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DEVELOPMENT OF A MODEL TO PREDICT SPORULATION OF *BREMIA LACTUCAE* IN LETTUCE

by

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A thesis submitted to the Faculty of
Graduate Studies and Research in partial fulfilment
of the requirement for the degree of
Masters of Science

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November, 1995

ISBN 0-612-12279-4

Canada

SUGGESTED SHORT TITLE

DEVELOPMENT OF A MODEL FOR SPORULATION FOR *BREMIA LACTUCAE* IN LETTUCE

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FOREWORD

This thesis consists of five chapters. Chapter I includes the introduction and presents general information about the nature and the objectives of the studies presented. Chapter II is the literature review and gives more detailed information about the subject of the present research. The materials and methods are explained in Chapter III and Chapter IV includes the results. The general discussion, Chapter V gives the synthesis of conclusion as well as suggestions for future research.

The studies were supervised by Dr. A. C. Kushalappa, Department of Plant Science, Macdonald Campus of McGill University and Dr. O. Carisse and Dr. G. Bourgeois from the Centre for Research and Development in Horticulture of Agriculture and Agri-Food Canada in Saint-Jean-sur-Richelieu (Quebec) for specific aspects of the research. However the candidate is fully responsible for the reported work.

ABSTRACT

DEVELOPMENT OF A MODEL TO PREDICT SPORULATION OF *BREMIA LACTUCAE* IN LETTUCE

Eli Tchervenivanova

M. Sc. **Plant Science**

The effect of temperature and duration of leaf wetness (DLW) on sporulation of *Bremia lactucae* was determined for lettuce cv. Ithaca. A single spore isolate of *B. lactucae* was produced and was identified using lettuce differential lines each of which had known gene for resistance. Potted lettuce plants were inoculated with the isolate and incubated at 15 C, at 100% relative humidity for 24 h and then at a relative humidity lower than 70%. Seven days later, the plants were submitted to five different temperatures (5, 10, 15, 20 and 25 C) and six durations of leaf wetness (4, 6, 8, 10, 12 and 14 h). The number of spores produced was determined at the end of each wet period. After 4 h of incubation no spores were observed at any of the temperatures. Highest number of spores was found at 10 and 15 C for more than 10 h of DLW. The rate of sporulation rapidly increased between 8 and 10 h for all the temperatures, including 25 C, where the amount of spores produced was very low. The observed number of spores was transformed into proportion of maximum sporulaton (PMS) by dividing each data by the maximum number of spores observed for each experiment. The Richards model was used to describe sporulation as a function of leaf wetness duration and the rate and maximum value expressed as a function of

temperature. This approach resulted in a three-dimensional equation that explained 87% of the variation in the PMS. Spore viability was also estimated for each temperature and DLW. It was zero after 6 h of incubation and reached almost the maximum after 10 h for all the temperatures. The sporulation model was validated under field conditions and it predicted high, medium, low, or no sporulation in 8 out of 11 times.

RESUMÉ

DÉVELOPPEMENT D'UN MODÉL DE PRÉVISION DE LA SPORULATION DU CHAMPIGNON *BREMIA LACTUCAE* SUR LAITUE

Eli Tchervenivanova

M. Sc. **Phytologie**

L'objectif de cette étude était de déterminer l'effet de la température et de la période de mouillure des feuilles sur la sporulation de *Bremia lactucae* utilisant le cultivar de laitue *Ithaca*. Un isolat obtenu par la germination d'une spore unique de *B. lactucae* fut identifié en utilisant différentes lignées de laitue dont chacune était pourvue de gènes de résistance connus. Des plants de laitues en pots furent inoculés avec cet isolat et incubés à une température de 15 C à 100% d'humidité relative durant 24 heures. A la fin de cette période, l'humidité relative fut réduite à 70%. Après 7 jours, les plants furent soumis à 5 températures différentes (5, 10, 15, 20 and 25 C) et six différentes périodes de mouillure sur les feuilles (4, 6, 8, 10, 12 and 14 heures). Le nombre de spores a été déterminées à la fin de chaque période de mouillure. Après 4 heures d'incubation, aucune spore n'a été observée à aucune des températures testées. Le plus haut niveau de sporulation fut estimé à 10 et à 15 C pour une période de mouillure des feuilles de plus de 10 heures. Entre 8 et 10 heures, le taux de sporulation a augmenté rapidement et ce pour toutes les températures aussi bien qu'à 25 C, où la quantité de spores était déjà très basse. Le modèle de Richards fut utilisé afin de décrire la sporulation en fonction de la période de mouillure des feuilles avec des valeurs pour les taux et l'asymptote maximale exprimés en fonction de la température. Cette approche donne lieu à une équation tri-dimensionelle qui expliquait 87% de la proportion du nombre maximal de spores.

La viabilité des spores fut aussi déterminée pour chacune des températures et périodes de mouillure des feuilles. Après 6 heures d'incubation la viabilité était nulle et elle atteignait presque le maximum après 10 heures d'incubation et ce pour toutes les températures. Le modèle de sporulation fut validé au champs et, 8 fois sur 11, il a prédit avec succès le niveau de sporulation (élevé, moyen, faible, ou nul).

ACKNOWLEDGEMENT

The research was conducted under the supervision of Dr. A. Kushalappa to whom I want to express my gratitude for providing the initial idea and background for the experiment and for his advice and help during my study.

I would like to thank also Dr. G. Bourgeois and Dr. O. Carisse from Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, for the invaluable help in development of the model for sporulation as well as the model for spore viability. Their advice and directions were highly appreciated.

My gratitude is expressed to some of the professors from the Department of Plant Science who shared part of their time and knowledge and provided me with valuable advice and suggestions regarding my research - Dr. T. Paulitz, Dr. M. Fortin and Dr. K. A. Stewart.

I am grateful to all the staff of the Plant Science Department for their technical advice. I am thankful also to all my colleagues in the epidemiology laboratory Nancy Lovering, Varghese Abraham and Patrick Stephenson for their help and assistance throughout the experiment.

