh & Mitchell, 1976
Balkan	Elenkov, 1979
Spitz Kool	Verma & Thakur, 1989
PI 246063, PI 246077, PI 245013	Hoser-Krause et al., 1991
Tromchuda cabbage "Algarvia" (ISA 207)	Caravalho & Monteiro, 1996
PI 245015, Geneva 145-1	Sherf and Macnab, 1986
B. oleraceae var *accephala* gr. *ornamentalis* (Decorative cabbage)	Vitanova, 1996
B. oleracea (Broccoli)	

calabrese, Grand Central	Natti et al., 1956
PI 231210, Italian Green Sprouting	Natti, 1958
Hyb. 1230 (Moran), Green surf (Moran), 2804 (Qualisal), Hyb. 2805 (Qualisal), Hyb. 2803 (Qualisal), GSV 82-4310 (Goldsmith), XPH 1117 (Asgrow), Hyb. 288 (Moran), AVX 7631 (Sun Seeds).	Laemmlen & Mayberry, 1984
PI 263056, PI 263057, PI 3573, PI 3574. PI 418984, PI 418985, PI 418986, PI 418987 PI 418988	Hoser-Krause et al., 1991
OSU CR 2 to OSU CR 8	Baggett and Kean, 1985
Citation, Excalibur, Nancy	Sherf and Macnab, 1986
B. napus (Rape)	
Hg Vestal	Jonsson, 1966
Eurora, Janetzki, Kubla, Lesira, Mogul, Primar, Rapot, Rapara, Sinus	Dixon, 1975
cultivar 78-22	Chang, 1981
Cresor	Kluczewski & Lucas, 1983
PI 199949, PI 263056	Thomas & Jourdain, 1992
Gulivar, Midas, Tower	Saharan, 1992a, b
RES 01-1-4, RES-02, RES-26	Nashaat et al., 1995; 1996
HNS3, HNS4, GSL 1, GSL 1501	Saharan, 1996
B. chinensis (Chinese cabbage)	
Bau chin 26, PHW 64707, PHW 64710, PHW 64722, PHW 64620	Niu et al., 1983
Hyb. 77M(3)-27, Hyb. 77M(3) - 35 Hyb. 82-46, Hyb. 82-46R, Hyb. 82-156, Hyb. 82-157	Anonymous, 1987a, b
B. nigra (Black mustard)	
PI 199948	Thomas & Jourdain, 1992
B. napa	
PI 418984, PI 418988, PI 418987, PI 418988	Thomas & Jourdain, 1992
B. rapa subsp. *rapifera*	
Long Blanc de croissy, Stanis, Jaune Boule d'or	Silue et al., 1996
Raphanus sativus (Radish)	
Okura	Shiraishi et al., 1974
Tokinoshi (All season)	Bonnet & Blancard, 1987
Bamba, Noir Lon d'Horloge, Rave à Forcer	Silue et al., 1996
Cheiranthus cheirii (Wallflower)	
Convent Garden blood Red	Greenhalgh & Dickinson, 1975

Table 29. Responses of phenylamide sensitive and insensitive isolates of *P. parasitica* to phenylamide fungicides (Reprinted from N.A. Moss, I.R. Crute, J.A. Lucas and P.L. Gordon. 1988. Requirements for analysis of host-species specificity in *Peronospora parasitica* (downy mildew). Cruciferae NewsLetter 13:114-116, by permission of the authors and the publisher)

Compound	Isolates	Fungicide μg/ml				Factor of Insensitivity
		0.05	0.05	5.0	50.00	
Metalaxyl	P005	32[a]	0	0	0	0
	P006	94	89	100	83	x1000
Cyprofuran	P005	84	79	0	0	
	P006	100	94	78	3	x10

a. Figures are reciprocals of mean latent periods (time from inoculation to sporulation expressed as a percentage of the untreated control).

The biotrophic nature of this pathogen implies a sophistication of nutritional requirement which can not be met upon the death of the host plant. *P. parasticia* was cultured on disinfected slices of swede root where aerial growth of mycelium was observed with conidiophores, conidia, antheridia, oogonia and oospores (Guttenberg and Schmoller, 1958). Ingram (1969) successfully established and maintained cultures of the fungus on callus tissues derived from (a) a mature leaf of cabbage, (b) a mature root/hypocotyl of rape, and (c) a seedling hypocotyl of swede. The infected calluses were incubated either at 22°C in the dark, or at 15°C with 12h fluorescent illumination photoperiod. To maintain the dual culture of the callus and the pathogen, sub-culturing through transfer of an explant of infected callus to fresh uninfected callus was necessary every 14 - 21 days. Calluses derived from the root/hypocotyl of rape grew faster than those from cabbage leaf, and were transferred directly when subculturing. About four weeks after inoculation of the rape callus, small nodules of new tissue were developed on the infected callus. These nodules or the whole callus were successfully maintained when transferred to fresh culture medium. Conidia of *P. parasitica* were produced on infected callus tissue maintained at 15°C and 12 h/photoperiod but production was much lower at 22°C in the dark. Such conidia were used to infect detached cotyledons or leaf callus of cabbage or rape.

Attempts were made with limited success by Guttenberg and Schmoller (1958) to culture the fungus in the absence of living plant tissue. They obtained visible mycelial

growth in filter, sterilized swede juice, but it ceased after 3 days when a yellow discoloration appeared suggesting that the medium was chemically unstable. Very limited mycelium was developed on swede seed-glucose agar and maize decoctions-glucose agar, but more success was achieved with an agar medium containing 2% beer wort + 0.1% phosphate in which hyphae and conidiophores were developed within and outside the agar substrate. Similar, but less growth was achieved in oatmeal agar and rice starch agar.

Asada and Ohguchi (1981) studied the behavior of downy mildew fungus of Japanese radish on modified Knop's medium. An isolate of *P. parasitica* from naturally infected leaves was cultured on modified Knop's medium and 0.1% streptomycin, using infected slices of radish root tissue as inoculum. Vigorous hyphal growth was observed spreading into the medium and numerous haustorium like bodies were formed. The production of which was favoured by low agar concentration, low pH(4) and high sucrose concentration (50 g/l). Growth was ceased 2 weeks after placing the tissue slices on the medium.

The response of *P. parasitica* to a liver medium was studied by McMeekin (1981). Washed, autoclaved 2mm cubes of liver were placed in a 9 cm petri dish and covered with 20 ml of a mixture of 0.01% tryptone (Difco: pancreatic digest of casin), 0.04% K_2HP0_4 and 2% agar: a 1/10 dilution of the K_2HP0_4 and tryptone. Ten μg/ml streptomycin (Calbiochem) were added to this medium to control bacterial growth. When the agar was solidified, drops of a suspension of conidia in water were placed on the piece of liver. The plates were incubated at 18°C. After 4 days the germ tubes in the control (without liver) disintegrate, but in a 5 mm diam zone around the liver pieces, 90% of the germ tubes grow towards the liver. They grow from the surface towards the bottom of the medium and formed large swellings or lobes within the agar. The lobed germ tubes reached their maximum size after 4 days. When the plate was flooded with sterile distilled water, either 4 or 7 days after the beginning of conidial germination, the lobes on the germ tubes maintained the same size.

b. Maintenance of *P. parasitica* isolates

Isolates of *P. parasitica* were maintained separately on cotyledons obtained from 6-days old seedlings, raised in soilless compost in a modified plant propagtor (35.5 cm x 21.6 cm x 18 cm), sited in the glasshouse (Nashaat and Rawlinson, 1994). The propagator was supplied with continuous filtered (spore-free) moist air at 18$\pm$2°C through a central flue conducting air from beneath the propagator to exhaust at two adjustable ventilators on the cover and the junction between the cover and the base (Jenkyn et al., 1973). Supplementary light was given to maintain a 16h photoperiod. Cotyledons and a short length of hypocotyl were detached and transferred to folded filter paper (Whatman 12.5cm, 113v) supports in glass jars (8cm diameter, 7 cm depth)

containing 20 ml sterile distilled water. Cotyledons were then inoculated in a sterile air flow with 5μl of conidial suspension on each half cotyledon with the aid of a micropipette. Conidial suspensions were prepared by tapping infected cotyledons to dislodge conidia into sterile distilled water; this minimized bacterial contamination. After inoculating the cotyledons, the glass jars were covered with clear plastic lids, sealed with parafilm and incubated in a growth cabinet at 16°C under 70μE/m $s^{2\ -1}$ irradiance with a 16h photoperiod for 7 days after which peak sporulation occurred.

c. Germplasm screening and evaluation

Genotypes are grown in propagators, (as described under isolates maintenance in subsection 12b) except that two adjacent 5 cm 'Jiffy-pots' for each line or cultivar are used as pots. The pots are placed on capillary matting to ensure a uniform water supply. Each propagator contains up to 13 accessions arranged as two randomized blocks (propagators) with each accession occurring only once in each propagator. Initially, nine to 15 seedlings per accession are grown in each propagator and these are thinned 6-days after sowing to 6 to 10 to decrease variability in growth. Sowing dates are staggered to produce seedlings at the required growth stage for inoculation at the same time. The average times required under these conditions to reach fully expanded cotyledons, first, and second true leaves are 7, 16, and 22 days, respectively (Nashaat and Rawlinson, 1994) .

Seedlings are inoculated by spraying them to run-off with a suspension of conidia (2.5 x 10 conidia/ml). The propagators are sealed after inoculation to allow the relative humidity to rise to 100%, and then incubated in growth cabinets under the conditions described for isolates maintenance. Infection phenotypes are recorded 7 to 9 days after inoculation (on cotyledons and leaves, respectively) using a 0-9 scale (Nashaat and Rawlinson, 1994).

According to Williams and Leung (1981), single seeds are grown in 12-pack pots and when the cotyledons have expanded, after five to seven days, single 0.01 to 0.02 ml drops of a freshly collected conidial suspension containing approximately 10^5 conidia/ml are placed on each half of the two cotyledons using a finely tipped glass pipette. As plants of each 12-pack are inoculated they are placed in glass or plastic boxes containing a 1 to 2 cm depth of warm water in the bottom. A tight fitting cover is placed on the box after the box is filled with plants. The box is then placed in a darkened incubator at 20°C for 8 to 16h. The atmosphere in the box will maintain the droplets on the cotyledons during which time germination and penetration will occur. After incubation, plants are placed on a lighted greenhouse bench at 20 to 25°C for five days then returned to humidity boxes at 20°C for 16-24h. Upon removal from the humid atmosphere, susceptible plants will have a profuse growth of *P. parasitica* conidiophores on the abaxial sides of the cotyledons, whereas resistant plants will

exhibit varying degrees of sporulations and tissue necrosis which can be evaluated on a 0-9 scale (Fig. 62). A rating of 1 is given to rapidly occurring (24 to 48h) hypersensitive necrotic flecking, without visible sporulation, found immediately under the droplets. Interaction phenotypes representing host-pathogen compatibility are expressed as increasing degree of sporulation on the abaxial side of the cotyledons and decreasing degree of necrosis associated with tissue colonization. It is important to use freshly produced inoculum collected by washing off spores from leaves with distilled water. Older plants may be inoculated by atomizing a suspension of conidia on the foliage and holding them at 100% RH for 8-16h.

Knight and Furber (1980) used descriptive key for the assessment of downy mildew disease prevalence and severity on winter oilseed rape varieties. A group of plants (10 plant/sample) chosen at random from a number of observation site assessed were as follows:

Percentage disease	Host - pathogen interaction
0.0	No infection
0.1	Traces of infection generally confined to lower leaves. One plant in ten or fewer with lesions
1	Some plants infected, but one or two lesions per plant
5	Most plants affected with about 5% of the lower leaf area affected
10	Most plants with about 10% of the lower leaf area affected. Up to 5% infection on the upper stem leaves and bracts
25	About 25% of the lower leaf area affected. Leaf area may appear to be half affected and half unaffected. Infections frequent on upper stem leaves and bracts (up to 10% area affected)
50	About 50% of the leaf area affected. Affected area appears to be greater than unaffected
75	About 75% of leaf area affected. Very little unaffected tissue observable
100	100% of leaf area affected

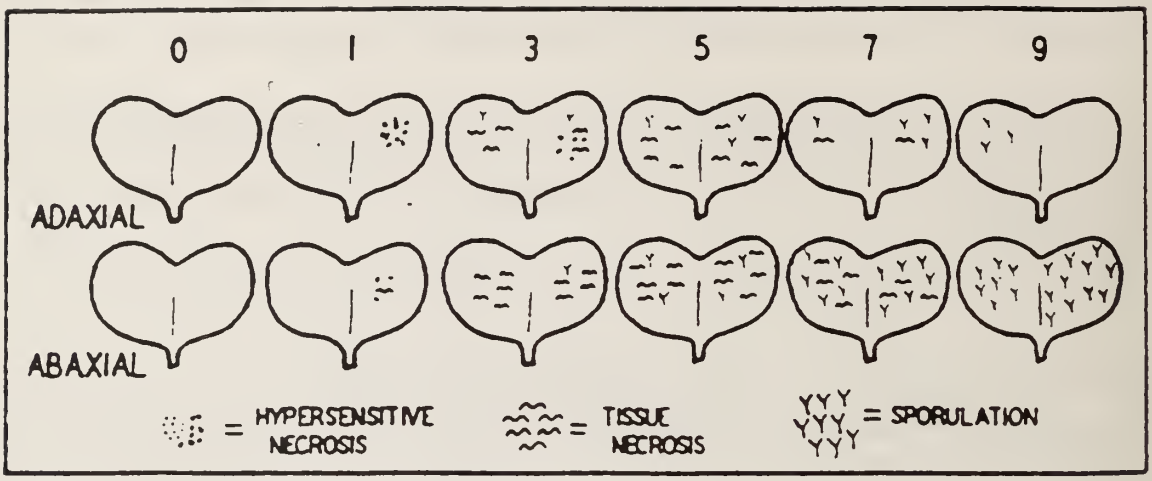


Fig. 62. Rating scale for downy mildew *(Peronospora parasitica)* interaction phenotypes on Chinese cabbage (Reprinted from P.H. Williams and H. Leung. 1981. Methods for breeding for multiple disease resistant Chinese cabbage. IN: Chinese cabbage. Proc. 1st Intern. Symposium, N.S. Talekar and T.D. Griggs (Editors): pp. 393-403, by permission of the authors and the publisher the Asian Vegetable Research and Development Center, Shanhua, Taiwan).

d. Preservation of *P. parasitica*

Brassica leaves infected with the pathogen were collected and conidia from such infected material were inoculated on cotyledons of a susceptible *Brassica* variety (Paul and Klodt-Bussmann, 1993). Cotyledons are put into plastic boxes on moist filter paper and incubated at 15°C and 70-80% RH. Under these environmental conditions conidia and conidiophores of the pathogens are ready to be harvested 6-days after inoculation. For each isolate 5 cotyledons colonized with fresh conidia were collected in a glass vial in 10% (v/v) glycerine which serves as a cryoprotectant in the suspension medium. For each isolate, 6 glass vials are filled with the conidial suspension and immediately stored in a freezer at -21°C. After a storage interval, samples are taken out from the freezer and thawed at room temperature (20°C). After thawing for 10 minutes, 2 ml of the conidial suspension are added to each petri-dish which contains 15% (w/v) water-agar and the percentage of conidial germination is assessed after 24h at 15°C. After a storage period of 8-days it was found that the highest germination rate of 73% occurred using 10% (v/v) glycerine.

To culture and preserve Japanese Radish downy mildew fungus, slices (1cm) of Japanese radish root cv Miyashige, were inoculated with conidia of the fungus collected from naturally infected leaves and incubated at 20°C for 6-8days (Ohguchi and Asada, 1981). Conidia produced on the infected slices were then collected to make a suspension and were used to inoculate other healthy slices. Numerous oospores are observed in these sliced tissues 6 days after inoculation.

The conidial viability of *P. parasitica* derived from cabbage was longest, up to 130 days, when the spores were stored in air dried soil at a constant temperature of 5°C. Conidia kept at -25°C and relatively dry on leaf disks (air dried at 20°C) maintained a relatively high rate of germination after 1 year or longer (Krober, 1970, 1981).