Finally I wish to thank my husband Charles Potter for his understanding and support during my study as well as my brother and my family for their encouragement.

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I. INTRODUCTION

The 1993 Canadian lettuce production represents 57,126 t for a total value of $ 30,222,000. More than 67% of this is grown in Quebec (Statistics Canada, 1995). Lettuce, *Lactucae sativa* L., is a plant of cool climate. Higher temperatures and dry conditions can cause bolting (CPVQ, 1978). The conditions favourable for the growth of lettuce plants are suitable for the development of downy mildew caused by the fungus *Bremia lactucae* Regel. This is a major disease both in production and storage of the lettuce crop. Downy mildew is a serious problem when lettuce is grown in cool and moist conditions which are frequently encountered in Quebec during the early and late periods of the growing season. The most common symptoms are the presence of light green to yellow leaf spots, which rapidly change into brown, necrotic lesions and soon can cover the whole leaf. Under high moisture conditions, these spots are covered by the white sporangiophores and sporangia of the fungus (Verhoeff, 1960; Dickinson and Crute, 1974). The disease can spread rapidly even in a short period of time. Only 3-4 hours are enough for the downy mildew spores to germinate and to cause infection in the temperature range of 10-15 C (Scherm and van Bruggen, 1993). Sporulation process occurs outside the

plant, and is highly sensitive to environmental conditions. It is influenced by an interaction of light, humidity and temperature. A period of darkness of about 2 h is necessary for minimum sporulation (Raffray and Sequeira, 1971). The process requires relative humidity higher than 90% and a temperature within the range of 5-24 C (Verhoeff, 1960; Powlesland, 1954).

The conventional way of managing the disease is mainly with fungicides. No resistant cultivars suitable for the climate of Quebec are available at the moment. The control of this disease by cultural practises can only slow down its development but cannot completely replace the use of fungicides. In Quebec, in order to prevent downy mildew epidemics, growers apply a total of 5-7 sprays at 7-10 day intervals, regardless of the presence of the disease or suitable conditions for infection (CPVQ, 1987).

Development of a reliable forecasting system is needed to time the first and subsequent fungicide applications in order to achieve optimal fungicide use. Forecasting systems can be empirical or fundamental (Campbell and Madden, 1990). The former doesn't explain the pathway of biological action and the latter does. Fundamental forecasting systems are based on infection, sporulation and/or dissemination processes of the pathogen.

An empirical forecasting system was developed in California by Scherm and van Bruggen (1994) based only on

environmental factors that influence infection. They found that days when infection occurred were those with morning leaf wetness of at least 3 h or more after sunrise. By applying their forecasting system they succeeded in reducing the fungicide sprays by 67%. They also determined that for the coastal area of California, where heavy rains are common and conditions suitable for sporulation are almost always present the sporulation process does not have a significant effect on disease epidemics. In the area of Quiebec the environmental conditions are not always favourable for sporulation and there is a fluctuation in the sporulation process. The forecasting system developed in California could be supplemented to suit Quebec conditions by the development of a model for sporulation based on duration of leaf wetness, temperature and host growth.

The objectives of this study were to develop a model for describing sporulation as a function of temperature and duration of leaf wetness and to evaluate the sporulation model under field conditions.

II. LITERATURE REVIEW

1. LETTUCE PRODUCTION

More than 60% of the lettuce growing area in Canada is in Quebec. In 1993, 2898 ha of lettuce were planted in Canada including 1745 ha in Quebec (Statistics Canada, 1995). The growing season for lettuce in Quebec starts early May (or end of April), depending on the cultivar and climatic conditions, and continues until the second half of September or the first frost.

Lettuce is divided into four groups based on morphological characters (George, 1985). The first group comprises the head lettuce which is subdivided into two categories, the crisphead lettuce and the butterhead. The crisphead is the most commonly grown lettuce in North America and the most widely known cultivars are Great Lakes, New York, Ithaca and Salinas.

Cultivated lettuce (*Lactucae sativa* L.) is a cool season, annual plant. The optimum temperature for plant growth is 15 to 18 C. Higher temperatures and dry conditions can cause premature flowering (bolting) (CPVQ, 1978). High temperatures can also affect seed germination. Germination is severely inhibited when temperatures exceed 26 C (Lovelock, 1972). Lettuce plants do not require too much fertilisation. During the period of head formation 1.5 kg of

20-20-20 NPK fertiliser mixture per 100 m^2 should be applied (CPVQ, 1980). Johansen et al. (1994) obtained maximum yield of marketable lettuce heads when nitrogen levels of 150 kg/ha were applied. They observed only small differences in the yield when N values were 100 and 200 kg per ha. The total nitrogen supply included the amount of mineral nitrogen within the rhizosphere.

Lettuce is well suited for greenhouses because of its requirement for lower temperatures and short growing periods. In springtime, when lettuce is grown in a greenhouse, the plants are ready for harvest only two months after sowing. During winter, three to four months of growth are necessary to provide sufficient yield.

Lettuce is affected by many diseases. Economically the most important ones are lettuce mosaic virus, big-vein disease, varnish spot, anthracnose, downy mildew and root-knot nematode (Paterson et al., 1986).

2. DOWNY MILDEW DISEASES

The downy mildews are induced by fungi that belong to the class Oomycetes and family Peronosporaceae. Their reproduction and spread are fast and they can cause heavy losses in short periods of time. The development and severity of downy mildew epidemics depend to a great extent on high relative humidity during cool or warm, but not hot periods. While wetness is the most important weather factor

during the infection period, temperature is the most important factor influencing the rate of colonisation (Royle, 1976).

The humidity as well as the diurnal period seem to play an important part in the dispersal of downy mildew sporangia. Cohen and Rotem (1971a) observed intensive dispersal of sporangia of downy mildew on cucumber (*Pseudoperonospora cubensis*) which started between 0600 and 0700 h, reached a peak around 0800 h and declined by noon. When sporangia were wetted they failed to release zoospores which explained the inability of their dispersal. Pegg and Mence (1972) obtained almost the same results for the dispersal of sporangia of *Peronospora viciae* on pea. The sporangial release occurred between 0600 and 0800 h with a peak at 0700 h (1-2 h after sunrise).