The pathogen is usually preserved by storing few sporulating cotyledons in small vials with tight lids inside a deep freezer (-25 to -30°C) for up to 6 months. Temperature fluctuations should be avoided during this period. For reviving the pathogen, the vials containing the sporulating cotyledons are taken out of the freezer and, with their lids kept tightly on, transferred immediately to a container containing icy water. The temperature of the container and its content is then allowed to rise gradually, within 1-2h, to 15°C. Thereafter, the vials are taken out of the container and conidial suspension for inoculating fresh cotyledons is prepared in the normal way (Nashaat and Rawlinson, 1994).

e. Artificial inoculation of excised cotyledons

Cotyledons of radish, 9 days after sowing, were placed face downwards on a damp

filter-paper in a transparent plastic box (Bonnet and Blancard, 1987). The box was then put in a growth chamber at 20°C day and 18°C night, relative humidity of 90%, and illumination of 2000 lux for 12h. With a micropipette, 50μl of *P. parastica* conidial suspension (16,000 sp/ml) were placed on the abaxial surface of the cotyledons. After 5 days, the conidia were collected by rubbing the cotyledons with a brush into 2 ml of water; the concentration of the suspension was then measured and adjusted using a hemacytometer. When 15-day-old plants were inoculated, an excellent correlation was observed between the number of conidia and symptoms on leaves.

f. Propagation of *P. parasitica* on cotyledons or true leaves of Japanese radish seedlings

Cotyledons and the true leaves of radish seedlings were subjected to hot water (50°C) treatment, or the roots were cut off to weaken resistance to *P. parasitica* (Ohguchi et al., 1989). Each of the 7-11 days-old cotyledons of cvs. Awa-ichigo, Sarakamuri and Daimaru-Shogoin, which had been treated with hot water, were put in a test tube (2.8 x 19 cm) containing 15 ml of distilled water. The upper surfaces of these cotyledons were inoculated with drops of conidial suspension of the fungus. The percentage of conidiophore formation on the cotyledons grown for one week at 20°C, 1000 lux after inoculation was highest on the 11 day old cotyledons from cv. Diamaru-Shogoin treated with hot water for 60 seconds. On the true leaf of 3 week old seedlings, the percentage was highest in cv Sarakamuri treated with hot water for 30 seconds. Since the cotyledons of cv. Shirokubi-miyashige, Heian-tokinashi and Daimaru-Shogoin, which had been grown for 4 to 6 days in a growth chamber (25°C; 5,000 lux) were very susceptible to hot water treatment, their roots were cut off. Cut surfaces of hypocotyls were wrapped with cotton wetted with sterilized distilled water or a modified Knop solution in order to keep the cotyledons from withering. The lower surfaces of cotyledons were inoculated with the suspension of conidia. The percentage of the conidiophore formation was highest on the 6-day-old cotyledon of cv. Shirokubi-miyashige. In the case of cv. Daimaru-Shogoin, the 4-day-old cotyledon was best suited. A dark treatment of 18 h of the infected cotyledons on the 6th day after inoculation stimulated conidiophore formation following synchronized formation of the conidia. Also, many conidiophores were formed on the infected cotyledon when moved into an incubator at 20°C after being stored in a refrigerator at 5°C for two weeks after the 3rd day after inoculation (Ohguchi et al., 1989).

g. Laboratory tests of fungicides

A susceptible cultivar of *Brassica* species must be used for the maintenance of *P. parasitica* (Channon and Hampson, 1968). Sow seeds of susceptible cultivar in boxes. Detach cotyledons bearing 4-5 mm petiole from the seedlings and lay them in a single layer on sterilized moist, crinkled filter paper in transparent plastic boxes. Add

sufficient sterilized tap water to maintain the filter papers adequately moist. Ten to fourteen day-old cotyledons are suitable for maintaining the cultures. Obtain conidia from an actively sporulating fungus on leaf or cotyledon. Seedlings can be inoculated with the aid of a small paint brush or spraying, or by dipping the cotyledons in the spore suspension. After inoculation, incubate the boxes of cotyledons in growth cabinet at 15°C with illumination. Supplementary light (400w mercury fluorescent lamps, 3 3/4 ft above the boxes and each illuminating an area of just under 11 sq ft) for 12h per day is essential for the survival of both infected and uninfected cotyledons.

To test the protectant action of fungicides, 10-14 day old seedlings are cut off at soil level and placed in Weldmesh Racks (14 seedlings per rack) with cut ends of the stems immersed in water in an enamel dish which support the racks (Channon and Hampson, 1968). Atomize 2 ml of the test chemical on the upper surfaces of leaves of seedlings in the racks. On the following day cut off the leaves with petioles and place on moist filter paper in three to four plastic boxes (3 1/3" x 1 7/8" x 7/8"). Inoculate these leaves with a drop (0.01 ml) of spore suspension containing approximately 1000 conidia and incubate at 15°C in an illuminated incubator. Record the number of leaves showing sporulation.

h. Fungicide resistance assay

An assay for resistance and sensitivity of *P. parasitica* to metalaxyl can be made using cauliflower seedlings of cv. Lawyna (Crute et al., 1985). Nutrient solution (25 ml) amended metalaxyl (Ridomil 25 W.P.) at a range of concentration of up to 100 μgml^{-1} was contained in 7 cm diameter glass crystallizing dishes and absorbed in an equal volume of vermiculite. Seed was sown into the dishes (30 - 40 per dish) and placed in a temperature controlled growth room (15°C, 12h photoperiod, 100 $\mu Em^{-2}S^{-1}$). To avoid problems with vapour activity, each dish was contained within a plastic "treacle pot'. Seedlings at the cotyledon stage, 7-10 days after sowing, were inoculated with the conidial suspension of the fungus and incubated under the same conditions. Observations were recorded for the presence or absence of sporulation 5 - 10 days after inoculation. A standard metalaxyl sensitivity isolate which was completely inhibited at $0.01\mu gml^{-1}$ was included in all tests. A modification of the method using cauliflower seed treated with metalaxyl (Ridomil 25 WP) at a rate of 1 g a.i. per kg clearly discriminated between resistant and sensitive isolates. The bioassay of the plant material revealed 15-20 μg 'metalaxyl equivalents' per g fresh weight in seedlings during the course of the test. Resistant isolates sporulated profusely on seedlings grown from treated and untreated seed while sensitive isolates only sporulated on the later (Crute et al., 1985).

i. Measuring systemic infection by the downy mildew pathogen

According to McMeekin (1971), seeds of brassicas were first surface sterilized in sodium hypochlorite (10% Commercial Clorox) for 5 minutes, and then placed about 1.5 cm apart on either 1-2% agar or sterilized glass wool moistened with distilled water in the bottom of a moist chamber (McMeekin, 1971). They were germinated in the dark at 20°C. Seven days later most seedlings were about 2 cm tall. The cotyledons and roots were excised from these seedlings. The hypocotyl, relatively free of starch granules and chloroplast, was placed with one end in 10 ml of test solution at the bottom of a Petri dish (5 cm diameter). Ten or more hypocotyls were kept upright in the dish by pushing them through a double layer of cheesecloth stretched over the dish, and held in place by a rubber band.

Inoculum was applied either on the upper tip or on the side of the hypocotyl. A 1 mm square piece of brassicas cotyledon covered with conidiophores was used as inoculum. The Petri dish bottoms, containing the inoculated hypocotyls were placed in a moist chamber lined with wet paper towelling. The moist chamber was placed in a 15°C incubator with a light intensity of 5 ft-c for about a week. The whole hypocotyl was removed from solution and fixed on a slide by 0.1% cotton or anilin blue in lactophenol (20% carbolic acid: 20% lactic acid: 40% glycerine: 20% distilled water). The hypocotyl was pressed evenly with another slide until it was flattened and then a cover slip was applied. After a few hours, the mycelium was stained and could be seen within the host. The length of time between inoculation and fixation determined the intensity of the stain in the mycelium within the host tissue. The cotton blue stained the protoplasm of the fungus, but not the cell wall. Only the youngest fungal growth at the time of fixation was deeply stained in the final preparation. If the test solution favoured or did not interfere with host or fungal growth, the fungus could grow from the point of inoculation to the lower tip of the hypocotyl that was immersed in the test solution for 4 to 5 days at 15°C. At this time the mycelium in the lower tip would stain dark blue, but the older mycelium at the point of inoculation took little stain. If the test solution was unfavourable, a "zone of inhibition" lacking fungal growth could be measured from the base of the hypocotyl to the point where fungal growth has stopped. Most of the mycelium was parallel to the stele. Solutions containing either antibiotic or sugars were tested with this method, and for a given concentration, the zone of inhibition was very consistent. It was possible to use this method without completely aseptic procedures, and not have a serious problem with rotting. However, streptomycin sulfate (0.25 μg/ml) reduced the rotting without appearing to affect host or the pathogen (McMeekin, 1971).

j. Methods of breeding for multiple disease resistance

To identify resistance to various chinese cabbage pathogens, Williams and Leung (1981) developed methods for screening large populations of seedling plants. Screening

of seedlings was preferred in the early stages of breeding programs because it takes less time and space. Plants which exhibited seedling resistance were later evaluated for mature plant resistance. Such procedures may involve simultaneous inoculation and incubation of one week old seedlings with *Plasmodiophora brassicae*, *Peronospora parasitica*, *Albugo candida*, *Phoma lingam* and *Alternaria brassicicola* or *A. brassicae*. This can be followed by a sequential inoculation with Turnip mosaic (TUMV), or/and *Erwinia carotovora* and/or *Xanthomonas campestris*. The interactions phenotypes of more than one pathogen on a single host can be relied on for evaluation (Fig. 63) by paying special attention to the following: (a) careful preparation, quantification and delivery of precise amounts of virulent inoculum, (b) careful cultivation of host "target tissues" of known physiological age, and (c) optional incubation conditions for disease development.

Single seeds were sown in 12-pack pots and when the cotyledons have expanded, after 5 to 7 days, single 0.01 to 0.02 ml drops of a freshly collected conidial suspension containing approximately 10^5 conidia/ml were placed on each of the two cotyledons with the aid of a finely tipped glass pipette (Williams and Leung, 1981). As plants of each 12-pack were inoculated they were placed in glass or plastic boxes containing a 1 to 2 cm depth of warm water. A tight fitting cover was placed on the box when it was filled with plants. The box was then placed in a darkened incubator set at 20°C for 8-16h. The atmosphere in the box maintained the droplets in the cotyledons during which time germination and penetration occurred. Plants were then transferred to the greenhouse bench at 20-25°C for 5 days, then returned to the humidity boxes at 20°C for 16-24h. By then, the susceptible plants had a profuse growth of conidiophores on the lower sides of the cotyledons, whereas resistant plants exhibited varying degrees of sporulation and tissue necrosis which was evaluated on a 0-9 scale as illustrated in Fig. 62. Williams and Leung (1981) also noted that it is important to use freshly produced inoculum collected by washing off spores from leaves with distilled water and that older plants may be inoculated by atomizing the foliage with the suspension of conidia and keeping them at 100% RH for 8-16h.

Chinese cabbage was grown under the above conditions and sequentially inoculated with four pathogens (Williams and Leung, 1981). Five days after sowing, seedlings were dipped in a spore suspension of *Plasmodiophora brassicae* spores and transplanted, then one to two days later inoculated with *Peronospora parasitica* conidia. Twelve days after sowing the plants were evaluated for downy mildew resistance and the susceptible plants removed. The remaining plants were then inoculated with turnip mosaic virus (TuMV) at 14 days, evaluated, reinoculated and rogued over the following 14 days. Surviving plants were then inoculated at 21 days after sowing for soft rot resistance. Two weeks later, 35 days after sowing, resistant plants could be removed from the pots and examined for club root. Plants withstanding all four diseases could then be potted and vernalized or treated with benlate fungicide and transplanted to the field. Further inoculations with TuMV,

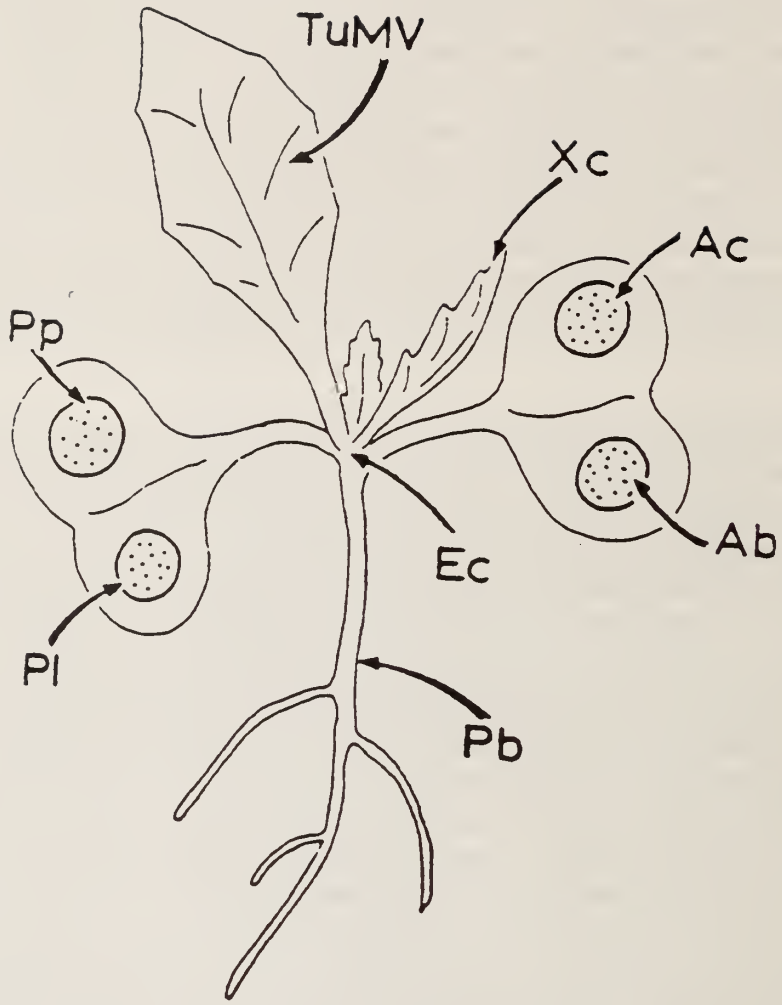


Fig. 63. Location of inoculum placement of eight pathogens in multiple disease screening of seedling Chinese cabbage. Pb = Plasmo*diophora brassciae,* Ec = *Erwinia carotovora,* Ac = *Albugo candida*; Pl = *Phoma lingam;* Ab = *Alternaria brassicae;* Pp = *Peronospora parasitica*; Xc = *Xanthomonas campestris;* and TUMV = Turnip mosaic virus (Reprinted from P.H. Williams and H. Leung. 1981. Methods for breeding for multiple disease resistant Chinese cabbage. IN: Chinese cabbage. Proc. 1st Intern. Symposium, N.S. Talekar and T.D. Griggs (Editors): pp. 393-403, by permission of the authors and the publisher the Asian Vegetable Research and Development Center, Shanhua, Taiwan).

Erwinia and *Peronospora* could be made in the field and plants not treated with benomyl fungicide could be planted in *P. brassicae* infested field plots.

The procedures for the sequential inoculations were reported to be essentially the same as those for individual inoculations except when TuMV inoculation was to be followed by *E. carotovora* or *P. brassicae* (Williams and Leung, 1981). The plants were maintained at 25°C instead of returning them to cooler temperatures for enhancement of virus symptoms. Inoculation with a combination of any of the four pathogens was possible by following the appropriate portions of the total sequence in Fig. 63. Plants can also be inoculated with other pathogens such as *Albugo candida*, *Alternaria* spp., *Phoma lingam* and *Xanthomonas campestris*. Inoculation with these pathogens were kept apart from those areas of the cotyledons and leaves which were occupied by *Peronospora* (Fig. 64). It allowed large F_2 and backcross progenies to be efficiently screened. It was possible to screen approximately 600 plants per m^2 for the four diseases every 35 days. If resistance to each of the above pathogens were controlled by independent single recessive genes, a theoretical minimum population of 256 plants would be needed to recover the four recombinants. It is likely that far larger populations would be screened to accommodate the differing heritabilities for each form of resistance. An important consideration in selecting dominant forms of resistance in the production of F_1 hybrid Chinese cabbages was that resistance to different pathogens can be introduced into the hybrid from different inbred parents.

k. Heterothallism and homothalliam

For such studies, isolates of *P. parastica* were collected from different host species and also from various geographical locations (Sherriff and Lucas, 1989b). The isolates were maintained on seedlings of susceptible cultivars of respective hosts. Cotyledons with spores were excised, placed in 50 ml sterile distilled water (SDW) and shaken gently to dislodge the conidia. The conidial suspension was then filtered through three layers of cotton gauze and centrifuged at 1500g. The conidial pellet was resuspended in SDW, centrifuged and finally resuspended in 1-2 ml SDW. Cotyledons of 7-day old susceptible seedlings, raised in a 9 cm pot, were drop inoculated with the conidial suspension with the aid of a Pasteur pipette. Inoculated seedlings were then sealed in a 13 x 21 cm propagator and transferred to a growth room (19 ± 1°C; 16 h d, osram cool white fluorescent tubes, photon flux density $70\mu ml^{-2}\ S^{-1}$). Conidia were harvested 5-7 days after inoculation.