The sporulation process is observed at the end of the latent period. Studies on the sporulation period were done for many downy mildews. Cohen and Rotem (1971b) studied sporulation on lesions of *Pseudoperonospora cubensis* on cucumber plants. They found that the highest numbers of sporangia per sporulation period were produced when plants were incubated at 15 C continuously or at 20 and 15 C day and night temperatures, respectively. The sporulation of downy mildews occurs via stomata and requires relative humidity greater than 90% (Royle, 1976). According to the same author wetness is unnecessary for sporulation, although

it does not inhibit the process. Free moisture on leaves is not necessary for the sporulation of onion downy mildew (Yarwood, 1937) but humidity must be continuously high, not only for the formation of the sporangiophores, but also for the maturation of the sporangia. The same author observed that on infected plants placed under favourable conditions for sporulation the fungus will sporulate only, or most abundantly, at night. He suggested that since this is an obligate parasite and depends on its living host for a food supply, the synthesis of certain organic materials during daylight are necessary for the nocturnal sporulation of the fungus. The effect of darkness, high RH and temperature on onion downy mildew (*Peronospora destructor*) had been studied by Hildebrand and Sutton (1984). The pathogen sporulated in a temperature range of 6-22 C in conditions of high RH and darkness. The visible signs of sporulation of *Plasmopara viticola* on grape leaves (Lalancette et al., 1988) were observed after 7 h of darkness at relative humidity >95% and temperatures of 15, 20, and 25 C. The largest number of spores was produced after 12 h at 20 C. Pegg and Mence (1972) obtained sporulation of *Peronospora vicae* in relative humidities between 91 and 100%. No sporangia were produced at relative humidities below 91% or when the plants were kept in continuous light during sporulation. Sporulation was observed between 4 and 20 C with a maximum between 12 and 16 C. The sporulation process was completely inhibited at 1

and 24 C.

Most authors agree that high moisture conditions are needed for infection and sporulation of downy mildews. For both processes, especially infection, a period of free moisture is needed, which is relatively short compared to the requirements of many other pathogens (Palti and Rotem, 1981).

3. DOWNY MILDEW IN LETTUCE

Downy mildew is induced by *Bremia lactucae* an obligate fungal pathogen. The fungus belongs to the order Peronosporales of the class Phycomycetes, and was first found and described by Regel (1843). The mildew is present to some extent in most areas where lettuce is grown and can cause considerable damage to plants during periods of prolonged leaf wetness (Paterson et al., 1986).

The mycelia and spores are hyaline. In the host the mycelium grows between the cell walls, producing club-shaped haustoria into the cells. The sporangiophores are nonseptate and profusely branched (Milbrath, 1923).

3.1 Disease life cycle

Downy mildew on lettuce has two cycles, sexual and asexual. The sexual cycle results in oospores which are thick walled survival spores. Soilborne oospores are the likely source of primary inoculum (Michelmore et al., 1988).

However most reports agreed that these oospores were rarely present in lettuce. For the reproduction and disease development the asexual cycle is more important. During its asexual cycle *B. lactucae* forms sporangiophores and sporangia.

3.2 Symptoms

The most common and easily recognisable symptom of the disease is the presence of light green to yellow leaf spots. On the lower side of the leaf these spots are covered by white tufts of sporangiophores with sporangia (Verhoeff, 1960). Visual symptoms on young seedlings cannot be seen until sporulation occurs, when both the upper and the lower surfaces of the cotyledons and leaves become covered by sporangiophores (Dickinson and Crute, 1974).

3.3 Dispersal and survival

The sporangia are the means by which the fungus is disseminated (Paterson et al., 1986). Oversummering of the sporangia of *B. lactucae* is unlikely because of their intolerance to heat (Marlatt et al., 1966). Systemic invasion has been observed on leaves, hypocotyl and roots of lettuce plants collected during the first 4 weeks after inoculation. No infection of taproots was observed in any of the infected plants. Wild (1946) found that there was almost no evidence of the seed-transmission of mildew. Only 0.025%

of 23,755 seedlings were infected with mildew.

3.4 Infection

Climatic conditions favouring sporulation are optimal for germination of the spores. Lettuce plants can be infected through open stomata as well as through the leaf epidermis (Paterson et al., 1986). The sporangia germinate directly by germ tubes or indirectly by the production of zoospores (Milbrath, 1923). Germ tube production is the rule for the genera *Bremia* and *Peronospora*. Powlesland (1954) demonstrated that *Bremia* spores are short-lived and show a much reduced germinating capacity even after three days. According to the observations made by Verhoeff (1960) the way in which *Bremia lactucae* enters into the leaf is similar to that found in various species of *Peronospora,* direct penetration of the epidermal cells within three hours of spore deposition under optimal conditions (10-22 C). The same author demonstrated that spores would germinate between -3 and 31 C with an optimum at 4-10 C, but the temperature during spore production affected this optimum. He also proved that detached spores of *B.lactucae* remained viable for one or two weeks at 21 C and for more than 50 days at 2-10 C. Crute and Dickinson (1976) studied the behaviour of *B. lactucae* on different lettuce cultivars. Many of the species did not show obvious symptoms but exhibited a hypersensitive response to invasion that was limited to a few cells. The

severity of the disease depends also on the age of the infected seedlings. The number of seedlings infected with *B. lactucae* was the same regardless of their age but the incidence of the pathogen in the main roots and in the hypocotyl was considerably less in the older seedlings (Dickinson and Crute, 1974).