For microscropic examination, cotyledons and leaf pieces were cleared by boiling for 2 minutes in a lactophenol-ethanol solution containing 10 g phenol, 20 ml glycerol, 10 ml lactic acid and 20 ml 96% (V/V) ethanol (Sherriff and Lucas, 1989b). Cleared cotyledons were rinsed in water and stored in 70% (V/V) glycerol and examined under a low powered microscope. Mature oospores were easily visible due to their brown

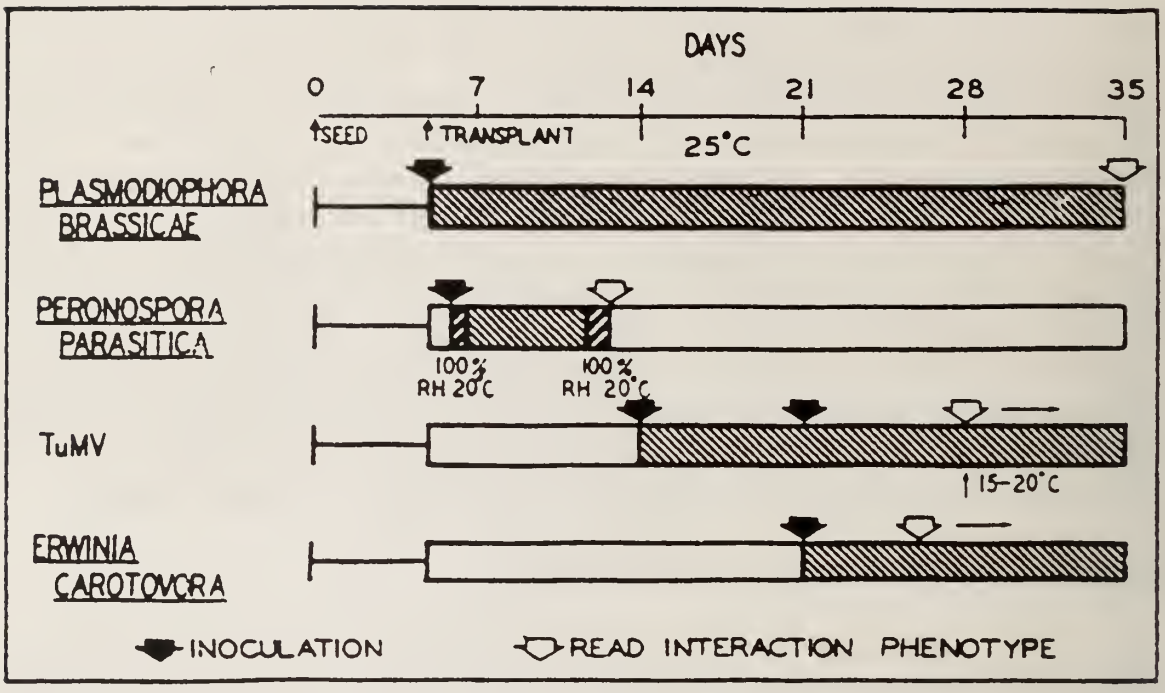


Fig. 64. Sequence for individual and multiple disease resistance screening in Chinese cabbage (Reprinted from P.H. Williams and H. Leung. 1981. Methods for breeding for multiple disease resistant Chinese cabbage. IN: Chinese cabbage. Proc. 1st Intern. Symposium, N.S. Talekar and T.D. Griggs (Editors): pp. 393-403, by permission of the authors and the publisher the Asian Vegetable Research and Development Center, Shanhua, Taiwan).

pigmentation; young oospores tended to take up and retain green pigments from host tissues during cleaning.

l. Seed-borne nature of *P. parasitica*

Jang and Safeeulla (1990c) studied the seed-borne nature of *P. parasitica* in *Raphanus sativus*. Four hundred seeds of test host cultivars were sown in field plots which were observed periodically for the occurrence of downy mildew disease. At the seed setting stage, seeds from infected plants were subjected to a maceration technique (Shetty et al., 1978). Seeds were placed in 250 ml of 10% NaOH for 24, 36, and 48 hours, at 22°C along with 0.5 g of Trypan blue stain. After the alkali treatment, the seeds were agitated in warm water (60 - 70°C) for 5 minutes. Hard seeds were softened by boiling in 5% NaOH for an additional 5 - 10 minutes. Seeds were then sieved, excess water drained off, and lactophenol was added to a beaker containing the treated seeds. The lactophenol completed the detachment of the embryo from the seed coat. The beaker with the embryos and the seed coats was placed in a water bath and heated with a low flame until the embryos were cleared. The embryos and seed coats were examined under a stereomicroscope. To determine the viability of the internally borne mycelium, a seedling symptom test was carried out. Four hundred seeds from the above samples were sown under controlled conditions in a glass house which was free from airborne inoculum. Before sowing, the seeds were surface sterilized. Such seeds were sown in pots containing steam sterilized soil. Daily observations were made following seedling emergence and the percentage of infected seedlings within each cultivar was recorded. The seeds from the first harvest were subjected to the alkali maceration technique to determine the rate of transmission of the pathogen in the seeds (Jang and Sefeeulla, 1990c).

To study pathogen infection through the stigma, unfertilized stigma of healthy plants were taken from test cultivars of the host (Jang and Safeeulla, 1990d). Unfertilized carpels were removed from healthy plants. The ovaries along with style and stigma were placed on the sporulating surface of infected leaves at 16°C for 3 days. At 12 h intervals such ovaries were fixed in acetic acid: alcohol (1:3) and subjected to the alkali maceration technique (Shetty et al., 1978). Another method was to spray unpollinated carpels with a conidial suspension, or dipping inflorescences of healthy plants in a container with a concentrated conidial suspension. Such treated carpels were covered with moist polyethylene bags to maintain humidity for 2-3 days. The carpels are then fixed in acetic acid: alcohol. They were dehydrated by boiling in alcohol: lactophenol (50:50) for 30 - 35 min. followed by maceration in 5% KOH solution for 24 h. The macerated carpels were washed in distilled water and treated with saturated chloral hydrate solution with 0.5% cotton blue for 24h. The clear ovaries were mounted in lactophenol on slides after squashing and then observed microscopically (Jang and Safeeulla, 1990d).

m. Conidial germination

To test the effect of temperature and relative humidity on conidial germination and germ tube growth, a conidial suspension was made by washing off the conidia from the donor host leaves into petri dishes (Lin, 1981). A fine stream of cold, sterilized, distilled water delivered by an atomizer was used for this purpose. The donor leaves were usually collected at 4 a.m. when sporulation was abundant. The conidial suspension was adjusted to the desired concentrations (5 x 10^3 cells/ml) by dilution with distilled water and then sprayed on to 1.25% water agar in petri dishes. After incubating the inoculated petri dishes separately at 4°C, 8°C, 12°C, 16°C, 20°C, 24°C, 28°C, 32°C and 36°C for 24h, germination and germ tube growths of conidia was determined by microscopic observation of 400 spores per plate. Three replications were used for each treatment. A conidium was considered germinated if the length of the germ tube exceeded the width of the conidium. To determine the effect of humidity on germination of conidia, three drops of conidial suspension were pipetted onto a clean glass slide placed in a petri dish containing saturated salt solution to obtain theoretical relative humidity of 0% ($CaCl_2$), 32 % ($CaCl_2$ $6H_2O$), 55% ($Ca(NO_3)_2$ $4H_2O$), 81% ($NH_{4)2}SO_4$)and 95% ($NAHPO_4$ $12H_2O$). The petrie dishes were sealed and incubated at 16°C for 24 h. Spore germination was then counted (Lin, 1981).

n. Sporulation

In the evening, diseased leaves were excised from 40 - 50 day old plants grown in the field (Lin, 1981). Excised leaves showing fresh symptoms were cut into several 0.5 x 0.5 cm^2 pieces. The pieces were first gently dipped into sterilized water to wash off the conidia borne on conidiophores. Six pieces were then placed on a slide with the abaxial surface upward. The slide was put in a petri dish containing two filter papers previously moistened with distilled water. After incubating the petri dishes at 4°C, 8°C, 12°C, 16°C, 20°C, 24°C, 28°C, 32°C, and 36°C, for 18 h, sporulation was determined by shaking the six pieces in 1 ml distilled water and counting the number of conidia with a haemacytometer under a microscope (Lin, 1981).

o. Discharge of conidia

Lin (1981) also measured the discharge of *P. parasitica* conidia from diseased leaves of the host. A leaf showing typical symptoms of downy mildew was selected and fixed on the hole of a spore collector so that the abaxial surface of the diseased leaf faced directly over one of the 24 slides attached to this collector. The surface of the slides were smeared with a layer of vaseline to intercept the falling conidia. Each slide automatically moved forward one position per hour, thus a 24 h spore collection was obtained. The collection was continued for three days beginning at 9 p.m. each day, and the periodic conidial discharge was determined by counting the conidia on slides

under a microscope. Temperature and relative humidity for each hour during conidia collection was also recorded to establish the relationship between the discharge of conidia and environmental parameters (Lin, 1981).

13. DISEASE MANAGEMENT

To manage downy mildew of crucifers, no single method or approach is considered feasible, effective, environmentally safe and economical. It is always essential to integrate the available methods for disease control.

a. Cultural practices

Cultural control of crucifers downy mildew disease is largely a matter of sanitation and of manipulating the environment to the advantage of the host and to the detriment of the pathogen. Since the pathogen survives in the form of oospores in the host tissues, removal, destruction and burning of the infected plant debris along with weeds has been suggested to restrict the source of primary inoculum (Butler, 1918; Vasudeva, 1958). In addition, clean, well-drained soils with two years of crop rotation using non-cruciferous crops was also recommended. Measures to reduce the relative humidity around the plants by adequate aeration and avoidance of dense sowing and controlling the growth of weeds also helped to reduce the disease (Butler, 1918; Conroy, 1960; Schmidt, 1960; Sherf and Macnab, 1986). Avoidance of continuous cropping of rape on the same field or adjacent to a field sown to rape in the previous year was also advised to reduce infection by *P. parasitica* (Downey and Bolton, 1996). The widespread cultivation of one or only a few cultivars of the same species may favour the disease. In India, the late sown crops of rapeseed-mustard were reported to have a higher incidence of downy mildew than the early (before October) or timely (by middle of October) sown crops (Kolte, 1985; Saharan, 1984, 1992a).

In the Lujskaya area, Lenengrad regions of the USSR, the level of downy mildew infection was reduced on cabbage plants transplanted between 26-30 June. Fertilizer containing 50% humus, 45% peat and 5% millein with 3.9 g ammonium nitrate, 4.3g Kcl, and 8.1 g superphosphate/100 added to 110 g organic matter applied to the soil reduced the percentage of diseased plants better than the organic manure alone (Kupryanova, 1957).

b. Seed treatment

Fungicidal seed treatment followed by a foliar spray is a common practice to control downy mildew of crucifers. Metalaxyl seed treatment at the rate of 0.3 - 0.6 g a.i. kg^{-1} reduced downy mildew infection on broccolli (Paulus and Nelson, 1977) and

rapeseed mustard (Kolte, 1985; Saharan, 1992a). A significant yield increase was observed when plants raised from such treated seed were sprayed once or twice with the same compound. Seed treatment with Apron SD 70 (35% metalaxyl and 35% captan) controlled downy mildew of cauliflower for more than 2 weeks after sowing (Crute, 1984). According to White et al. (1984) seed treatment with Apron SD 70 (1 g metalaxyl kg. $^{-1}$) gave complete control of downy mildew on cauliflower inoculated 10 days after sowing . Following seed treatment, metalaxyl was detectable in the cotyledons, true leaves and roots of cabbage seedlings up to 4 weeks after sowing. An effective and economical schedule for control of downy mildew of mustard through fungicidal seed treatment and/or spray application has been worked out under Indian conditions. Seed treatment with Apron SD 35 (2 g metalaxyl a.i. kg^{-1} seed) along with two foliar applications of Ridomil MZ72 at 30 days intervals gave the best control of downy mildew on mustard along with an increase in yield (Mehta et al., 1996; Table 30). The maximum cost-benefit ratio was obtained when mustard seeds were treated with Apron SD35 followed by three spays with Mancozeb.

c. Soil treatment

The use of systemic fungicides such as prothiocarb (Dynone) @ 5g m^{-2} before sowing and fosetylaluminium (Aliette) @ 10 g m^{-2} as soil drench gave excellent disease control on cauliflower (Ryan, 1977) (Table 31). Both these fungicides were as effective as eight sprays of dichlofluanid (Ryan, 1977). Prothiocarb also reduced infection of radish leaves and bulbs when applied @ 0.1% as a drench (4 litres m^{-2}) at 50% seedling emergence and was much more effective than sprays of dichlofluanid, zineb, captafol and maneb (Anonymous, 1974).

Granular applications of metalaxyl prior to sowing was shown to be an effective control of downy mildew on broccoli (0.56 and 1.2 kg a.i. ha^{-1}) and on cauliflower (0.28 kg a.i. ha$^{-1)}$. In cauliflower, pre-sowing incorporation or a single post-sowing drench (1.5 kg a.i. ha^{-1}), or three high volume sprays (0.8 g a.i. litre^{-1}) of metalaxyl gave much better disease control than nine sprays of dichlofluanid applied during a 6-8 week period (Chiu, 1959).

d. Compost treatment

In the UK, metalaxyl, milfuran + manganese zinc dithiocarbamate, or propamocarb incorporated in the compost provides good control of downy mildew on module-raised cauliflowers in early summer plantings. In summer cauliflowers, good control was achieved by drenching the compost with propamocarb, fosetyl-aluminium foliar sprays and by applying a dichlofluanid foliar spray programme (Davies and Wafford, 1987).

e. Foliar spray of fungicides

During the period from the mid-1940's to the mid-1960's, control of downy mildew of crucifers rested on frequent applications of sprays or dusts of fungicides such as chloranil (spergon), copper based materials and zineb (Channon, 1981). These materials were subsequently superseeded by other non-systemic fungicides like captafol, daconil, dichlofluanid, propineb, bordeaux mixture, copper oxychloride, mancozeb, ziram, chlorothalonil and fentin hydroxide (Butler, 1918; Butler and Jones, 1949; Kolte, 1985; Saharan and Chand, 1988; Sherf and Macnab, 1986; Vasudeva, 1958). The list of fungicides found effective against downy mildew of crucifers at different locations is given in Table 32. Captafol, mancozeb, difolatan, copper oxychloride, dichlofluanid, propineb and metalaxyl have been found to be superior to other fungicides on a large number of crucifers at several locations. The time of application of fungicides and numbers and interval of sprays depend on the duration and type of crop species grown (Channon et al., 1970; Kolte, 1985; Saharan and Chand, 1988; Saharan, 1992a; Verma et al., 1994; Whitewell and Griffin, 1967).

i) Brassica vegetables: In the UK, dichlofluanid gave excellent control of the disease on the cotyledons of cabbage and cauliflower. Dichlofluanid and propineb reduced the level of early mildew infection and increased the size and dry weight of cauliflower plants (Channon et al., 1970; Whitewell and Griffin, 1967).

In the Irish Republic, downy mildew of *Brassica* crops, especially cauliflower, has been controlled by fosetyl aluminum, metalaxyl + mancozeb, cyprofuram and propamocarb (Ryan et al., 1984).

In South Africa, during the initial years of containerized seedling production of cabbage, mancozeb (dithane M-45), chlorothalonil (Bravo), metalaxyl (Ridomil) and metalaxyl plus mancozeb (Ridomil MZ) provided adequate control of downy mildew disease. Later on, cymoxanil plus mancozeb consistently provided the most effective control against downy mildew. Oxadixyl plus mancozeb, cupric hydroxide and chlorothalonil gave significantly better protection than mancozeb (Brophy and Laing, 1992).

In Australia, neutralized phosphonic acid sprays applied onto cauliflower in the field within 3 weeks of harvest reduced downy mildew under storage conditions. Two applications of 2.4 kg a.i./h, 21 and 7 days before harvest reduced the curd infection development at the post-harvest stage in storage. There was no effect of phosphonic acid on crop appearance and maturity. The maximum phosphonate residue in curds at harvest was 12 μg/g which was considered a safe limit (McKay et al., 1992).

In Thailand, the best control of Chinese cabbage downy mildew was obtained with

Table 30. Efficacy, economics and spray schedule of fungicides against downy mildew of mustard (Mehta, Saharan and Kaushik, 1986)

Fungicides	Concentration (%)	Spray No.	Percent Disease Intensity	Percent Disease Control	Percent Increase In Yield	Cost: Benefit Ratio
Dithane M-45	0.2	4	28.4	42.4	28.5	1:2.20
Kavach	0.2	4	28.2	35.0	23.3	1:1.57
Ridomil MZ-72	0.25	3	9.1	81.3	49.3	1:1.21
*Apron SD-35+ Ridonmil MZ-72	0.25	2	15.8	68.5	34.2	1:1.11
Apron SD-35+ Dithane M-45	0.2	3	22.9	47.4	22.1	1:2.11
Apron SD-35+ Kavach	0.2	3	21.9	49.6	20.4	1:1.29
Apron SD-35	-	-	33.6	41.0	15.9	1:20.62
Control	-	-	49.7	-		
LSD 0.05	-	-	4.1			

*Apron SD-35 as seed treatment @ 2 g a.i. kg^{-1} seed

Ridomil 25 WP @ 2 kg/h (Yang et al., 1983). Three sprays at weekly intervals beginning from 28 days after transplanting gave 65% more marketable yield.

In India, four sprays with difolatan (0.3%), daconil (0.1%), dithane M-45 (0.2%), Ridomil (0.2%) or aliette (0.1%) at intervals of 8-10 days were most effective for controlling downy mildew of radish (Sharma and Sohi, 1982; Sharma, 1983) (Table 33). Root yield was significantly higher in sprayed plots. There was a significant reduction in the apparent infection (r) and the basic infection rate (R) of downy mildew in treated plots.

ii) Brassica oilseeds: For the control of downy mildew of mustard, difolatan, mancozeb and metalaxyl have been found to be very effective at different locations (Table 34) in India. An effective and economical schedule has been worked out under Indian conditions for the control of downy mildew of mustard through seed treatment and/or spray with fungicides. Three sprays of Ridomil MZ-72 (Metalaxyl and Mancozeb @ 0.25%) at an interval of 20 days starting from 40 days after sowing gave maximum disease control (82%) along with >49% increase in yield; seed treatment with Apron SD-35 (metalaxyl @ 2 g a.i. kg^{-1} seed) along with two foliar applications of Ridomil MZ-72 at 30 day intervals were relatively less effective (Tables 35, 36). These treatments were quite effective in reducing staghead formation in mustard (Table 37). When mancozeb (dithane M-45) and chlorothalonil (Kavach) were sprayed three times following seed treatment with Apron SD-35, disease control of around 47% and 49% respectively was achieved. The maximum cost-

Table 31. Efficacy of fungicidal treatments on the severity of downy mildew of cauliflower (Reprinted from E.W. Ryan. 1977. Control of cauliflower downy mildew *(Peronospora parasitica)* with systemic fungicides. Proc. Ninth British Insecticide and Fungicide Conference, Brighton, Volume 1 and 2, Research Report, London, U.K., Sessions 6B, pests and disease of vegetables: pp. 297-300, by permission of the author and the publisher British Crop Protection Enterprises, Loughborough, U.K.)

Fungicides	Method of Application	Rate of Application	Disease severity index			
			Walk-in tunnels		Low tunnels	
			March 28	April 13	April 13	April 25
Dichlofluanid	Foliar spray	8@1g/10m^2	1.4	2.8	1.6	2.3
Aliette	Foliar spray	3@1g/10m^2	1.7	1.9	1.7	1.9
Prothiocarb	Foliar spray	3@1g/10m^2	1.6	2.1	1.5	1.7
Aliette	Soil treatment	5g/m^2	1.2	2.6	1.0	2.0
Aliette	Soil treatment	10g/m^2	0.7	1.8	0.3	1.2
Aliette	Soil treatment	20g/m^2	0.0	0.8	0.0	0.6
Prothiocarb	Soil treatment	5g/m^2	0.6	1.4	0.2	1.0
Prothiocarb	Soil treatment	10g/m^2	0.2	1.1	0.0	0.9
Prothiocarb	Soil treatment	20g/m^2	0.0	0.5	0.0	0.7
Control	-	-	2.4	3.7	2.0	3.0
LSD 5%			0.72	0.76	0.78	0.56

O = No disease; 5 = very severe disease

benefit ratio was either with four sprays of mancozeb or seed treatment with Apron SD-35, followed by three spays of mancozeb (Mehta et al., 1996). Absorption of metalaxyl increased, up to 30 days, when applied as seed treatment, thereafter it gradually declined and was not detectable after 60 days of sowing (Table 38). The maximum residue (average 9.03 ppm) of metalaxyl was found to be one day after spraying (Table 39). The metalaxyl on mustard plants was almost undetectable 15-30 days after spraying (Table 40). The safe waiting period for metalaxyl was calculated to be 62 and 8 days for seed treatment and for foliar application, respectively (Table 41). No metalaxyl was detected in mustard seedlings raised from seeds obtained from these treatments (Mehta, 1993) (Table 42).

f. Biological control

i) Plant extracts as fungitoxicant: Garlic juice or aqueous extracts of garlic was reported to be toxic to *P. parasitica* which causes downy mildew of radish (Ark and

Table 32. Fungicides found effective against downy mildew of crucifers

Fungicide	Rate of Application	Reference
CABBAGE		
spergon spray (48% a.i.)	4 lbs/100 gallons	Borders, 1953
spergon dust (4.8% a.i.)	30 lbs/acre	
kolophygon dust (30% sulfur and 1% phygon)	30 lbs/acre	
Parzate Dust (6.5% a.i.)	30 lbs/acre	
Dithane Z-78 spray (65% a.i)	2 lbs/100 gallons	
Dithane Z-78 dust (6.5% a.i.)	30 lbs/acre	
yellow cuprocide	1 lb/100 gallons	Foster 1947[b]
Dithane B-11	1 lb/100 gallons	
Spergon (Wittable)	4 lbs/100 gallons	
Dow Seed treatment	2 lbs/100 gallons	
Fermate	2 lbs/100 gallons	
Phygon	1/4 lb/100 gallons	
Phenanthraquinone	1 lb/100 gallons	
Bordeaux mixture	1:1:10	Anonymous, 1938, Wiese, 1927
Spergon (Chloranil) (5 and 10% a.i.)		Epps, 1955
Dithane Z-28 (Zineb) (3.9% a.i.)		
Phygon XL(dichlone) (1.0% a.i.)		
Copper No. 30 (4.0% a.i.)		
Thiram (5.0% a.i.)		
Manzate (Maneb) (4.2% a.i.)		
Vancide F995W (6.0% a.i.)		
Vancide 51ZW (6.0% a.i.)		
Ethyl B-622 (4.0% a.i.)		
Metalaxyl	1.12kga.i./ha	Jaworski et al., 1982
Captafol (0.25% a.i.)		Channon & Hampson,1968
Daconil 2787	-	
Dichlofluanid	-	
Propineb	-	
Zineb	-	
Maneb	-	
Mancozeb	-	
Quintozene	-	
Quinomethionate	-	
Copper oxychloride	-	
Triphenyl tin hydroxide	-	
Dichlone	-	
Chloranil	4 lbs. 48%/100 gallons	Anonymous, 1953
Nabam - Zinc Sulphate	l lb/100 gallons	
Phygon XL-N	l lb/100 gallons	
Difolatan 4F (Captafol)	0.2%	Apandi, 1980
Polyram (Metiram)	0.2%	
Dithane Z-78	0.2%	Ciferri, 1953
Dichlofluanid	0.05-0.2% a.i.	Channon et al., 1970

Aspor	0.3%	Nakov, 1968
Maneb	0.2%	
Perotsin	0.3%	
Nickel sulphate spray	0.05-0.2%	Keyworth, 1967
Polycarbacin spray	0.4%	Vasileva, 1976
Cymoxanil + mancozeb (6+70% a.i.)	200g/100 litres	Brophy & Laing, 1992
Cymoxanil + chlorothalonil (6+50% a.i.)	200g/100 litres	
Oxadixyl (8% a.i.)	80ml/100 litres	
Oxadixyl + mancozeb (8%+56% a.i.)	330g/100 litres	
Propamocarb + HCl (72% a.i.)	120ml/100 litres	
Propamocarb + mancozeb (72 + 80% a.i.)	60ml/100 litres+ 75g/100 litres	
Metalaxyl (Ridomil WP) (25% a.i.)	50g/100 litres	
Metalaxyl + Mancozeb (25 + 80% a.i.)	50g/100 litres	
Fosetyl - Al - Mancozeb (44 + 26% a.i.)	350g/100 litres	
Chlorothalonil (50% a.i.)	100ml/100 litres	
Mancozeb (80% a.i.)	200g/100 litres	
Copper oxychloride (80% a.i.)	400g/100 litres	
Cupric hydroxide (72% a.i.)	200g/100 litres	
CGA - 48988 soil application	23 mg a.i./M	Gabrielson & Getzin, 1979

BROCCOLI

Agrimycin	0.1 lb/acre	Natti et al., 1956
Spergon SL	2 lbs/acre	
Streptomycin	50 ppm	Altman, 1958
Agri-strep	3 lbs/acre	Natti, 1957
Agri-strep + Glycerol	3 lbs/acre	
Copper-Zinc	6 lbs/acre	
Copper-Manganese	6 lbs/acre	
Spergon SL	3 lbs/acre	
Manzate	4 lbs/acre	
Thioneb	6 lbs/acre	
Captan 50W	6 lbs/acre	
Vancide M	4 lbs/acre	
Kemate 50%	6 lbs/acre	
Manzate + Agri-strep	4+0.4 lbs/acre	Natti, 1957, 1959
Copper-zinc + Agri-strep	4+0.4 lbs/acre	Natti, 1957
Agrimycin 500	4.6 lbs/acre	
CGA-1-82 50 WP	2 lbs/5 ft. band soil application	Johnston & Springer, 1977
CGA-38140 50WP	2 oz/acre 14 days after seeding	
CGA-48988 (Metaxadine)	1 or 2 oz/100 lbs seed	Paulus et al., 1978

CAULIFLOWER

Dichlofluanid 50WP	1 ½ lbs/100 gallons	Whitewell & Griffin, 1967
Captafol 85WP	3 lbs/100 gallons	
Zineb 70WP	3 lbs/100 gallons	
Propineb 70WP	3 lbs/100 gallons	
Daconil 2787 75WP	3 lbs/100 gallons	

Dichlofluanid	0.05-0.2% a.i.	Channon et al., 1970
Fosetyl aluminium	-	Ryan et al., 1984
Metalaxyl + Mancozeb	-	
Cyprofuram	-	
Propamocarb	-	
Phosphonic acid	2.4 kg a.i./h	McKay et al., 1992
RAPESEED-MUSTARD		
Polyram M	2 lbs/100 gallons	Perwaiz et al., 1969
Melprex	1.5 lbs/100 gallons	
Bordeaux mixture	4:4:50 (0.8%)	
Cuprovit	2 lbs/100 gallons	
Dithane M-45	2 lbs/100 gallons	
Dithane M-45	0.3%	Bains & Jhooty, 1979
Dithane Z-78	0.3%	
Blitox-50	0.3%	
Difolatan 80	0.2%	Chauhan & Muheet, 1976
Ziram	0.2%	
Dithane M-45	0.2%	
Thiovit	0.2%	
Difolatan	0.2%	Saharan, 1984; 1992a
Dithane M-45	0.2%	
Dithane Z-78	0.2%	
Blitox 50	0.3%	
Ridomil	0.2%	
Bristan	0.1%	
Apron SD-35	0.2% seed treatment	
Metalaxyl	0.2%	
Kavach	0.2%	Mehta et al., 1996
Radomil MZ-72	0.25%	
Apron SD 35 seed treatment	2 g a.i./kg seed	
+ Dithane M-45 spray	0.2%	
Apron SD 35 seed treatment	2 g a.i./kg seed	
+Radomil MZ-72 spray	0.2%	
Apron SD 35 seed treatment	2 g a.i./kg seed	
+ Difolatan spray	0.2%	
Apron SD 35 seed treatment	2 g a.i./kg seed	
+ Kovach spray	0.2%	
RADISH		
Difolatan seed treatment or spray	0.3%	Sharma & Sohi, 1982
Daconil seed treatment or spray	0.1%	
Dithane M-45 seed treatment or spray	0.2%	
Ridomil seed treatment or spray	0.1%	
Aliette seed treatment or spray	0.1%	
Blitox seed treatment or spray	0.2%	
Captan seed treatment or spray	-	
Copper oxinate seed treatment or spray	-	
Delan seed treatment or spray	-	
Dathane Z-78 seed treatment or spray	-	
Macuprax seed treatment or spray	-	

STOCK		
Zineb	8 lbs/100 gallons	Jafar, 1963
Strepto spray + glycerol	500 ppm + 1%	
Trioneb	8 lbs/100 gallons	
Bordeaux mixture	5:5:50	
Fongarid (CGA 38140)	0.05%	Trimboli & Hampshire, 1978
Zineb	0.13%	
CAMELINA SATIVA		
Brestan	-	Zarzycka & Kloczowska, 1964
Polyram-M	-	
Sadoplon	-	
Copper oxychloride	0.4%	Zarzycka & Kloczowska, 1967

Thompson, 1959).

ii) Antagonists for biocontrol: Bacteria were observed on the mycelium, conidiophores and conidia of *P. parasitica* on *Lepidium graminifolium* (Nicolas and Aggery, 1940). This was associated with a reduction in conidial germination.

g. Host resistance

Many sources of resistance to downy mildew of crucifers have been identified in major host species from various parts of the world. Information is also known on the genetics of the host parasite interaction. Efforts are being made to breed downy mildew resistant cultivars in various crucifers through conventional and biotechnological techniques.

h. Integrated disease management

In the quadrangle of integrated control (chemical-cultural-biological-host resistance) of downy mildew of crucifers, biological control has not been exploited at the field scale. Breeding for resistance has only succeeded in some crucifers. Chemical control of the disease may not always be reliable as resistance has been developed in *P. parasitica* to metalaxyl, which at one stage proved outstandingly effective in the control of downy mildew (Brophy and Laing, 1992; Crute et al., 1985). Thus there is clearly a need to breed sources of host resistance that would counter pathogenic variation. It is also possible that differential sources of host resistance could be useful in programs of integrated control if they were deployed together with fungicides; this would potentially prolong the effectiveness of both control procedures (Silue et al., 1996). Other methods involve sanitation, field practices like sowing time, plant density, and the judicious use of nutrition and irrigation so that inoculum levels will not build

Table 33. Efficacy of fungicidal sprays on downy mildew of radish (Reprinted from S.R. Sharma and H.S. Sohi. 1982. Effect of fungicides on the development of downy mildew and white rust of radish. Indian J. Agric. Sci. 52:521-524, by permission of the authors and the publisher Indian Council of Agricultural Research, New Delhi, India)

Fungicides	Disease index (%)		Apparent infection rate (r)		Basic infection rate (R)		Yield of roots (kg/plot)	
	A	B	A	B	A	B	A	B
Aliette	NT	6.37	NT	0.078	NT	0.59	NT	20.45
Blitox	17.07	12.27	0.108	0.088	2.36	0.93	18.45	22.02
Captan	17.18	11.03	0.115	0.083	3.08	0.79	19.85	19.65
Copper oxinate	24.46	8.25	0.128	0.083	4.60	0.81	18.85	18.47
Deconil	3.70	3.40	0.055	0.051	0.28	0.21	23.15	24.45
Delan	19.75	8.22	0.123	0.083	4.04	0.81	19.67	19.17
Difolatan	5.88	4.10	0.079	0.053	0.81	0.23	25.67	24.06
Dithane M-45	7.35	7.55	0.084	0.071	0.99	0.48	22.37	24.12
Dithane Z-78	13.67	10.30	0.109	0.092	2.56	1.10	21.52	21.61
Macuprax	26.82	13.20	0.130	0.101	4.84	1.53	17.92	18.43
Control	55.79	19.51	0.171	0.109	13.06	1.93	17.30	17.73
SEM±	1.74	0.942					1.156	1.020
CD at 5%	5.049	2.722					3.356	2.947

A = December 1979 - February 1980; B = June - September 1980; NT = Not tested

Table 34. Efficacy of fungicidal treatments on the downy mildew of mustard in India (Saharan, 1984, 1992a)

Fungicide	Concentration %	Percent Disease Intensity		
		Durgapura	Hisar	Pantnagar
Defolatan	0.2	10.65	9.80	15.09
Dithane M-45	0.2	8.31	12.00	10.79
Dithane Z-78	0.2	-	14.80	14.50
Blitox	0.2	15.00	14.80	16.77
Ridomil	0.2	16.25	8.0	-
Control	-	23.00	24.80	16.80
C.D. 5%	-	-	5.90	5.69

Table 35. Efficacy and spray schedule of fungicides against downy mildew of mustard during 1991-92 and 1992-93 crop seasons (Mehata, Saharan and Kaushik, 1996)

Treatments	Conc. %	No. of Sprays	+ Percent Disease Index (DAS) 1991-92 (60)	1991-92 (90)	1992-93 (60)	1992-93 (90)	Average (60)	Average (90)	+ Percent Disease Control 1991-92 (60)	1992-93 (90)	Average
Dithane M-45	0.2	4	2.7	26.2	11.3	30.7	7.0	28.4	39.7	45.2	42.4**
Kavach	0.2	4	3.5	28.2	-	-	3.5	28.2	35.0	-	35.0
Difolatan	0.2	4	2.7	22.4	-	-	2.7	22.4	48.2	-	48.2
Ridomil MZ-72	0.25	3	0.2	7.8	4.7	10.3	4.0	9.1	82.0	81.6	81.8**
*Apron SD-35+ Ridomil MZ-72	*- 0.25	2	0.0	12.9	12.3	18.7	6.1	15.8	70.3	66.6	68.5**
*Apron SD-35+ Dithane M-45	0.2	3	0.4	22.9	-	-	0.4	22.9	47.4	-	47.4
*Apron SD-35+ Kavach	0.2	3	1.2	21.9	-	-	1.2	21.9	49.6	-	49.6
*Apron SD-35+ Difolatan	0.2	3	1.8	20.9	-	-	1.8	20.9	51.8	-	51.8
* Apron SD-35	*-	-	2.2	27.3	15.3	40.0	8.7	33.6	36.9	28.6	32.7**
Unsprayed (control)	-	-	4.8	43.4	23.7	56.0	14.2	49.7	-	-	-

+ Average of four replicates
*Seed treatment @ 2. g a.i. kg^{-1} seed
( ) DAS: Days after sowing
**: Two years mean

up too rapidly.

Butler (1918) reported a long time ago that the disease can be controlled in young crucifer plants by a mulch of sawdust saturated with copper sulphate placed around the base of the plants.

In the Shanghai region of China, a combination of seed treatments, direct seeding, application of fertilizer, and 2-3 fungicide sprays at the first peak infection period decreases the incidence of *P. parasitica* in Chinese cabbage and increases yield by 10 - 18% (Shao et al., 1991).

14. FUTURE STRATEGIES AND PRIORITIES OF DOWNY MILDEW DISEASE MANAGEMENT

With the globalization of agriculture, there is significant increase in the movement of crucifer germplasm and in the cropping patterns of this important commodity all over the world. The absence of strict measures on the restriction of movement of germplasm and the intensive cultivation of these crops has resulted in large scale perpetuation, build-up and dissemination of *Peronospora parasitica* virulences on cruciferous species all over the world. The information gathered in this monograph indicates that some gaps still exist in the complete comprehension of this disease and this is indicated below:

a. Disease epidemiology

Factors governing disease initiation, development and consequent progression are not completely understood. There is need for more information on the role of initial inoculum in disease development, in the area of changing host susceptibility over time and in oosporic multiplication. Time should always be included as one of the variables in the study of the relationship between pathogen development and host or environmental conditions. Multilocational trials with staggered dates of planting can be helpful in analyzing disease development in relation to environmental conditions and to develop disease prediction models.

b. Physiological specialization

To analyze the virulence pattern of *P. parasitica*, identification and standardization of host differentials is necessary. The relationship between pathogenicity on wild hosts and crop plants needs further study since wild hosts may act as a theatre for increased genetic variation in the pathogen. The use of modern techniques like RAPD fingerprinting may distinguish between pathotypes or even separate clonal population. PCR amplification of ITS (internal transcribed spacer) regions may be useful both for identification of isolates and in estimating their similarity. Ultimately this type of sequence analysis may reveal the evolutionary relationship between different species, genera or higher taxa. The studies on variation between isolates obtained from different geographic regions might explain the variability with respect to virulence and to other characters such as fungicide sensitivity.

c. Genetics of resistance

The search for new sources of resistance is always of high priority. The understanding of population biology and genetics (genetic diversity, relative fitness in geographically separated population, good knowledge of host pathogen variation, availability of reliable markers like virulence/avirulence mating type, allozymes,

Table 36. Comparative yield increase and cost benefit ratio of fungicides used against downy mildew of mustard (Mehta, Saharan and Kaushik, 1996)

Treatments	+Average yield/ plot* (kg)	% increase in yield over control	Cost: benefit** ratio Rs.: Rs. Ps.
Dithane M-45	1.164	28.5	1:2.20
Kavach	1.116	23.3	1: 1.57
Ridomil MZ-72	1.352	49.3	1:1.21
***Apron SD-35 + Ridomil MZ-72	1.216	34.2	1:1.11
Apron SD-35+ Dithane M-45	1.115	22.1	1:2.11
Apron SD-35+ Kavach	1.090	20.4	1:1.29
Apron SD-35	1.050	15.9	1:20.62
Unsprayed (control)	0.950	-	-

\+ Average of four replicates

* Plot size: 2.0 x 2.1m^2

** Based on prelavent market price in 1992

Raya: Rs.800/-Q
Dithane M-45: 152/kg
Ridomil MZ-72:950/kg
Kavach: 333/kg
Apron SD-35: 2782/kg
Labour - 5 labour/spray/hectare @ Rs.40/ - per labour

*** Seed treatment @ 2 g a.i. kg^{-1} seed

mitochondrial and nuclear DNA RFLPS) should lead to a more effective management strategy for disease control.