3.5 Sporulation

The sporulation process of *B. lactucae* does not differ significantly from those observed in all downy mildews. It is highly dependant on environmental conditions like humidity, temperature and light. Van Bruggen (1991) observed sporulation on lettuce cotyledons five days after inoculation at 18 and 22 C. From another field experiment, the same authors concluded that the process of sporulation occurred during night time and infection resulted the same morning if prolonged leaf wetness was recorded (Scherm and van Bruggen, 1995).

Heavy infection of lettuce seedlings was induced in 24 h of incubation after inoculation with *B. lactucae* in an atmosphere of high relative humidity followed by 7-10 days under ordinary conditions (Powlesland, 1954). Lebeda and Reinink (1991) reported first occurrence of sporulation on the cotyledons of the susceptible lettuce cultivars five days after incculation while examining the sporulation intensity on four lettuce cultivars differing in their field

resistance.

Temperature and relative humidity, like in most downy mildews, are important factors for the sporulation process of *B. lactucae*. According to Verhoeff (1960) there was sporulation only when leaves were covered by a film of water. However, mycelial growth can continue in the leaf even when the humidity is not high. The infected leaf, turns yellow and withers. Powlesland (1954) obtained sporulation with relative humidity ranging from 100% down to a point between 80 and 90% RH using saturated salt solutions. No sporulation occurred at relative humidities as high as 90 and 95% when H_2SO_4 was used. He also tested the viability of spores exposed to 20 C for periods up to 24 h at various relative humidities. A significant drop in spore viability was observed only after 2 h of exposure at RH below 50%. The same author also recorded sporulation on lettuce seedlings at temperatures from 4 to 20 C, with a great decrease at 4 C and no sporulation at 1-2 or at 25 C.

According to Yarwood (1937) light has an inhibiting effect on the production of sporangiophores of *B. lactucae*. He obtained a large number of new sporangiophores with spores within 11 hours at 22 C but only on plants that were kept in dark. In his opinion, the production of sporangia might be influenced by changes in metabolism of the host. Verhoeff (1960) made a similar observation. The exposure to continuous light delayed the production of sporangiophores,

but did not prevent it. He also found that longer the plants were in the dark, prior to the wet period, the more rapidly sporulation developed. Raffray and Sequeira (1971) confirmed the inhibitory effect of light on sporulation of *B. lactucae*. Furthermore, they established that continuous light for 24 h inhibited sporulation completely. Maximum sporulation after 24 h of 95% RH was obtained with a minimum of 6 h of darkness, but they observed some sporulation even after 2 h of darkness.

Dickinson and Crute (1974) established a relationship between seedling age and sporulation of *B. lactucae*. The sporulation and the spread of the pathogen were limited in older seedlings.

3.6 Race identification

B. lactucae is among the plant pathogens whose genotype has been studied in great detail. It is hetero-thallic with two mating types, and also exhibits secondary homothallism. In a survey, the majority of isolates were found to be self-sterile and of two sexual compatibility types, designated B1 and B2, but some isolates contained the determinants of both compatibility types (Michelmore and Ingram, 1982). The interaction between lettuce and *B. lactucae* is based on a gene-for-gene relationship. The thirteen single dominant genes for resistance in the lettuce plant are matched by specific avirulence genes in the pathogen (Ilott et al.,

1989; Michelmore et al., 1988; Norwood et al., 1993). The identification of the different races can be distinguished in a gene-for-gene system and is based on the genes for resistance that the respective races can overcome. Surveys in the United Kingdom for virulence to five resistance genes identified over half the theoretically possible virulence phenotypes. In contrast, populations in California (Ilott et al, 1987) and Australia (Trimboli and Crute, 1984) consisted of only a few virulence phenotypes and sexual reproduction seemed to be unimportant, even though both sexual compatibility types were present in California. Oospores of *B. lactucae* were also observed in cultivated lettuce from commercial fields in New York and two compatibility types were detected (Yuen and Lorbeer, 1987). To determine the virulence phenotype of *B. lactucae* isolates, Michelmore and Crute (1982) used differential series of cultivars containing specific resistance factors. The inoculated lettuce seedlings were tested for sporulation seven, nine and twelve days after inoculation. Four major pathotypes of *B. lactucae* have been identified in California. Most of the ninety-seven Californian isolates tested were pathotype IV and only two were pathotype I (Schettini et al, 1991). Datnoff et al. (1994) tested isolates of *B. lactucae* obtained from infected lettuce coming from California into Florida and also from naturally infected lettuce fields in Florida. They established that 72% of the isolates coming

from California were pathotype IV and 28% were pathotype III.

4. DOWNY MILDEW CONTROL

To slow down the infection rate of the downy mildew epidemic control practices are used such as application of fungicides and use of resistant varieties (Berger, 1977). Downy mildews are difficult to control because symptoms develop very rapidly under suitable conditions. Even during a short moisture period infection and sporulation can occur.

4.1 Cultural practices and breeding for resistance

Cultural control of downy mildew fungi depends mainly on sanitation and control of the environment to the disadvantage of the pathogen. Two typical cultural practices include the physical removal of infected plants or organs and moisture reduction. These cultural measures are more efficient when applied on young, highly susceptible foliage in the nurseries, but even then they can only slow down the development of the disease (Palti and Rotem, 1981).

Breeding for resistant varieties is another way of controlling downy mildew on lettuce. Breeding work has been most active in the Netherlands, United Kingdom and United States (Crute and Dixon, 1981). Several lettuce cultivars have been reported to be resistant to downy mildew when tested in comparison with others. Crisp genotypes had less

mildew and a slower rate of disease development than other types of lettuce. It was confirmed that the three lettuce cultivars (Iceberg, Batavia blonde de Paris and Grand Rapids) had low field susceptibility (Crute and Norwood, 1981). However, because of the great variability of *B. lactucae*, strong resistance to lettuce downy mildew is difficult to achieve (Gustafsson et al., 1985). The number of races which might arise and overcome resistance is extremely large. In the Netherlands every one to two years a new race of the fungus appeared (Johnson et al. 1978). To determine levels of partial resistance Eenink (1981) screened 756 lettuce genotypes. The experiments in the field revealed significant differences for partial resistance which can be used for breeding of high quality lettuce cultivars.