On the cellular level in relationship to molecular studies, the development of an axenic culture system would help in studies of heredity. Different resistance

Table 37. Efficacy of fungicides against staghead of mustard due to combined infection of white rust and downy mildew (Mehta, Saharan and Kaushik, 1996)

Treatments	No.of Sprays	+ Staghead** incidence (%)	+ Staghead** length (cm)	+ Staghead** score
Diathane M-45	4	8.8 (16.2)	7.9	2.1
Kavach	4	2.4 (8.9)	8.9	1.4
Ridomil MZ-72	3	1.6 (6.4)	5.4	1.5
*Apron SD-35 + Ridomil MZ-72	2	5.2 (12.0)	8.1	2.0
*Apron SD-35+ Dithane M-45	3	2.6 (9.2)	1.9	1.6
*Apron SD-35+ Kavach	3	2.9 (9.9)	3.7	1.7
*Apron SD-35	-	13.9 (19.0)	10.4	2.3
Unsprayed (control)	-	25.8 (30.4)	14.5	3.4
LSD (0.05)		(2.7)		

( ) Angular transformed values
\+ Average of four replicates
* Seed treatment @ 2 g a.i.kg^{-1} seed
** Two years mean (91-92, 92-93)

mechanisms should be characterized in more detail at the histological and cellular level.

d. Molecular aspects

On the molecular level, a major emphasis should be placed on the development and improvement of methods for isolation of RNA and DNA, isozyme analysis, use of RFLP analysis for assessment of genetic variation, development of genetic maps, research of transposable elements and plasmids, development of fungal transformation system, i.e., availability of vectors with suitable markers, and methods for introducing DNA. The

Table 38. Persistence of metalaxyl in mustard foliage after seed treatment (Mehta, 1993)

Treatment	Days of sampling	Average residue Level (ppm)	* Range (ppm)	Dissipation (%)	SD ±
Apron SD-35	7	1.81	1.58-2.02	0.00	0.23
@ 2 g a.i./kg seed	15	3.46	3.03-3.80	+191.46	0.37
	30	9.08	8.63-9.84	+242.42	0.54
	40	5.82	5.57-6.01	35.90	0.18
	60	0.00	-	100.00	-

* Average of three replicates.

Table 39. Persistence of metalaxyl in foliage of mustard after foliar application (Mehta, 1993)

Treatments	Average residue level* (ppm)				
Days	1	5	10	15	30
** Foliar spray-I	9.03	0.68	0.28	0.0	0.0
Range	7.94-10.08	0.49-0.80	0.25-0.32	-	-
Dissipation (%)	0.00	92.46	96.89	100.0	100.0
Foliar spray - II	10.37	0.54	0.29	0.0	0.0
Range	9.57-11.10	0.51-0.57	0.29-0.30	-	-
Dissipation (%)	0.00	94.79	97.20	100.0	100.0
SD± I Spray	0.830	0.169	0.040	-	-
II Spray	0.812	0.034	0.005	-	-

* Average of three replicates

** Foliar spray: 40, 70 days after sowing @ 0.25%

Table 40. Persistence of metalaxyl in mustard foliage after seed treatment and foliar sprays (Mehta, 1993)

Treatments	Average residue level* (ppm)				
Days	1	5	10	15	30
** Foliar spray-I	8.21	0.49	0.26	0.0	0.0
Range	7.89-8.76	0.47-0.52	0.23-0.29		
Dissipation (%)	0.00	94.03	96.83	100.0	100.0
Foliar spray - II	9.45	0.69	0.27	0.0	0.0
Range	8.64-10.22	0.59-0.80	0.25-0.30	-	-
Dissipation (%)	0.00	92.69	97.14	100.0	100.0
SD± I Spray	0.382	0.028	0.034	-	-
II Spray	0.673	0.121	0.029	-	-

** Foliar sprays : 60 & 80 days after sowing @ 0.25%
* Average of three replicates.

Table 41. Safe period and residue half life values of Metalxyl in mustard (Mehata, 1993)

Treatment	RL-50 (Days)	SWP (Days)
Seed treatment	17.57	62.33
Foliar Spray 1	2.08	8.69
Foliar Spray 2	1.79	7.85

RL-50 = Residue half life
SWP = Safe waiting period

Table 42. Translocation of metalaxyl residues into mustard seed following different treatments at harvest (Mehta, 1993)

Treatment	Average residue (ppm)
Seed-treatment* (Apron SD-35) (T_1) 60 DAS	ND
Foliar sprays** Ridomil MZ-72) (T_2) 40, 70 DAS	ND
Seed treatment + Foliar spray (T_3) 60, 80 DAS	ND

ND: Not Detectable
* @ 2 g a.i. kg-1 seed

DAS: Days after sowing
** @ 0.25 per cent

cloning of avirulence and resistance genes will contribute to the elucidation of the molecular basis of host-pathogen specificity and from the practical point of view, be helpful in the design of integrated control strategies.

The concentrated effort to identify loci in *Arabidopsis thaliana* associated with specific resistance to *P. parasitica* should enable cloning of host resistance genes in the near future. The eventual isolation and functional analysis of avirulence genes from crucifers downy mildew will be of particular interest, given the apparently separate phylogeny of this group from other plant pathogenic fungi.

e. Biochemical aspects of resistance

The elucidation of the biochemical background of biotrophy, the establishment of an intracellular interface with host cells, and the role of different infection structures should be topics of future research. Also we understand very little of the biochemical mechanisms involved in the hypersensitive reaction and in various types of resistance. There is a need to gather information concerning the effects of the downy mildew fungus on respiration, photosynthesis, and the translocation, accumulation and transfer of carbohydrates in infected host tissues. The role of hormonal disturbances in pathogenesis, and the basis of systemic versus local lesion infection needs more study.

Genetical and histo-cytological descriptions of interactions, and the availability of methods for growing parasites alone and in combination with their hosts, are largely lacking.

f. Disease management

There is good information on the efficacy of fungicides against downy mildew pathogen. Efforts should continue to search for low cost effective chemicals which can provide economically significant disease control. The possibility of biocontrol agents need to be explored. Study of integrated disease control strategies may be very useful. However, integration of all the means of control needs to be done for each crop and for each geographical region.

15. REFERENCES

Achar, P.N. 1992a. Dose-response relationship of *Peronospora parasitica* in *Raphanus sativus*. Phyton (Buenos Aires) 53: 89-94.

Achar, P.N. 1992b. Yield loss by *Raphanus sativus* from systemic infection by *Peronospora parasitica*. Phyton (Buenos Aires) 53: 89-94.

Altman, J. 1958. Studies on the control of plant diseases with antibiotics with particular reference to streptomycin. Dis. Abstr. 19: 201.

Anonymous. 1938. Plant diseases. Notes contributed by the Biological Branch. Agric. Gaz., N.S.W. 17: 669-673.

Anonymous. 1949. [Plant diseases in Denmark in 1949. Annual survey of data collected by the State Phytopathological Service, Lyngby.] Tidsskr. Planteavl. 55: 1-81.

Anonymous. 1953. Annual Report of the Agricultural Experiment Stations, Florida, for the year ending June 30, 1952: 310 p.

Anonymous. 1955. Plant disease survey for the twelve months ending 30th June 1954. Twenty-fourth Annual Report N.S.W., Dept. Agric., Biological Branch-Division of Science Services: p. 27.

Anonymous. 1957. Annual Report of the Senior Research Officer, Plant Pathology, 1955. Annual Report, Dept. Agric., Kenya, 1955. 2: p.15.

Anonymous. 1960a. Index of plant diseases in the United States. USDA Agric. Handbook No. 165: pp. 97-106.

Anonymous. 1960b. Plant disease survey for the twelve months ending 30th June 1959. Twenty-ninth Annual Report N.S.W. Dept. Agric., Biological Branch - Division of Science Services: pp. 42-43.

Anonymous. 1962. Quarterly report for July-September, 1962 of the Plant Protection Committee for the South East Asia and Pacific region. F.A.O. Publ., Bangkok, Thailand: 19 p.

Anonymous. 1964. New or uncommon plant diseases and pests. Plant Path. 13 (1): 45-46.

Anonymous. 1969. Plant Pathology. Report Dept. Agric. Fiji. 1968: pp 29-30

Anonymous. 1972a. Plant pests of importance to the Caribbean. F.A.O. Caribban Pl. Prot. Commission: pp. 29.

Anonymous. 1972b. A list of plant diseases, insect pests, and weeds in Korea. Korean Soc. Pl. Prot: pp 424.

Anonymous. 1974. Plant Pathol. Annual Report for 1974. ADAS Science Service, MAFF: 180.

Anonymous. 1983. Diseases of vegetables. Research Report 1982. Horticulture. Dublin, Irish Republic. An. Foras Taluntais: pp. 44-45.

Anonymous. 1985. Diseases of oilseed rape. Rothamested Experimental Station Report, 1984. Harpenden, Herts, U.K.: pp. 124-125.

Anonymous. 1987a. *In vitro* studies in downy mildew resistance in Chinese cabbage. Progress report 1985. Asian Vegetable Research and Development Centre, Shanhua, Taiwan: pp. 39-42.

Anonymous. 1987b. Resistance of Chinese cabbage hybrids to downy mildew under field conditons. Progress Report 1985. Asian Vegetable Research and Development Centre. Shanhua, Taiwan: pp. 37-39.

Apandi, E.S. (1980). [*Peronospora parasitica* control on cabbage seedlings (*Brassica oleracea* L.) In the nursery.] Bulletin Penlitian Horticultura. 8: 3-5.

Ark, P.A. and **J.P. Thompson**. 1959. Control of certain diseases of plants with antibiotics from garlic (*Allium sativum*L.). Plant Dis. Reptr. 43: 276-282.

Asada, Y. and **I. Matsumoto.** 1969. Formation of lignin-like substance in the root tissue of Japanese radish plant infected by downy mildew fungus. Ann. Phytopathol. Soc. Japan 35: 160-167.

Asada, Y. and **I. Matsumoto.** 1971. Microspectrophotometric observations on the cell walls of Japanese radish (*Raphanus sativus*) root infected by *Peronospora parasitica*. Physiol. Plant Pathol. 1: 377-383.

Asada, Y. and **I. Matsumoto.** 1972. The nature of lignin obtained from downy mildew-infected Japanese radish root. Phytopathol. Z. 73: 208-214.

Asada, Y. and **T. Ohguchi.** 1981. Behaviour of downy mildew fungus of Japanese radish on modified knop's medium. Ann. Phytopathol. Soc. Japan 47: 71-74.

Asada, Y., T. Ohguchi and **I. Matsumoto.** 1975. Lignin formation in fungus-infected plants. Review Plant Prot. Res. 8: 104-113.

Awasthi, R.P., N.I. Nashaat and **S.J. Kolte**. 1995. Interaction between *Peronospora parasitica* and *Albugo candida* in relation to development of stagheads in *Brassica juncea*. National Symposium on Detection of Plant Pathogens and their Management, Faizabad, India, January 18-20, 1995: p. 36.

Awasthi, R.P., N. Nashaat, A. Heran, S. Kolte and **U. Singh**. 1997. The effect of *Albugo candida* on the resistance to *Peronospora parasitica* and vice versa in rapeseed-mustard. ISHS Symposium on Brassicas, Tenth Crucifer Genetics Workshop, September 23-27, 1997, Rennes, France.

Baggett, J.R. and **D. Kean.** 1985. Clubroot resistant broccoli breeding lines OSU CR-2 to OSC CR-8. Hort. Sci. 20: 784-785.

Bains, S.S. 1989. Relationship between detoppings and mixed infections of *Albugo candida* and *Peronospora parasitica* on inflorescences of *Brassica* species. Plant Dis. Res. 4: 148-150.

Bains, S.S. and **J.S. Jhooty.** 1978. Increased susceptibility of virus-infected *Brassica juncea* to *Peronspora parasitica*. Plant Dis. Reptr. 62: 1043-1046.

Bains, S.S. and **J.S. Jhooty.** 1979. Mixed infections by *Albugo candida* and *Peronospora parasitica* on *Brassica juncea* inflorescence and their control. Indian Phytopathol. 32: 268-271.

Bains, S.S. and **J.S. Jhooty.** 1983. Host range and morphology of *Peronospora parasitica* from different sources. Indian J. Mycol. Plant Pathol. 13: 372-375.

Bains, S.S. and **J.S. Jhooty.** 1985. Association of *Peronospora parasitica* with *Albugo candida* on *Brassica juncea* leaves. Phytopathol. Z. 112: 28-31.

Bains, S.S. and **S.S. Sokhi.** 1986. Increased levels of mixed infections by *Albugo candida* and *Peronospora parasitica* on *Brassica juncea* inflorescence of de-topped plants. Plant Dis. Res. 1: 55-58.

Bains, S.S., S.S. Sokhi and **J.S. Jhooty.** 1981. Outbreak of *Peronospora parasitica* on cauliflower curds in Punjab. Indian Phytopathol. 34: 389-390.

Bedlan, G. 1987. [The most important diseases of radishes.] Pflanzenschutz. (Wien) 3: 9-10.

Bedlan, G. 1989. [Diseases of Chinese cabbage and their control.] Pflanzenschutz (Wien) No. 3: 2-3.

Baudys, E. 1928. [Phytopathological notes IV]. Ochrana Rostlin, viii, 6: 151-162.

Bertus, A.L. 1968. Downy mildew of stocks. Agric. Gaz. N.S.W. 79: 178-179.

Bhatt, D.D. 1966. J. Sci. Tri-Chandra Coll. Sci. Assoc. 2: 13-20.

Bjorling, K. 1944. [Some fungus diseases of colza and white mustard.] Vaxtskyddsnotiser vaxtskyddsanst. Stockh. 7: 73-77.

Boning, K. 1936. [Investigations on horseradish disease and their control] Angew Bot. 18: 482-494.

Bonnet, A. and **D. Blancard.** 1987. Resistance of radish (*Raphanus sativa* L.) to downy mildew, *Peronospora parasitica*. Cruciferae NewsLetter No. 12: 98-99).

Borders, H.I. 1953. Preliminary report on fungicides tests for control of downy mildew of cabbage seedlings in South Georgia. Plant Dis. Reptr. 37: 363-364.

Borg, A. 1952. [Some further parasitic attacks on the oil camelina.] Vaxtskyddsnotiser, Stockh.: pp. 20-23.

Brophy, T.F. and **M.D. Laing**. 1992. Screening of fungicides for the control of downy mildew on container-grown cabbage seedlings. Crop Prot. 11: 160-164.

Butler, E.J. 1918. Fungi and diseases in plant. Thaker Spink & Co., Calcutta, India: pp 297-300.

Butler, E.J. and **S.G. Jones.** 1949. Plant Pathology, McMillan, London.

Buzza, G.C. 1995. Plant Breeding. IN: Brassica oilseeds. D.S. Kimber and D.I. McGregor (Eds.). Commonwealth Agricultural Bureau International: pp. 153-175.

Caravalho, T., and **A. Monteiro.** 1996. Preliminary study on the inheritance of resistance to downy mildew (*Peronospora parasitica* (Pers. Ex. Fr.) at cotyledon stage in Tranchuda cabbage "Algarvia". Curciferae NewsLetter. 18:104.

Chandrasrikul, A. 1962. Tech. Bull. Dept. Agric. Bangkok. 6:23 p.

Chang, I.H., N.L. Shih and **W.F. Chiu.** 1964. [A preliminary study on the physiological differentiation of the downy mildews (*Peronospora parasitica* (Pers.) Fr.) of Chinese cabbage and other cruciferous vegetables in the vicinity of Peking and Tientsin.] Acta Phytopathol. Sinica 7: 33-44.

Chang, I.H., R.F. Xu and **W.F. Chiu.** 1963. [On the primary sources of infection of the downy mildew of Chinese cabbage caused by *Peronospora parasitica* (Pers.) Fr. and the limited systemic infection of seedlings.] Acta Phytopathol. Sinica 6: 153-162.

Chang, Z.M. 1981. Evaluation of rape downy mildew and white rust. Fukien Agric. Sci. Technol. No. 5: 8-10.

Channon, A.G. 1981. Downy mildew of *Brassicas*. IN: The downey mildews, D.M. Spencer (ed.). Academic Press, London: pp. 321-339.

Channon, A.G. and **R.J. Hampson**. 1968. Laboratory tests of fungicides against *Peronospora parasitica* (Pers. Ex Fr.) Fr. Ann. Appl. Biol. 62: 23-33.

Channon, A.G., R.J. Hampson, M. Gibson and **M.K. Turner**. 1970. Field tests of dichlofluanid against *Brassica* downy mildew (*Peronospora parasitica*). Plant Pathol. 19: 151-155.

Chaudhury, R.G. and **R.N. Verma.** 1987. Preferential pathogenesis of white rust pustules by *Peronospora parasitica* in mustard. Indian J. Hill Farm. 1: 29-34.

Chauhan, L.S. and **A. Muheet**. 1976. Control of downy mildew of rapeseeds and mustard. Pesticides 10: 38-39.

Chaurasia, S.N.P., U.P. Singh and **H.B. Singh.** 1982. Preferential parasitism of *Peronospora parasitica* on galls of *Brassica campestris* caused by *Albugo cruciferarum*. Tans. Br. Mycol. Soc. 78: 379-381.

Chiu, W.F. 1959. On problems of three destructive diseases of the Chinese cabbage. Bull. Agric. Sci. 9: 314-315.

Chorin, M. 1946. Downy mildew on cauliflower curds. Palest. J. Bot. Res. Ser. 2: 258-259.

Chou, C.K. 1970. An electron-microscrope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* (Fr.) Tul. on cabbage cotyledons. Ann. Bot. 34: 189-204.

Chu, H.T. 1935. [Notes on the penetration phenomena and haustorium formationof *Peronospora brassicae* Gaum.] Ann. Phytopathol. Soc. Japan 2: 150-157.

Ciferri, R. 1953. [Five years of experiments with fungicides with a zinc ethylene bisdithiocarbamate base.] Notiz, Malatt. Piante 23 N.S.: 3-19.

Ciferri, R. 1961. Riv. Patol. Veg. Pavia Ser., 3; 1: 333-348.

Connors, I.L. 1967. An annotated index of plant diseases in Canada and fungi recorded on plants in Alaska, Canada and Greenland. Res. Branch Can. Dept. of Agric. Pub. 1251: 3818.

Conroy, R.J. 1960. Control cauliflower diseases. Agric. Gaz. N.S.W. 71: 462-468.

Constantinescu, O. 1989. *Peronospora* complex on compositae. Sydowia 41: 79-107.

Cook, R.J. and **E.J. Evans.** 1978. Buildup of diseases with intensification of oilseed rape in England. Proc. 5th Intern. Rapeseed Congr. Malmö, Sweden 1: 333-337.

Corda, A.C.J. 1837. Icones fungorum hucusuque cognitorum. 1:20.

Crute, I.R. 1984. Downy mildew of *Brassicas*. 35th Annual Report 1983. National Vegetable Research Station, Wellesbourne, Warwick, U.K.: p. 76.

Crute, I.R., J.M. Norwood and **P.L. Gordon**. 1985. Resistance to phenylamide fungicides in lettuce and *brassica* downy mildew. Proc. Bordeaux Mixture Centenary Meeting, September 5-7, 1985: pp. 311-314. Brit. Crop Prot. Council Monograph. No. 31.

Crute, I.R. and **P.L. Gordon.** 1986. Downy mildew of brassicas. Annual Report. 1986. National Vegetable Research Station, Wellesbourne, Warwick, U.K. pp. 54-55.

Da, Costa, M.E.A.P. and **E. De S. Da Camara.** 1953. [Some Portuguese mycological species II.] Protug. Acta Biol. 4: 162-176.

Darpoux, H. 1945. [A contribution to the study of the disease of Oleaginous plants in France.] Ann. Epiphyt. N.S. 11: 71-103.

Davies, J.M.L. and **J.D. Wafford.** 1987. Control of downy mildew in module-raised cauliflowers. Monograph - Brit. Crop Prot. Council No. 39: 379-386.

Davis, J.J. 1929. Notes on parasitic fungi in Wisconsin 15, 16, 17. Trans. Wisconsin Acad. Sci. Arts and Lett. 24: 269-302.

Davis, K.R. and **R. Hammerschmidt.** 1993. *Arabidopsis thaliana* as a model for plant pathogen interactions. APS Press, St. Paul, U.S.A.

Davison, E.M. 1967. Some aspects of the biology of *Peronospora parasticia* and related organisms. Ph. D. Thesis, Univ. Of Bristol, England.

Davison, E.M. 1968a. Cytochemistry and ultrastructure of hypae and haustoria of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 613-621.

Davison, E.M. 1968b. Development of sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 623-631.

Davison, E.M. 1968c. The distribution of substances in sporangiophores of *Peronospora parasitica* (Pers. ex Fr.) Fr. Ann. Bot. 32: 633-647.

De Bruyn, H.L.G. 1935a. [The influence of manure on attack of cabbage by *Peronospora parasitica*.] Tijdschr. Plantenziekten 41: 57:64.

De Bruyn, H.L.G. 1935b. Heterothallism in *Peronospora parasitica*. Phytopathology 25:8 (abstr.).

De Bruyn, H.L.G. 1937. Heterothallism in *Peronospora parasitica* - Genetica 19: 553-558.

De Carvalho, T. 1948. [Preliminary report on the diseases observed in plants and insects with phytopathological annotations.] Colonia de Mocambique, Reparticao de Agricultura, Seccao de Micologia: 84 p.

D'Ercole, N. 1975. [*Peronospora* disease of cauliflower in north central Italy.] Informatore Fitopatol. 25: 21-23.

Dias, M.R. de S. and **E. De S. Da Camara.** 1953. Agronomia Lusit. 15:17-37.

Dickinson, C.H. and **J.R. Greenhalgh.** 1977. Host range and taxonomy of *Peronospora* on crucifers. Trans. Br. Mycol. Soc. 69: 111-116.

Dickson, M.H. and **R. Petzoldt.** 1996. Seedling of downy mildew resistance in *B. oleracea*. Cruciferae NewsLetter, 18:108.

Dippenaar, B.J. 1934. Downy mildew of cabbage and related plants. Farming in South Africa 9: 279-280.

Dixon, G.R. 1975. The reaction of some oil rape cultiars to some fungal pathogens. Proc. 8th British Insecticide and Fungicide Confr. 2: 503-506.

Doidge, E., A.M. Bottomley, J.E. Vander Plank and **G. D. Pauer.** 1953. A revised list of plant disease in South Africa. Science Bulletin No. 346 (Botany and Plant Pathology series No. 16): p. 19.

Doughty, K.J., R.N. Bennett, N.I. Nashaat, S. Schrijvers, G. Kiddle, B.J. Pye, S.E. Mitchell and **R.M. Wallsgrove**. 1995. The response of oilseed rape (*Brassica napus* L.) seedlings to *Peronospora parasitica* and *Alternaria brassicae* following treatment with salicylic acid or methyl jasmonate. Proc. 9th International Rapeseed Congress, Cambride, July 4-7, 1995, 3:971-973.

Downey, R.K. and **J.L. Bolton.** 1961. Production of rape in western Canada. Publ. Dept. Agric. Canada No. 1021: 19 p.

Dzhanuzakov, A. 1963. [Specialization and variability in some Peronosporaceous fungi.] Bot. Zh. U.S.S.R. 47: 862-867.

Ebrahimi, A.G., P.A. Delwiche and **P.H. Williams.** 1976. Resistance in *Brassica juncea* to *Peronospora parasitica* and *Albugo candida*, Race 2. Proc. Amer. Phytopathology Soc. 3: 273 (abstr.).

Eddins, A.H. 1943. Control downy mildew of cabbage with Spergon and Fermate. Florida Agric. Exp. Stn. Press Bull. 589: 4 p.

Ehrlich, M.A. and **H.G. Ehrlich.** 1966. Ultrastructure of the hyphae and haustoria of *Phytophthora infestans* and hyphae of *Peronospora parasitica*. Can. J. Bot. 44: 1495-1504.

Elarosi, H. and **M.W. Assawah.** 1959. Downy and powdery mildews of certain ornamental plants in Alexandria region. Anexandria J. Agric. Res. 7: 253-268.

Elenkov, E. 1979. [Breeding disease resistant varieties of vegetable crops.] Rastitelna Zashchita 27: 13-16.

Epps, W.M. 1955. Fungicides for downy mildew control on heading cabbage. Plant Dis. Reptr. 39: 89-90.

Etebarian, H.R. 1989. [Studies on downy mildew of radish and its chemical control.] Iranian Agric. Sci. 20: 23-31.

Farlow, W.G. 1883. Enumeration of the Peronosporeae of the United States. Bot. Gaz. 8: 305-315, 327-337.

Farlow, W.G. 1884. Addition to the Peronosporeae of the United States. Bot. Gaz. 9: 37-40.

Felton, M.W. and **J.C. Walker**. 1946. Environal factors affecting downy mildew of cabbage. J. Agric. Res. 72: 69-81.

Fernandez, R.M. 1973. Serie Agricola Academia de ciencias de Cuba, Instituto de Investigaciones Tropicoles. No. 27: 78 p.

Firman, I.D. 1975. Information Document South Pacific Commission, No. 38: 7+3 p.

Foister, C.E. 1948. Report of the Annual Conference of the Crytogamic section, 1947. Trans. Bot. Soc. Edingurgh 35: 103-107.

Foster, H.H. 1947a. Reaction of species and varieties of cruciferae to artificial inoculation of cabbage downy mildew. Phytopathology 37: 433 (abstr.).

Foster, H.H. 1947b. The control of cabbage downy mildew through the use of sprays. Phytopathology 37: 712-720.

Fraymouth, J. 1956. Haustoria of the Peronsporales. Trans. Br. Mycol. Soc. 39: 79-107.

Gabrielson, R.L. and **L.W. Getzin.** 1979. Systemic pesticides for control of downy mildew and insects on cabbage transplants grown for seed production. Plant Dis. Reptr. 63: 131-135.

Gardner, M.W. 1920. *Peronospora* in turnip roots. Phytopathology 10: 321-323.

Gaumann, E. 1918. Uber die Formen der *Peronospora parasitica* Ein Beitrag Zur Spezies frage bei parasitischen Pilzen. Beih Bot. Zentralblatt. 35: 395-533.

Gaumann, E. 1923. [Contributions toward a monograph of the genus *Peronospora corda*.] Beitrage zur kryptogamenflora de schweiz 5: 360 p.

Gaumann, E. 1926. [On the specialization of downy mildew (*Peronospora brassicae* Gaum.) on cabbage and related species.] Landw. Jahrb. der schewiz 40: 463-468.

Glaeser, G. 1970. [The occurrence of important causes of injury to cultivated plants in Austria in 1969.] Pflanzenschutzberichte 41: 49-62.

Gonzalez, F.R. 1924. [*Peronosporaceae* at present known to occur in the Iberian flora.] Bol. R. Soc. Espan. Hist. Nat. 24: 305-312.

Gram, E. and **S. Rostrup.** 1924. [Survey of diseases of agricultural and horticultural cultivated plants in 1923.] Tidsskr. for Planteavl. 30:361-414.

Gram, E. and **A. Weber.** 1952. Plant diseases in orchard, nursery and garden crops. R.W.G. Dennis, (ed.), MacDonald & Co. Ltd., London.

Greenhalgh, J.R. and **C.H. Dickinson.** 1975. Differential reactions of three crucifers to infection by *Peronospora parasitica* Pers. Ex Fr.) Fr. Phytopathol. Z. 84: 131-141.

Greenhalgh, J.R. and **N.D. Mitchell.** 1976. The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivated forms of *Brassica oleracea*. New Phytol. 77: 391-398.

Gupta, D.K. and **K.C.B. Chaudhary.** 1987. *Brassica oleracea* var. *capitata* - a new host of *Peronospora parasitica* (Pers.) DeBary. Intern. J. Trop. Plant Dis. 5: 219-220.

Guttenberg, H. Von and **H. Schmoller.** 1958. [Culture tests with *Peronospora brassicae*). Arch. Mikrobiol. 30: 268-279.

Hammarlund, C. 1931. [Shorter mycological notices II. A giant form of *Peronospora brassicae* Gaumann (=*P. parasitica* (Fries) Tulasne) on *Raphanus sativus* f. *radicula*.] Botaniska Notiser 5: 392-393.

Harter, L.L. and **L.R. Zones.** 1923. Cabbage disease. U.S. Dept. Agric. Farmer's Bull. No. 1351: 28 p.

Hartman, H., J.C. Sutton and **R. Proctor.** 1983. Effects of atmospheric water potentials, free water and temperature on production and germination of sporangia in *Peronospora parasitica*. Can. J. Plant Pathol. 5: 70-74.

Hill, C.B., Crute, I.R., C. Sherrife and **P.H. Williams.** 1988. Specificity of *Albugo candida* and *Peronospora parasitica* pathotypes towards rapid-cycling *Brassicas*. Cruciferae NewsLetter No. 13: 112-113.

Hiura, M. and **H. Kanegae.** 1934. Studies on the downy mildews of cruciferous vegetables in Japan. I. Trans. Sapporo Nat. Hist. Soc. 13: 125-133.

Holliday, P. 1980. Fungus diseases of tropical crops. Cambridge Univ. Press, London: pp. 305-306.

Holub, E.B., J.L. Beynon and **I.R. Crute.** 1994. Phenotypic and genotypic characterization of interactions between isolates of *Peronospora parasitica* and accessions of *Arabidopsis thaliana*. Mol. Plant Microb. Interactions.

Hoser-Krauze, J., E. Lakowska-Ryk and **J. Antosik**. 1991. Resistance to some *Brassica oleracea* L. plant introductions to downy mildew (*Peronospora parasitica*). Cruciferae NewsLetter No. 14/15: 144-145.

Hoser-Krauze, J., E. Lakowska-Ryk and **J. Antosik**. 1995. The inheritance of broccoli (*Brassica oleracea botrytis*L) leaf resistance to downy mildew - *Peronospora parasitica* (Pers. Ex. Fr.). J. Appl. Genet. 36: 27-33.

Ingram, D.S. 1969. The susceptibility of *Brassica* callus to infection by *Peronospora parasitica*. J. Gen. Microbiol. 58: 391-401.

Jafar, H. 1963. Studies on downy mildew (*Peronospora mattiolae*) Roumequere, Gaumann) on stocks (*Matthiola incana* R. Br.). New Zealand J. Agric. Res. 6: 70-82.

Jang. P. and **K.M. Safeeulla.** 1990a. Factors affecting germination of *Peronospora parasitica* in radish. Proc. Indian Acad. Sci. Plant Sci. 100: 113-116.

Jang, P. and **K.M. Safeeulla.** 1990b. Production and viability of *Peronospora parasitica* in radish. Proc. Indian Acad. Sci., Plant Sci. 100: 117-122.

Jang. P. and **K.M. Safeeulla.** 1990c. Seed-borne nature of *Peronospora parasitica* in *Raphanus sativus*. Proc. Indian Acad. Sci., Plant Sci. 100: 225-258.

Jang, P. and **K.M. Safeeulla.** 1990d. Modes of entry, establishment and seed transmission of *Peronospora parasitica* in radish. Proc. Indian Acad. Sci., Plant Sci. 100: 369-373.

Jaworoski, C.A., S.C. Phatak and **A. Csinos.** 1982. Effect of metalaxyl on Phycomyceteous fungi and yield of pepper, tomato and cabbage transplants. J. Amer. Soc. Hort. Sci. 107: 911-913.

Jenkins, J.E.E. 1963. Downy mildew of cauliflower curds. Plant Pathol. 12-13: 46.

Jenkins, J.E.E. 1964. New or uncommon plant diseases and pests. Plant Pathol. 13: 45-46.

Jenkyn, J.F., J.M. Hirst, and **G. King.** 1973. An apparatus for the isolated propagation of foliar pathogens and their hosts. Ann. app. Biol. 73: 9-13.

Johnston, A. 1963. A preliminary plant disease survey in Hong Kong. Plant Prod. and Prot. Div. FAO, Rome: 32 p.

Johnston, S.A. and **J.K. Springer.** 1977. Fungicide evaluation for downy mildew of broccoli, 1976. Fungicide Nemticide Tests 32: 76.

Jones, W. 1944. Downy mildew disease of cauliflower seed plants. Sci. Agric. 24: 282-284.

Jonsson, R. 1966. [*Peronospora* on oil yielding *Brassicas*. Methods for testing resistance in winter rape and their results.] Sver. Utsädestör Tidskr. 76: 54-62.

Kadow, K.J. and **H.W. Anderson** 1940. A study of horseradish diseases and their control. Illinois Agric. Expt. Stn. Bull. 469: 531-583.

Karwasra, S.S. and **G. S. Saharan.** 1982. Two new diseases of Chinese cabbage (*Brassica pekinensis* L.). Cruciferae NewsLetter No. 7: 58-59.

Keyworth, W.G. 1967 (1968). Plant Pathology. Report National Vegetable Research Station, Wellesbourne, Warwick, U.K. 18: 75-83.

Khristov, A. 1979. [Diseases of turnip in Bulgaria.] Rastitelna Zaschita. 27: 27-28.

Klemm, M. 1938. [The most important diseases and pests of colza and rape.] Dtsch. Landw, Pr., 75, 19: 239; 20: 251-252.

Kluczewski, S.M. and **J.A. Lucas.** 1982. Development and physiology of infection by the downy mildew fungus *Peronospora parasitica* (Pers. ex Fr.) Fr. in susceptible and resistant *Brassica* species. Plant Pathol. 31: 373-389.

Kluczewski, S.M. and **J.A. Lucas.** 1983. Host infection and oospore formation by *Peronospora* parasitica in agricultural and horticultural *Brassica* species. Trans. Brit. Mycol. Soc. 81: 591-596.