At the moment no resistant varieties are available for use by the growers in Quebec. The most widely grown cultivar Ithaca presently represents 95% of the Iceberg lettuce in the province. The cultivar has been the Quebec standard for 35 years and it is highly susceptible to *B. lactucae*.

4.2 Fungicide control

Chemical control is, to date, the most effective and most economical measure to protect crops from downy mildew diseases. The acylalanine, organic systemic fungicide, metalaxyl (Ridomil) has been introduced for commercial use

to control downy mildew on lettuce for more than 15 years. In Quebec this fungicide was registered only in 1995. This is the most effective downy mildew fungicide at present in common use. It has excellent curative action by blocking the formation of secondary haustoria and mycelial growth inside the leaf and can be used in extended spray schedules (Schwinn, 1981). Cornford and Pitt (1985) tested the susceptibility to Ridomil of 9 isolates of *B. lactucae* collected from various sites in United Kingdom. The development of the fungus was completely inhibited by the fungicides. But more recent studies (Crute, 1987; Schettini et al., 1991; Wicks et al., 1993) showed insensitivity of some *B. lactucae* isolates to acylalanine fungicides.

The most commonly used protective fungicide in Quebec is Maneb, a dithiocarbamate. Because Maneb is a surface protectant fungicide and cannot reach the pathogen once it is in the host tissue, its use is very limited in rainy weather or prolonged high relative humidity. Growers in Quebec apply fungicides at 7-10 day intervals to manage the disease regardless of suitable conditions for its development (CPVQ, 1987). A forecasting system could help to time the first and subsequent fungicide applications.

An effort has been made to integrate disease management practices. Fungicide sprays have been combined with field resistance to lettuce downy mildew (Crute, 1984). In a glasshouse lettuce crop the effective control of *B. lactucae*

was achieved by a combination of environmental and fungicidal control (Morgan, 1984).

5. DISEASE FORECASTING

5.1 What is a forecasting system

Disease forecasting systems function by predicting disease development based on weather, host, or pathogen conditions. Forecasting can be based on: crop, disease, pathogen, environment, or a combination of these. Forecasting systems developed from experiments in laboratories, greenhouse, growth chamber or field are fundamental in nature. They should be simple and based on one component of the disease cycle (Madden and Ellis, 1988).

A forecaster improves disease control by reducing the number of pesticide applications and should result in better timing of the application when the risk of disease is high. In this way it reduces the environmental pollution and the uncertainty in disease management as well as the risk of pathogen tolerance to pesticides.

Several forecasting systems (BOTCAST, BLIGHT-ALERT) have been developed to time the fungicide applications in managing botrytis leaf blight on onion which are based on weather and the infection cycle of the pathogen (Sutton et al., 1986; Vincelli and Lorbeer, 1989).

Another forecaster known as DOWNCAST was used for timing fungicide sprays to control downy mildew on onion.

The system correctly predicted sporulation on 111 of 119 nights during the growing season (Jesperson and Sutton, 1987).

Based on forecast of morning leaf wetness, the number of fungicide applications against downy mildew on lettuce in California was significantly reduced (Scherm et al., 1995). The total number of sprays was decreased by 67% relative to the calendar-based schedule, with no difference in disease intensity.

5.2 Factors necessary for the development of a forecasting system

Forecasting models have been developed based on models for various processes of the pathogen. The most successfully used disease cycle component in forecasting is infection. Temperature, wetness duration or high relative humidity are important variables that control infection. Other disease cycle components are survival of inoculum, sporulation and dispersal (Madden and Ellis, 1988). These have been used singly or in combination to time fungicide application.

5.3 Modelling approach

5.3.1 Models of epidemic development

A model is a simplified representation of reality. Based on general approach of model development, models can be empirical and mechanistic. The empirical models are

developed to describe and observe relationship between two or more variables. The model does not have to incorporate any information previously known about the dependent and independent variables. The second type of models are developed by starting with a concept or theory instead of data. Mechanistic models must eventually be tested on real data. Models can be classified based on how they are used. A predictive or forecasting model is used to predict the development and increase of future disease based on some set of independent variables (Campbell and Madden, 1990). Modelling epidemics has several objectives - description, prediction and explanation.

There are several phases in the fungal life cycle: infection, sporulation and dispersal. Each phase can also be split into subphases. When the analytic approach is used, the influence of external factors on specific phases of the pathogen life cycle and their linkage must be known very well (Hau, 1988). The epidemiologists are interested in the rate of change, rather than in direct state of the process. Rates are usually derived from states and are expressed as the difference between two states over the time between these states (Zadoks and Schein, 1979).

When the amount of the disease present is determined at several times, the result can be represented as a disease progress curve and the most common type of models used to describe it are growth curve models. To apply this model

several descriptive parameters should be estimated, such as the rate of disease progress. Exponential, monomolecular, logistic, Gompertz, and log-logistic are models with three or fewer parameters. None of these models was developed specifically for application in plant pathology and their parameters do not always have a strict biological meaning (Campbell and Madden, 1990). Berger (1981) compared the Gompertz and logistic model for goodness of fit for over 100 disease progress curves. Better statistical fit was obtained with Gompertz model for all the disease progress curves for nine pathosystems. The Richards model gives more complete description of the epidemic, because unlike other models it includes also a value for a shape parameter. Venus and Causton (1979) obtained better fit of the data when the Richards model was used in comparison to the polynomial functions.