Knight, C. and **M.J. Furber.** 1980. Winter oilseed rape disease variety reaction. Nat. Inst. Agric. Bot. 15: 276-280.

Kobel, F. 1921. [The problem of host selection by parasitic fungi.] Naturw. Wochenschr. 36: 113-118.

Koch de Brotus, L. and **C. Boasse.** 1955. Publ. Minist. Gonad. Agric. Montevideo, 106: 65 p.

Koch, E. and **A. Slusarenko.** 1990. *Arabidopsis* is susceptible to infection by a downy mildew fungus. Plant Cell 2: 437-445.

Kolte, S.J. 1985. Diseases of annual edible oilseed crops Vol II. Rapeseed-mustard and sesame diseases. CRC Press, Boca Raton, Florida, USA.

Kolte, S.J., R.P. Awasthi and **Vishwanath.** 1986. Effect of planting dates and associated weather factors on staghead phase of white rust and downy mildew of rapeseed and mustard. Inidan J. Mycol. Plant Pathol. 16: 94-102.

Kolte, S.J. and **A.N. Tewari.** 1980. Note on the susceptibility of certain Oleiferous *Brassicae* to downy mildew and white blister diseases: Indian J. Mycol. Plant Pathol. 10: 191-192.

Kontaxis, D.G., K.S. Mayberry and **V.E. Rubatzky.** 1979. Reaction of cauliflower cultivars to downy mildew in Imperial Valley. California Agric. 33: 19.

Krober, H. 1969. [Degree of infestation of cabbage turnip by *Peronospora parasitica* (Pers.) Fr. and of tobacco by *P. tabacina* Adam as related to concentration of conidia.] Phytopathol. Z. 66:180-187.

Krober, H. 1970. [Longevity of conidia of several *Peronospora* species under different environmental conditions.] Phytopathol. Z. 69: 64-70.

Krober, H. 1981. [Germination and longevity of conidia of some Peronosporaceae kept under different conditions.] Zeitschrift für Pflanzenkrankheiten Und Pflanzenschutz 88: 510-517.

Kruger, W. 1991. A review on assessment of diseases in oilseed rape - comparison of various methods. I0BC/WPRS Bull. 14: 91-111.

Kuhnholtz, L.M. and **J.M. Gastuad.** 1943. [Plant Pathological Notes.] Ann. Epiphyt. N.S. 9: 207-219.

Kupryanova, Mme. V.K. 1957. [Measures for the control of cabbage diseases.] Plant Prot. Moscow 5: 28-29.

Laemmlen, F.F. and **K.S. Mayberry.** 1984. Broccoli resistance to downy mildew. California Agric. 38: 17.

Langdon, R.F.N. 1948. Records of Queensland fungi v. Pap. Dept. Biol., Univ. Qd, 2: 9 pp.

Leather, R.I. 1967. A catalogue of some plant diseases and fungi in Jamaica. Bull. Minist. Agric. Lands Jamaica 61: 92 p.

LeBeau, F.J. 1945. Systemic invasion of cabbage seedlings by the downy mildew fungus. J. Agric. Res. 71: 453-463.

Lebeda, A. and **F.J. Schwinn.** 1994. The downy mildews - an overview of recent research progress. J. Pl. Dis. & Prot. 101: 225-254.

Lehoczky, J. 1957. [The occurrence of some economically significant parasitic micro-fungi. 1.] Kertesz. Szolesz. (Ann. Acad. Hort. Vitic. Budapest) 21: 3-14.

Leung, H. and **P.W. Williams.** 1983. Cytoplasmic male sterile *Brassica campestris* breeding lines with resistance to club root, turnip mosaic and downy mildew. Hort. Sci. 18: 774-775.

Lin, C.Y. 1981. Studies on downy mildew of Chinese cabbage caused by *Peronospora parasitica*. IN: Chinese cabbage. Proc. First Intern. Symp. Asian Vegetable Research Development Center, Shanhua, Taiwan: pp. 105-112.

Lin, C.Y. and **T. C. Liang.** 1974. [The discharge of conidia of *Peronospora brassicae* from kohlrabi and the polyethylene cover as a protection to seedlings against infection of this pathogen.] Bull. Agric. Res. 3: 41-48.

Lindquist, J.C. 1946. [Argentine species of the genus *Peronospora*.] Physis, B. Aires, 15: 13-20.

Lo, T.T. 1961. Plant Ind. Ser. Chin-AM Jt. Comm. Rur. Reconstr. 23: 52 p.

Lucas, J.A., J.B.R. Hayter and **I.R. Crute**. 1994. The downy mildews: host specificity and pathogensis. IN: pathogensis and host specificity in plant diseases (Ed. K. Kohmoto). Elsevier Science Ltd. Oxford. Vol. 2.

Lucas, J.A., I.R. Crute, C. Sherriff and **P.L. Gordon**. 1988. The identification of a gene for race-specific resistance to *Peronospora parasitica* (downy mildew) in *Brassica napus* var. *oleifera* (oilseed rape). Plant Pathol. 37: 538-545.

Lucas, J.A. and **C. Sherriff.** 1988. Pathogenesis and host specificity in downy mildew fungi. Experimental and Conceptual Plant Pathology. R.S. Singh, U.S. Singh, W.M. Hess and D.J. Weber. (Eds.) Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi, India.

Macek, J. 1961. [Contribution to the study of the family Peronosporaceae in Yugoslavia.] Zast. Bilja (Plant Protection Beograd): pp. 65-66; 101-104.

Masheva, S., G. Antonova and **D. Bahariev.** 1996a. Seasonal variability in the *Peronospora parasitica* (Pers.) Fr. population. Cruciferae NewsLetter. 18: 120.

Masheva, S., G. Antonova and **D. Bahariev.** 1996b. Pathogenicity of two isolates *Peronospora parasitica* with different district origin. Cruciferae NewsLetter. 18:118.

Matsumoto, I. and **Y. Asada.** 1984. [A role of lignification-inducing factor in resistance induction demonstrated in Japanese radish downy mildew and cucumber anthracnose.] ann. Phytopathol. Soc. Japan 50: 483-490.

Matsumoto, I., T. Ohguchi, M. Inoue and **Y. Asada.** 1978. Lignin induction in roots of Japanese radish by an homogenate of downy mildew-infected root tissue. Ann. Phytopathol. Soc. Japan 44: 22-27.

McGuire, J.V. and **B.S. Crandall.** 1967. USDA Intern. Agric. Develop. Service: 157 p.

McIntosh, A.E.S. 1951. Vegetable diseases. Annual report of the department of agriculture, Malaya, for the year 1949: p. 55.

McKay, A.G., R.M. Floyd and **C.J. Boyd.** 1992. Phosphonic acid controls downy mildew (*Peronosopora parasitica*) in cauliflower curds. Aust. J. Expt. Agric. 32: 127-129.

McKee, R.K. 1971. Plant Pathology Research Division - Report Res. & Tech. Work Minist. Agric. N. Ireland 1970: pp 105-113.

McMeekin, D. 1960. The role of the oospores of *Peronospora parasitica* from cabbage and radish. Phytopathology 50: 93-97.

McMeekin, D. 1969. Other hosts for *Peronospora parasitica* from cabbage and radish. Phytpathology 59: 693-696.

McMeekin, D. 1971. Measuring systemic infection of crucifers by downy mildew. Plant Dis. Reptr. 55: 877-878.

McMeekin, D. 1981. The response of *Peronospora parasitica* to a liver medium. Mycologia 73: 551-554.

Mehta, N. 1993. Epidemiology of white rust and downy mildew disease complex in mustard and residual toxicity of fungitoxicant. Ph.D. Thesis, C.C.S. Haryana Agricultural University, Hisar, Haryana, India: 154 p.

Mehta, N. and **G.S. Saharan.** 1994. Morphological and pathological variations in *Peronospora parasitica* infecting *Brassica* species. Indian Phytopath. 47: 153-158.

Mehta, N., G.S. Saharan and **C.D. Kaushik.** 1996. Efficacy and economics of fungicidal management of white rust and downy mildew complex in rape-mustard. Indian J. Mycol. Pl. Pathol. 26: 243-247.

Mehta, N., G.S. Saharan and **O.P. Sharma.** 1995. Influence of temperature and free moisture on the infection and development of downy mildew on mustard. Pl. Dis. Research 10: 114-121.

Mehta, N., G.S. Saharan and **S. Babber.** 1995. Sequence of events in the pathogenesis of *Peronospora* and Albugo on mustard. J. Indian Bot. Soc. 74: 299-303.

Moore, W.C. 1959. British parasitic fungi. Cambridge University Press: pp. 9, 236.

Morris, M.J. and **P.S. Knox-Davies.** 1980. *Raphanus raphanistrum* as a weed host of pathogens of cultivated cruciferae in the western cape province of South Africa. Phytophylactica 12: 53-55.

Moss, N.A., I. R. Crute and **J.A. Lucas.** 1994. Laboratory production of oospores of *Peronospora parasitica* (crucifer downy mildew) and the recovery and characterisation of sexual progeny from crosses between isolates with different host specificity. Plant Pathology 43:713-725.

Moss, N.A., J.A. Lucas and **I. R. Crute.** 1991. Evidence for differential response to isolates of *Peronospora parasitica* (downy mildew) in *Brassica rapa.* Test AgroChem. cv. 12. Ann. appl. Biol. 118: 96-97 (supplement).

Moss, N.A., I.R. Crute, J.A. Lucas and **P.L. Gordon.** 1988. Requirements for analysis of host species specificity in *Peronospora parasitica* (downy mildew). Cruciferae NewsLetter No. 13: 114-116.

Muir, J.C. 1948. Trinidad and Tobago Administration Report of the Director of Agriculture for the year 1946: 28 p.

Mujica, F. and **C. Vergara.** 1960. Balm tec. No. 6. Dipt. Invest. Agric. Chili. 6:60 p.

Muller, A.S. 1950. A preliminary survey of plant diseases in Guatemala. Plant Dis. Reptr. 34: 161.

My, H.T. 1966. A preliminary list of plant diseases in south Vietnam. Directorate of Research, Saigon. P. 142.

Nakov, B. 1968. [Chemical means for the control of *Peronospora parasitica* on cabbage.] Rastit. Zasht. 16: 25-30.

Nakov, B. 1972. [The effect of ecological factors on the dissemination of *Peronospora parasitica* (Pers.) Fr. on cabbage.] Nauch. Trudov. Vissh. Selskostop. Inst. V. Kolarov; Plovdiv. 21: 109-116.

Nashaat, N.I. 1997. Downy mildew. IN: Compendium of Brassica Crop Diseases. (Ed. S.R. Rimmer) American Phytopathological Society (In Press).

Nashaat, N.I.and **R.P. Awasthi.** 1995. Evidence for differential resistance to *Peronospora parasitica* (downy mildew) in accessions of *Brassica juncea* (mustard) at the cotyledon stage. J. Phytopathology 143:157-159.

Nashaat, N.I., A. Heran and **S. Mitchell**. 1996. Resistance to downy mildew and its interaction with white rust in rapeseed-mustard. 2nd International Crop Science Congress, New Delhi, India, November 17-24, 1996: p. 199.

Nashaat, N.I., S. Mitchell and **R.P. Awasthi.** 1995a. Genetic analysis of a noval factor for resistance to *Peronospora parasitica* in *Brassica napus* spp. *Oleifera*. Indian J. Mycol. Pl. Pathol - 25:55.

Nashaat, N.I., S.E. Mitchell and **R.P. Awasthi**. 1995b. Inheritance of two new factors for resistance to downy mildew *(Peronospora parasitica)* in oilseed rape *(Brassica napus* spp. *oleifera)*. Proc. 9th International Congress, Cambridge, July 4-7, 1995, 4:1283-1285.

Nashaat, N.I. and **C.J. Rawlinson.** 1994. The response of oilseed rape (*Brassica napus*) ssp. olcifera) accessions with different gluconsinolate and erucic acid contents to four isolates of *Peronospora parasitica* (downy mildew) and the identification of new sources of resistance. Plant Pathology. 43: 278-285.

Natti, J.J. 1957. Control of downy mildew of broccoli with antibiotics and fungicides. Plant dis. Reptr. 41: 780-788.

Natti, J.J. 1958. Resistance of broccoli and other crucifers to downy mildew. Plant Dis. Reptr. 42: 656-662.

Natti, J.J. 1959. Control of downy mildew of broccoli with fungicides and fungicide-spreptomycin combination sprays. Plant Dis. Reptr. 43: 735-740.

Natti, J.J., G.E.R. Harvey and **C.B. Sayre.** 1956. Factors contributing to the increase of downy mildew of broccoli in New York State and its control with fungicides and agrimycin. Plant Dis. Reptr. 40: 118-124.

Natti, J.J., M.H. Dickson and **J.D. Atkin.** 1967. Resistance of *Brassica oleracea* varieties to downy mildew. Phytopathology 57: 144-147.

Neumann, P. 1955. [Diseases of seedlings and young plants of our Brassicae.] Pflanzenschutz 7: 39-44.

Nicolas, G. and **B. Aggery.** 1940. [On some *Peronospora* species parasitized by bacteria.] Rev. Mycol., N.S. 5: 14-19.

Niu, X.K., H. Leung and **P.H. Williams.** 1983. Sources and nature of resistance to downy mildew and turnip mosaic in Chinese cabbage. J. Amer. Soc. Hort. Sci. 5: 775-778.

Ocfemia, G.O. 1925. The occurrence of the white rust of crucifers and its associated downy mildew in the Phillippines. Philipp. Agric. 14: 289-296.

Ohguchi, T. and **Y. Asada.** 1975. Dehydrogenation polymerization products of p-hydroxycinnamyl alcohols by isoperoxidases obtained from downy mildew-infected roots of Japanese radish *(Raphanus sativus)*. Physiol. Plant Pathol. 5: 183-192.

Ohguchi, T. and **Y. Asada**. 1981. Oospore formation of Japanese radish downy mildew fungus. Ann. Phytopathol. Soc. Japan 47: 75-77.

Ohguchi, T. and **Y. Asada.** 1989. [Behavior of downy mildew fungus of Japanese radish on various media. II. Culture from the infected tissue.] Memoirs Coll. Agric., Ehime Univ., 33: 201-209.

Ohguchi, T. and **Y. Asada**. 1990. Morphology of intercellular hypha of downy mildew in Japanese radish. Ann. Phytopathol. Soc. Japan 56: 300-308.

Ohguchi, T. and **Y. Asada**. 1991. Formation of haustoria and granules on the haustoria of downy mildew fungus, *Peronospora parasitica*, on Japanese radish. Ann. Phytopathol. Soc. Japan. 57: 1-8.

Ohguchi, T., Y. Yamashita and **Y. Asada**. 1974. Isoperoxidases of Japanese radish root infected by downy mildew fungus. Ann. Phytopathol. Japan 40: 419-426.

Ohguchi, T., K. Yoshida, M.Y. Ismail and **Y. Asada**. 1989. [A laboratory method for propagation of downy mildew fungus (*Peronospora parasitica*) using the cotyledon or true leaf of Japanese radish seedlings.] Ann. Phytopathol. Soc. Japan 55: 561-566.

Orian, G. 1951. Division of Plant Pathology. Report Dept. Agric. Mauritius, 1949: 66-72; 1950: 80-85.

Pai, C.K. 1957. [Notes on the Pernosporaceae in northeastern China.] Acta Phytopathol. Sinica 3: 137-154.

Park, M. 1932. Ceylon Adm. Rep. Agric. 1931. D103-D111.

Parker, J.E., V. Szabo, B.J. Staskawicz, B.J. Lister, C. Dean, M.J. Daniels and **J.D. Jones.** 1993. Phenotypic characterization and molecular mapping of the *Arabidopsis thaliana* locus, RPP5, determining disease resistance to *Peronospora parasitica*. The Plant J.

Paul, V.H. and **E. Klodt-Bussmann.** 1993. First results from simple methods of preservation of *Peronospora parasitica*. I0BC/WPRS. Bull. 16: 25-28.

Paulus, A.O. and **J. Nelson.** 1977. Systemic fungicides for control of Phycomycetes on vegetable crops applied as seed treatments, granular or foliar spray. Proceedings of the Ninth British Insecticide and Fungicide Conference, Brighton, England, 21-24 November, Volume 3, London, U.K., Session 6B, Pests and Diseases of Vegetables: pp. 929-935.

Paulus, A.O., M. Snyder, J. Gafney, J. Nelson and **H. Otto.** 1978. New systemic fungicide controls downy mildew of broccoli. California Agric. 32: 12-13.

Peleg, J. 1953. Outbreaks and new records. Israel. F.A.O. Plant Prot. Bull. 1-4: 60-61.

Peregrine, W.T.H. and **Siddiqi.** 1972. Phytopathological Papers No. 16. Comm. Mycol. Inst. Kew. U.K.: p. 51.

Persoon, C.H. 1796. Observations mycologicae sur descriptions tan novorum quam notibilium fungorum exhibitae. Part 1: 115p.

Perwaiz, M.S., S.M. Moghal and **M. Kamal.** 1969. Studies on the chemicl control of white rust and downy mildew of rape (Sarson), W. Pak. J. Agric. Res. 7: 71-75.

Pimenova, Mme A. and **I. Maslennikov.** 1955 [Diseases and pests of cabbage seedlings and their control.] Orchard and Garden 6: 30-33.

Pinckard, J.A. 1942. The mechanism of spore dispersal in *Pernospora tabacina* and certain other downy mildew fungi. Phytopathology 32: 505-511.

Ponce, G.F. and **Z.C. Mendoza.** 1983. [Fungal diseases and insect pests of rapeseed in the high valleys of Mexico.] Revista Chapingo 8: 53-61.