5.3.2 Models for germination and infection

Response surface models were developed to describe germination and infection of *B. lactucae* on lettuce in relation to temperature and leaf wetness duration (Scherm and van Bruggen, 1993). For the purpose a third-order polynomial and a modified Gompertz function were used. Models are used for many pathogens to characterise their behaviour in relation to the environmental factors (Eisensmith and Jones, 1981; Hildebrand and Sutton, 1984;

Magboul et al., 1992;). An infection model for *Cercospora carotae* on carrot was developed by Carisse and Kushalappa (1990). Growth chamber studies demonstrated that infection can occur after 12 h of leaf wetness for the tested temperatures (16-32 C). However severe infection was achieved at 20-28 C for more than 24 h of leaf wetness. A polynomial function and the Richards model were evaluated to describe infection as a function of temperature and leaf wetness. Better fit of the data was achieved with the Richards model. Infection of grape berries by *Botrytis cinerea* (Botrytis bunch rot of grape) was observed after 4 h of wetness at temperatures 12-30 C. A multiple regression model was used to describe the infection as a function of the interaction of wetness duration and temperature (Broome et al., 1995). Lalancette et al., (1988) developed a model to describe the infection efficiency of *Plasmopara viticola* on grape. At temperatures of 15-25 C, incidence increased rapidly during the first 4 h of wetness. The Richards model fit to the averaged or pooled data accounted for 84% and 73% of the variation in the infection efficiency respectively.

5.3.3 Models for sporulation

A computer based simulator was used by Magarey et al. (1991) for management of grape downy mildew (*Plasmopara viticola*). A model for sporulation predicted sporangia formation if the average temperature is initially greater

than 13 C for a minimum of 4 dark hours. Once initiated sporulation may continue if the above conditions continue and temperature is higher than 11 C. Lalancette et al (1988) developed a sporulation model in relation of temperature and high relative humidity for *Plasmopara viticola*. The Richards function predicted 90% of the variation in number of sporangia. Carisse et al.(1992) studied the influence of temperature (16-32 C) and duration of moist period on sporulation of *Cercospora carotae* on carrot leaves. The maximum number of spores was obtained after 96 h of leaf wetness and at 28 C. Two types of multiple regression models were evaluated.

III. MATERIALS AND METHODS

GROWTH CHAMBER EXPERIMENTS

1. Collection and maintenance of inoculum of *Bremia lactucae*.

Lettuce plants showing downy mildew symptoms were collected at random from the Agriculture and Agri-Food Canada Experimental Station in Saint-Clotilde, Quebec. The plants were placed in moist plastic bags to sporulate in a growth chamber at 15 C and 14 h light. Sporulation was observed the following day on most of the lesions. The spores and the sporangiophores were examined under a microscope to ensure that these were spores of *B. lactucae*. *B. lactucae* inoculum was maintained on lettuce plants, cultivar Cobham Green, which possess no known genes for resistance to the pathogen and is completely susceptible. Because of slow and non uniform growth of lettuce seedlings it was replaced by cv. Ithaca, which is highly susceptible to *B. lactucae*, produces uniform cotyledons and is widely grown in Quebec.

For maintenance of *B. lactucae*, plants were grown in magenta plastic boxes 6 x 6 x 10 cm lined with two layers of filter paper. The boxes and the filter papers were autoclaved to prevent contamination. Ten lettuce seeds were placed in each box and were watered once or twice, depending

on the conditions, with half strength Hoagland solution (4ml/box) (Scherm, personal communication). The boxes were closed with a plastic lid, sealed to prevent evaporation of the water solution and kept in an incubator at 15 C and 14 h light. At the cotyledon stage, 7-10 days later, the plants were inoculated with a spore suspension of *B. lactucae* by using a Gilmet pipette (two drops of 20 µl per cotyledon). The boxes were closed again, sealed to create high relative humidity inside and returned to the incubator. About seven days later profuse sporulation was present on both sides of the cotyledons. Sporangia were washed from cotyledons and the suspension was used for monospore isolation.

2. Single spore production

Spore suspension of *B. lactucae* was prepared from newly sporulated lettuce cotyledons. The spore concentration was adjusted to 20 000 sp/ml with a hemocytometer. Samples of 50 µl of suspension were plated on petri dishes with water agar and incubated at 15 C in dark. After 6 h, most of the spores germinated with short germ tubes. The germinated spore and part of the water agar was carefully removed without damaging the germ tube with a tiny needle and placed on detached lettuce leaves. The leaves were put in petri dishes lined with two layers of moist filter paper, sealed and incubated at 15 C with 12 h photoperiod. After about 10-12 days, sparse sporulation was observed on one of the

leaves. This was considered as a single spore isolate. The sporangia were collected and multiplied as above (Section 1) for further studies.

3. Identification of the isolate of *Bremia lactucae*

The isolate of *B. lactucae* was identified by using 15 differential varieties (lettuce lines which had known genes for resistance) (Schettini et al., 1991). Seeds were sown on moist filter paper in magenta boxes (15 seeds/box) which were sealed and placed in a growth chamber at 15 C and 14 h photoperiod. At the cotyledon stage the plants were inoculated with the isolate by placing 20 μl of spore suspension with a Gilmet pipette on the leaves (Michelmore and Crute, 1982). Inoculated seedlings were placed back in the growth chamber and kept at the above mentioned conditions. Presence or absence of sporulation was recorded 10 and 14 days after inoculation.

4. Plant production for the experiment

Lettuce plants (cv. Ithaca) were grown in 15 cm plastic pots filled with a potting mixture Pro-Mix-Bx containing peat moss, perlite, vermiculite, dolomite and calcitic limestone (six seedlings/pot). The plants were kept in a growth cabinet adjusted to 14 h photoperiod, 18/15 C day and night temperatures, respectively, and an ambient relative humidity of 60-75%. The seedlings were watered every other

day and were irrigated once with a soluble fertilizer mixture 15-15-18 (NPK, Plant Products Co. Ltd, Branton, Ont) prepared at 5 g/L water.

5. Plant inoculation and incubation for sporulation

A spore suspension of *B. lactucae* was prepared by shaking leaves of newly sporulated lettuce plants into a glass flask filled with distilled water with 0.02% solution of Tween 20. The spore concentration was adjusted to 3 x 10^4 sporangia/ml by using a hemocytometer. The plants were inoculated at the two true leaf stage (approx. 14 days old) using an automatic spray chamber (Incom Int., Research Instrument Mfg. Co. Ltd, Guelph, Ont). The spray nozzle was adjusted to 45 cm height from the canopy level. It moved horizontally, and the speed of the trolley was adjusted to 0.5 km/h to provide an adequate amount of inoculum on the leaf surface (Mudita and Kushalappa, 1991). For the determination of spore viability of the suspension two water-agar plates were placed on the spray bench, next to the plants for inoculation and were then incubated at 15 C for 24 h in dark. One hundred spores were observed microscopically for germination. Spores were considered to have germinated when the germ tube was longer than the width of the spore. After inoculation each pot was enclosed in plastic bags previously misted with water to insure leaf wetness necessary for infection, and incubated in dark in a

growth chamber maintained at 15 C. A wire frame kept the bags from touching the plants. Twenty four hours later the bags were removed and the plants were left at the same temperature and 14 h light supplied by fluorescent and incandescent lamps providing a light intensity of 250 μmoles m^{-2} s^{-1}.

Seven days after inoculation the plants were enclosed again in moist plastic bags to provide leaf surface wetness and were incubated in growth chambers set at different temperatures for different durations as required by the experimental design.

The temperature inside the plastic bags was also measured to see if there was any difference between the temperature at which the growth chamber was preset and that inside the plastic bag. No differences in temperature were observed between them at night. During day time the difference in the temperatures was less than 1 C, so we did not consider it in our results for the preliminary experiment.

6. Experimental design

6.A. Sporulation in alternating dark and light

A preliminary experiment was conducted to determine the effect of temperature, light/darkness and duration of leaf wetness required for sporulation. The experiment consisted of six temperatures (5, 10, 15, 20, 25 and 30 C) and five

durations of leaf wetness (12, 24, 48, 72 and 96 h). The light period for the first duration of leaf wetness was 10 h followed by 2 h of dark. For the next durations of leaf wetness the dark period was 10 h.

6.B. Sporulation at continuous dark

The inoculated lettuce plants were transferred to growth chambers set at different temperatures (5, 10, 15, 20, 25 C) in darkness. Three pots, for each temperature, were removed at random for observation after 4, 6, 8, 10, 12 and 14 h of incubation. Thus, the experiment consisted of a total of 30 treatment combinations of temperature and duration of leaf wetness (DLW). The whole experiment was conducted twice.

7. Quantification of sporulation.

The first true leaves from each of the inoculated six plants per pot were removed and put in a glass flask filled with 20 ml of distilled water and 0.02% Tween 20. The flask was agitated for 15 min using an orbit shaker and then the number of spores in the suspension was counted with a hemacytometer (four counts). The leaf area was measured with an areameter (Paton Electronic Planimeter). From these data the number of spores/mm^2 leaf area was calculated [(number of spores/ml of suspension) x (20 ml of spore suspension)/(leaf area in mm^2)].

8. Test for spore viability

A spore suspension from each of the 30 treatments was spread on petri dish with water agar and incubated at 15 C for 12 h in the dark. About 50 to 100 sporangia for each suspension were observed for germination. The spore was considered germinated if the length of the germ tube was longer than the width of the spore.

9. Data analyses and model development

9.1 Model for sporulation

The observed data on the number of spores per mm^2 leaf area at different temperatures and durations of leaf wetness were transformed to proportion of maximum sporulation (PMS) by dividing each data by the maximum number of spores observed for each experiment.

We used a split-plot design where the main plot was the temperature and the subplot was the duration of leaf wetness with three replicates. Analyses of variance were carried and a F test was used to determine if the data from both experiments could be pooled. No significant differences between the two experimental replicates were established, therefore all the following analyses were carried out with pooled data from the two experimental runs on the average data from the three replicates for each duration of leaf wetness. No analysis was performed for data from the preliminary experiment. The results from the first and

second experimental replicate are presented in Appendix 1 and 2, respectively.

The Richards function was used to express sporulation as a function of temperature and leaf wetness duration (eq.1).

$$PMS = k(1+(y_0^{1-m} - 1)e^{-rW})^{1/(1-m)} \quad \text{(eq. 1)}$$

where k is the upper asymptote, y_0 is the initial value of PMS, m is the shape parameter, r is the rate parameter and W is the duration of leaf wetness.

The model development consisted of several steps. First, we found y_0 and W_0 (initial leaf wetness duration) by using the linearized form of the Gompertz function:

$$-\ln(-\ln(y)) = b_0+b_1W \quad \text{(eq. 2)}$$

where y is the proportion of maximum sporulation (PMS), b_0 and b_1 are regression coefficients, and W is the duration of leaf wetness. This was needed to initialize the nonlinear (NLIN) procedure for Richards function. First we obtained the initial coordinates (y_0, W_0) for each temperature separately. Then the Gompertz function was used again to determine the initial coordinates for the pooled temperatures.

In the second step the upper asymptote (k) was determined by regressing the maximum sporulation observed for each temperature against a third order polynomial of

temperature (T) using eq. 3:

$$k = b_0 + b_1T^3 + b_2/T^2 \qquad \text{(eq. 3)}$$

where b_0, b_1 and b_2 are regression coefficients.

The Richards function (eq. 1) was then run with the estimated values for y_0, W_0 (eq. 2) for the pooled temperatures and k, the upper asymptote from equation 3. Different values for the shape parameter m were tried: $0<m<1$; $m=1$; $m>1$. Values for m equal to zero produced monomolecular-type function, those equal to one produced Gompertz function and those equal to 2 produced logistic-type function. To achieve the best possible fit at all temperatures the NLIN procedure of SAS for Richards function (eq. 1) was run for each temperature separately and different values of the m parameter were obtained. This was done first by running the best value for the m parameter for the temperatures 10, 15 and 20 C, because at these temperatures high levels of sporulation were observed. Then the same m parameter was established for all temperatures. The best value for m was estimated based on comparison of coefficients of determination, residuals and fit of the data to the model. The Richards function was run again with the same m value at all temperatures.