Porter, R.H. 1926. Preliminary report of surveys for plant diseases in East China. Plant Dis. Reptr. Supplement 46: 153-166.

Preece, T.F., G. Barnes and **J.M. Bayley.** 1967. Junctions between epidermal cells as sites of appresorium formation by plant pathogenic fungi. Plant Pathol. 16: 117-118.

Preston, N.C. 1929. Part I. Report of the Advisory Mycologist April 1928 March 1929 - Rept. Advisory Dept. Harper Adams Agric. Coll. New Port, Salop 4: 4-8.

Putto, B.L. and **K.C.B. Choudhary.** 1984. Two new hosts of *Peronospora parasitica*. F.A.O. Plant Prot. Bull. 30: 78.

Quanjer, H.M. 1928. [The influence of potash deficiency on the susceptibility of cauliflower to *Peronospora parasitica*.] Tijdschr, Over Plantenziekten 34: 254-256.

Raabe, A. 1939. [Investigations on parasitic fungal diseases of colza and rape.] Zbl. Bakt. Abt. 2, C1-3: p. 35-52.

Ramsey, G.B. 1935. *Peronospora* in storage cabbage. Phytopathology 25: 955-957.

Ramsey, G.B. and **M.A. Smith.** 1961. Downy mildew. IN: Market diseases of cabbage, cauliflower, turnips, cucumber, melons and related crops. USDA Handbook No. 184: pp. 6-7.

Rao, V.G. 1968. The genus *Peronospora corda* in India. Nova. Hedwigia 16: 269-282.

Rawlinson, C.J., K.J. Doughty, C.H. Bock, V.J. Church, G.F.J. Milford and **J.K. Fieldsend.** 1989. Diseases and responses to disease and pest control on single- and double-low cultivars of winter oilseed rape. Aspects Appl. Biol. 23: 393-400.

Rayss, T. 1938. [A new contribution to the study of the Palestinian mycoflora.] Palest. J. Bot. J. Ser. I 2: 143-160.

Ryan, E.W. 1977. Control of cauliflower downy mildew *(Peronospora parasitica)* with systemic fungicides. Proc. Ninth British Insecticide and Fungicide Conference, Brighton, England, Volumes 1 and 2, Research Reports, London, U.K., Session 6B. Pests and diseases of vegetables: pp. 297-300.

Ryan, E.W., W.P. Staunton and **J.C. Cassidy.** 1984. Diseases of vegetables. Res. Report 1983. Horticulture, Dublin, Irish Republic An Foras Taluntais: 41-42.

Rydl, R. 1968. [Cabbage mould on kale.] Uroda 16: 186-187.

Sadowski, C. 1987. Susceptibility of selected cultivars and lines of winter rapeseed to downy mildew. Proc. 7th Intern. Rapeseed Congr., Poznan, Poland, 11-14 May, 1987. 5: 1229-1234.

Saharan, G.S. 1984. A review of research on rapeseed mustard pathology in India. Annual Workshop AICORPO ICAR, Jaipur, 6-10 August 1984.

Saharan, G.S. 1992a. Management of rapeseed and mustard diseases. IN: In Advances in oilseeds research. D. Kumar and M. Rai (eds.). Sci. Pub. Jodhpur, India, Vol. 1, Chapt. 7: 152-188.

Saharan, G.S. 1992b. Disease resistance. IN: Breeding oilseed Brassicas. K.S. Labana, S.S. Banga and S.K. Banga (eds). Narosa Pub. House. New Delhi, India. Chap. 12: 181-205.

Saharan, G.S. 1996. Studies on physiologic specialization, host resistance and epidemiology of white rust and downy mildew disease complex in rapeseed - mustard. Final Report of an Ad-hock Research Project ICAR, Dept. Plant Pathology, CCS HAU, Hisar, India. 105 pp.

Saharan. G.S. and **J.N. Chand.** 1988. Diseases of oilseed crops. Haryana Agric. Univ., Hisar: p. 268.

Saharan, G.S. and **P.R. Verma.** 1992. White rust. A review of economically important species. IDRC, Ottawa, Canada. 65 pp.

Samuel, G. 1925. Annual Report of the lecturer on plant pathology. Reprinted from Report Minist. Agric., S. Australia for the year ending 30th June 1925: 2 pp.

Samuel, G. 1925. Rep. Dep. Agric. S. Australia for year ending 30th June, 1924: pp 76-78.

Sansome, E. and **F.W. Sansome.** 1974. Cytology and life history of *Peronospora parasitica* on *Capsella bursa-pastoris* and of *Albugo candida* on *C. bursa-pastoris* and on *Lunaria annua*. Trans. Br. Mycol. Soc. 62: 323-332.

Savulescu, T. 1948. [The species of *Peronospora Corda* of Rumania.] Sydowia 2: 255-307.

Savulescu, T. and **T. Rayss.** 1930. [Contribution to the knowledge of the Peronosporaceae of Rumania.] Ann. Mycol. 18: 297-320.

Schmidt (Trude). 1960. [The cabbage *Peronospora* properly controlled.] Pflanzenarzt. 13: 120-121.

Schrenk, H.V. 1905. On the occurrence of *Peronospora parasitica* on cauliflower. Mo. Bot. Gard. Annual Report 16: 121-124.

Semb, L. 1969. [Cabbage downy mildew.] Jord. Avling. 12: 32-35.

Sequeira, P. and **A. Monteiro.** 1996. Heterothallism and homothallism in Portugues isolates of *Peronospora parasitica* (Pers. ex Fr.) Fr. Curciferae NewsLetter 18: 126-127.

Shao, X.H., Q.Q. Chen and **F.Q. Zhang.** 1990. [Research on relation between some ecological factors and *Peronospora parasitica* (Pers.) Fr. on Chinese cabbage.] Acta. Agric., Shanghai 6: 78-81.

Shao, X.H., Q.Q. Chen, F.Q. Zhang, Z.N. Zhu, C.C. Wu and **C.Z. Lu.** 1991. [Studies on occurrence and control of Chinese cabbage downy mildew caused by *Peronospora parasitica* in Shanghai region.] Acta. Agric., Shanghai 7: 74-78.

Sharma, A.D. and **R.L. Munjal.** 1977. The genus *Peronospora* in Himachal Pradesh. Indian J. Mycol. Plant Pathol. 7: 135-138.

Sharma, B.R., J.S. Dhiman, J.C. Thakur, A. Singh and **K.L. Bajaj.** 1991. Multiple disease resistance in cauliflower. Adv. Hort. Sci. 5: 30-34.

Sharma, S.R. 1983. Effect of fungicidal spray on white rust and downy mildew diseases and seed yield of radish. Gartenbauwissenschaft 48: 108-112.

Sharma, S.R. and **H.S. Sohi.** 1982. Effect of fungicides on the development of downy mildew and white rust of radish. Indian J. Agric. Sci. 52: 521-524.

Shaw, C.G. 1981. Taxonomy and evolution. IN: The Downy Mildews. D.M. Specer (Ed.) Acad. Press, London: pp. 17-29.

Sherf, A.F. and **A.A. Macnab.** 1986. Downy mildew. IN: Vegetable diseases and their control. Wiley, New York: pp. 269-273.

Sherriff, C. and **J.A. Lucas.** 1989a. Cytogenetic study of heterothallic and homothallic isolates of *Peronospora parasitica*. Mycol. Res. 92: 302-310.

Sherriff, C. and **J.A. Lucas**. 1989b. Heterothallism and homothallism in *Peronospora parasitica*. Mycol. Res. 92: 311-316.

Sherriff, C. and **J.A. Lucas.** 1990. The host range of isolates of downy mildew, *Peronospora parasitica* from Brassica crop species. Plant Pathol. 39: 77-91.

Shetty, H.S., A.K. Khanzada and **S.B. Mathur.** 1978. Procedures for detecting seedborne inoculum of *Sclerospora graninicola* in pearl millet. Seed Sci. Technol. 6: 935-941.

Shiraishi, M., K. Sakamoto, S. Yoshida and **Y. Asada.** 1974. [An electron microscope study of Japanese radish leaves infected with *Peronospora parasitica*.] Memoirs Coll. Agric., Ehime Univ. 19: 137-168.

Shiraishi, M., K. Sakomoto, Y. Asada, T. Nagatani and **H. Hidaka.** 1975. [A scanning electron microscopic observation on the surface of Japanese radish leaves infected by *Peronospora parasitica* (Fr.) Fr.] Ann. Phytopathol. Soc. Japan 41: 24-32.

Silué, D., N.I. Nashaat and **Y. Tirilly**. 1996. Differential responses of *Brassica oleracea* and *B. rapa* accessions to seven isolates of *Peronospora parasitica* at the cotyledon stage. Plant Dis. 80:142-144.

Singh, S.B., D.V. Singh and **B.S. Bais.** 1980. *In vivo* cellulase and pectinase production by *Albugo candida* and *Peronospora parasitica*. Indian Phytopathol. 33: 370-371.

Snyder, W.C. and **K.F. Baker.** 1943. Diseases of seed cabbage in California. Plant Dis. Reptr. 27: 394-398.

So, I.Y., S.H. Lee, H.M. Kim and **W.H. Lee.** 1981. [Disease incidences in radish and Chinese cabbage I. Major diseases in radish and Chinese cabbage grown in alpine areas in Jeonbug Province.] Korean J. Plant Prot. 20: 135-145.

Soonthronpoet, P. 1969. Tech Docum. F.A.O. Pl. Prot. Comm. S.E. Asia, 70: 23 p.

Stell, F. 1922. Some common diseases of kitchen garden crops. Proc. Agric. Soc. Trinidad and Tobago 12: 779-785.

Sutic, C. and **R. Klijajic.** 1954. [A contribution to the knowledge of the parasitic flora of *Deliblatska pescara.*] Zasht. Bilza. (Plant Prot. Beograd). 24: 104-108.

Swingle, W.T. 1889. A list of Kansas species of *Peronosporaceae*. Trans, Kansas Acad. Sci. 11: 63-87.

Swingle, W.T. 1890. First addition to the list of Kansas Peronosporaceae. Trans. Kansas Acad. Sci. 12: 129-134.

Tham, F.Y., J.A. Lucas and **Z.A. Wilson.** 1994. DNA fingerprinting of *Peronospora parasitica*, a biotrophic fungal pathogen of crucifers. Theoretical & Applied Genetics 88: 490-496.

Thind, K.S. 1942. The genus *Peronospora* in the Punjab. J. Indian Bot. Soc. 21: 197-215.

Thomas, C.E. and **E.L. Jourdain.** 1990. Evaluation of broccoli and cauliflower germplasm for resistance to Race 2 of *Peronospora parasitica*. Hort. Sci. 25: 1429-1431.

Thomas, C.E. and **E.L. Jourdain.** 1992. Resistance to race 2 of *Peronospora parasitica* in U.S. Plant Introductions of *Brassica oleracea* var. *capitata*. Hort. Sci. 27: 1120-1122.

Thompson, B.D. and **P. Decker.** 1955. The occurrence of two types of hypocotyl discoloration of harvested radishes. Plant Dis. Reptr. 39: 416-417.

Thornton, J.D. and **R.C. Cooke.** 1974. Changes in respiration, chlorophyll content and soluble carbohydrates of detached cabbage cotyledons following infection with *Peronospora parasitica* (Pers. ex. Fr.) Fr. Physiol. Plant Pathol. 4: 117-125.

Thung, T.H. 1926a. [Observations on *Peronospora parasitica* on cabbage.] Tijdschr. Over Plantenziekten. 32: 161-179.

Thung, T.H. 1926b. *Peronospora parasitica* (Pers.) De By attacking cabbage heads. Phytopathology 16: 365-366.

Timina, L.T., N.N. Korganova and **E.G. Dobrorutskaya.** 1989. [Downy mildew on radish roots.] Zashchita Rasenii (Moskua) No. 10: 39.

Tor, M., E.B. Holub, E. Brose, R. Musker, N. Gunn, C. Can, P.R. Crute and **J.L. Beynon.** 1993. Map position of four loci in *Arabidopsis thaliana* associated with isolate specific recognition of *Peronospora parasitica*. Molecular Plant - Microbe Interactions.

Townsend, G.R. 1935. Everglades Experimental Station. Report Florida Expt. Stn. 1933-4: pp. 86-112.

Trimboli, D.S. and **F. Hampshire**. 1978. Control of downy mildew of stocks with fongarid. Apps. News Letter 7: 9-10.

Uknes, S., B. Mauch-Mani, M. Moyer, S. Potter, S. Williams, S. Dincher, D. Chandler, A. Slusarenko, E. Ward and **J. Ryals.** 1992. Acquired resistance in Arabidopsis. Plant Cell 4: 645-656.

Vasileva, E.D. 1976. [Damage by *Peronospora* disease to cabbage and the effectiveness of polycarbacin in its control.] Nauchnye Trudy Leningradskogo S-kh Instituta. No. 297: 91-100 (RU) from Referativnyi Zhurnal (1977) 155: 1299.

Vasudeva, R.S. 1958. Diseases of rape and mustard. IN: Rape and Mustard. Singh, D.P. (ed.). Indian Central Oilseed Committee, Hyderabad, India: pp. 77-86.

Verma, P.R., G.S. Saharan and **B.K. Goyal.** 1994. *Peronospora parasitica* (Pers. ex Fr.) Fr. (downy mildew) on crucifers: Introduction, bibliography and subject index. Saskatoon Res. Stn. Tech. Bull. 1994 - 02: 51 p.

Verma, T.S. and **P.C. Thakur.** 1989. Comparative field resistance of cabbage collection to downy mildew at seedling stage. Indian J. Plant Prot. 17: 79-80.

Viegas, A.P. and **A.R. Teixeira.** 1943. [Some fungi of Brazil Phycomycetos.] Bragantia, S. Paulo 3: 223-245.

Vishunavat, K. and **S.J. Kolte.** 1993. Brassica seed infection with *Peronospora parasitica* (Pers. ex. Fr.) Fr. and its transmission through seed. Indian J. Mycol. Pl. Pathol. 23: 247-249.

Vitanova, G. 1996. Sensitivity of the decorative species from the family Brassicaceae to downy mildew (*Peronospora parasitica*). Cruciferae NewsLetter. 18:132.

Vladimirskaya, M.E., M.H. Ilyina and **I.K. Klinkovskaya.** 1975. [Forecasting disease incidence on cabbage crops as influenced with their cultivation practices.] Mikologiya. I. Fitopatologiya 9: 130-132.

Wager, H. 1889. Ann. Bot. 14: 263-279.

Wager, H. 1900. On the fertilization of *Peronospora parasitica*. Ann. Bot. 14: 263-279.

Wang, C.M. 1944. [Physiological specialization in *Peronospora parasitica* and reaction of hosts.] Chin. J. Sci. Agric. 1: 249-257.

Wang, T.M. 1949. Studies on the mechanism of resistance of cruciferous plants to *Peronospora parasitica*. Phytopathology 39: 541-547.

Waterhouse, G.M. 1973. Peronosporales. IN: The Fungi 4B. Ainsworth, G.C., F.K. Sparrow and A. Sussman (Eds.). Academic Press, London: pp. 165-185.

Waterson, J.M. 1940. Report of the plant pathologist, 1939. Report Dept. Agric., Bermuda, 1939: pp. 19-31.

Weber, C.F. 1932. Some diseases of cabbage and other crucifers in Florida. Florida Agric. Expt. Stn. Bull. 256: pp. 17-22.

White, J.G., I.R. Crute and **E.C. Wynn.** 1984. A seed treatment for the control of *Pythium* damping-off diseases and *Peronospora parasitica* on brassicas. Ann. Appl. Biol. 104: 241-247.

Whitewell, J.D. and **G.W. Griffin.** 1967. Chemical control of downy mildew in seedling cauliflower. Proc. Fourth British Insecticide Fungicide Confr., Brighton, UK: pp. 239-242.

Wiese, E. 1927. [A fungus disease of wallflowers and stocks.] Gartenwelt 31: 486.

Williams, P.H. 1985. Crucifer genetics co-operative resource book. Downy mildew. University of Wisconsin, Madison: p. D5-7.

Williams, P.H. and **H. Leung.** 1981. Methods of breeding for multiple disease resistant Chinese cabbage. IN: Chinese Cabbage. Proc. 1st Intern. Symposium. N.S. Talekar and T.D. Griggs (eds.): pp. 393-403. The Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Yang, C.Y., R.L.F. Hu and **U. Pupipat.** 1983. Control of Chinese cabbage downy mildew (*Peronospora parasitica*) by metalaxyl (Ridomil) and other fungicides. Intern. Symp. on Pesticides in Developing Countries - Present and Future, Ibaraki, Japan. Tropical Agricultural Research Center: pp. 125-130.

Yuen, J.E. 1991. Resistance to *Peronospora parasitica* in Chinese cabbage. Plant Disease. 75: 10-13.

Yerkes, W.D. and **C.G. Shaw.** 1959. Taxonomy of the *Peronospora species* on Cruciferae and Chenopodiaceae. Phytopathology 49: 499-507.

Yuen, J.E. 1991. Resistance to *Peronospora parasitica* in Chinese cabbage. Plant Dis. 75: 10-13.

Zarzycka, H. 1970. [Investigations on sources of infection of camelina by *Peronospora camelinae*.] Acta Mycol., Warszawa 6: 7-19.

Zarzycka, H. and **T. Kloczowska.** 1964. [Results of investigations on the control of downy mildew of False Flax (*Camelina sativa*).] Biul, Inst. Ochr, Rosl., Poznan 26: 231-248.

Zarzycka, H. and **T. Kloczowska.** 1967. [Investigations on the control of *Peronospora parasitica* of false flax.] Biul. Inst. Hodowl. Aklim. Rosl. 6: 146-159.

16. SUBJECT INDEX