The next step was to determine the rate parameter estimates. This was achieved by regressing the r obtained

from Richards function for each temperature with the same m value against a second order polynomial of temperature:

$$r = b_o + b_1T + b_2T^2 \qquad \text{(eq. 4)}$$

in which b_0, b_1, and b_2 are regression coefficients.

The final step consisted of replacing the rate and the asymptote parameter functions (eq. 3 and 4) together with the estimated values for y_0 and W in the Richards model (eq. 1) to receive the predicted values for proportion of maximum sporulation as a function of T and DLW.

All the analyses for the Richards model and for the estimation of the parameters were performed by using SAS procedure (SAS Institute Inc., 1987).

8.2 Model for spore viability

The analyses for spore viability were performed on the average of temperatures for each replicate. Based on a F test we pooled the data from both experiments. The ANOVA test showed that the temperature was not significant for spore viability with $P < 0.08$. Further analyses were carried out only for the duration of leaf wetness. We pooled the data from both experiments so that each replication was an average of five temperatures with total of six replications. We used the logistic model to determine spore viability (SV) as a function of leaf wetness (eq. 5).

$$SV = k/(1+y_0e^{-rW}) \qquad \text{(eq. 5)}$$

where k is the upper asymptote, y_0 is the initial value, r is the rate parameter and W is the duration of leaf wetness. The logistic function gave a god fit to the observed data. The initial parameter (y_0) was adjusted close to zero, and we tried different values for the r parameter.

FIELD EXPERIMENT

1. Location and experimental setting

During the summer of 1994, an experimental plot of lettuce was established at the Horticulture centre of Macdonald campus, McGill University at St. Anne de Bellvue. Lettuce seedlings were grown in a growth chamber and at the 2-3 true leaf stage (about 20 days) the seedlings were transplanted in the field and watered by hand when necessary. The size of the plot was 4 X 4 m and consisted of 10 rows with a spacing of 0.4 m between rows and the same distance within.

2. Validation of sporulation in the field

Lettuce seedlings, grown as described for model development, were used to validate the model under field conditions. The plants were inoculated and incubated as indicated before. During the sporulation period, the pots with infected lettuce seedlings (6 seedlings/pot) were

placed in the field among other lettuce plants, at 2000 h. The following morning at 0900 h or at the end of leaf wetness period the plants were observed for the presence of downy mildew growth or spores. If sporulation occurred, the pots were removed and the number of sporangia per mm^2 of total leaf area were determined with a hemocytometer. From this the observed PMS in the field was determined by dividing the sporulation observed in the field to the maximum observed sporulation in the field.

3. Meteorological data collection

The weather data on ambient temperature, relative humidity and leaf wetness were recorded at 1-min intervals with a datalogger (model CR-10, Campbell Scientific Canada Corp.) and saved as hourly averages. The data logger and two temperature/RH sensors (Vaisala) were placed in a Stevenson shelter 0.5 m above the ground in the middle of the lettuce plot. The leaf wetness was measured with two sensors (model 237) placed within the plant canopy at the leaf height, one sensor at horizontal position and the other one tilted at 45^0 to simulate leaf orientation.

4. Prediction of sporulation in the field

The predicted proportion of maximum sporulation (PMS) was calculated by replacing the temperature duration of leaf wetness and relative humidity measured in the field in the

model for sporulation. The leaves of the plants were considered wet when the values measured by the leaf wetness sensors were ≥3.0. Because there were two leaf wetness sensors, we took the average data from both. The average temperature was calculated only for the hours where the DLW ≥3.0. The duration of relative humidity was estimated by calculating only the hours were the relative humidity was above or equal to 88%. Again the readings from the two sensors measuring relative humidity were averaged. The predicted sporulation was compared to the amount of sporulation observed in the field.

IV. RESULTS

1. Identification of the isolate

Reaction of the isolate of *Bremia lactucae* used in this study is presented in Table 1.

Table 1. Reaction of an isolate of *Bremia lactucae*, collected from the Montreal area to *Dm* genes of lettuce differential series.

Cultivar/line	Dm gene	Reaction to Dm gene
Lednicky	1	-
UCDM2	2	+
Dandie	3	-
R4/T57	4	+
Valmaine	5/8	-
Sabine	6	+
LSE57/15	7	(+)
UCDM10	10	-
Capitan	11	-
Empire	13	+
UCDM14	14	(+)
PIVT1309	15	+
LSE 18	16	(+)
Hilde	R12[b]	+
Cobham Green	0	+

\+ Compatible reaction, profuse sporulation;
\- Incompatible reaction, no sporulation;
(+) Sparse sporulation - sporulatin was clearly observed with naked eye, but it was infrequent and leaf necrosis was noticed.

Profuse sporulation was observed on seven and no sporulation was observed on five lettuce lines out of fifteen. The lines containing main gene for resistance Dm7,

Dm14 and Dm16 showed infrequent or sparse sporulation. According to our results the pathotype that we identified has different virulence phenotype than isolates detected in the United States, in both California and Florida. We consider that as a different pathotype and we named it pathotype Mac 1 (Mac stands for Macdonald campus).

2. Effect of temperature and DLW on sporulation

No sporulation was observed before 4 h of duration of leaf wetness (DLW) at any temperature (Fig. 1). However conidiophores were observed under a dissecting microscope at 10 and 15 C. Even after 6 and 8 h, the number of spores was considerably low. The rate of sporulation rapidly increased between 8 and 10 h of DLW for all the temperatures even at 25 C, where the amount of spores was always very low. A high proportion of maximum sporulation was observed at 10 and 15 C, after 10 h of DLW. After 10 h the rate of spore formation decreased significantly, particulary for 10 and 15 C.

The analysis of variance carried out separately for each temperature and pooled data showed a high significance of both temperature and duration of leaf wetness. The coefficient of determination (R^2) for the pooled data of both experiments was 0.90.

3. Estimation of the parameters for the Richards model

The estimated values of the parameters for the Gompertz

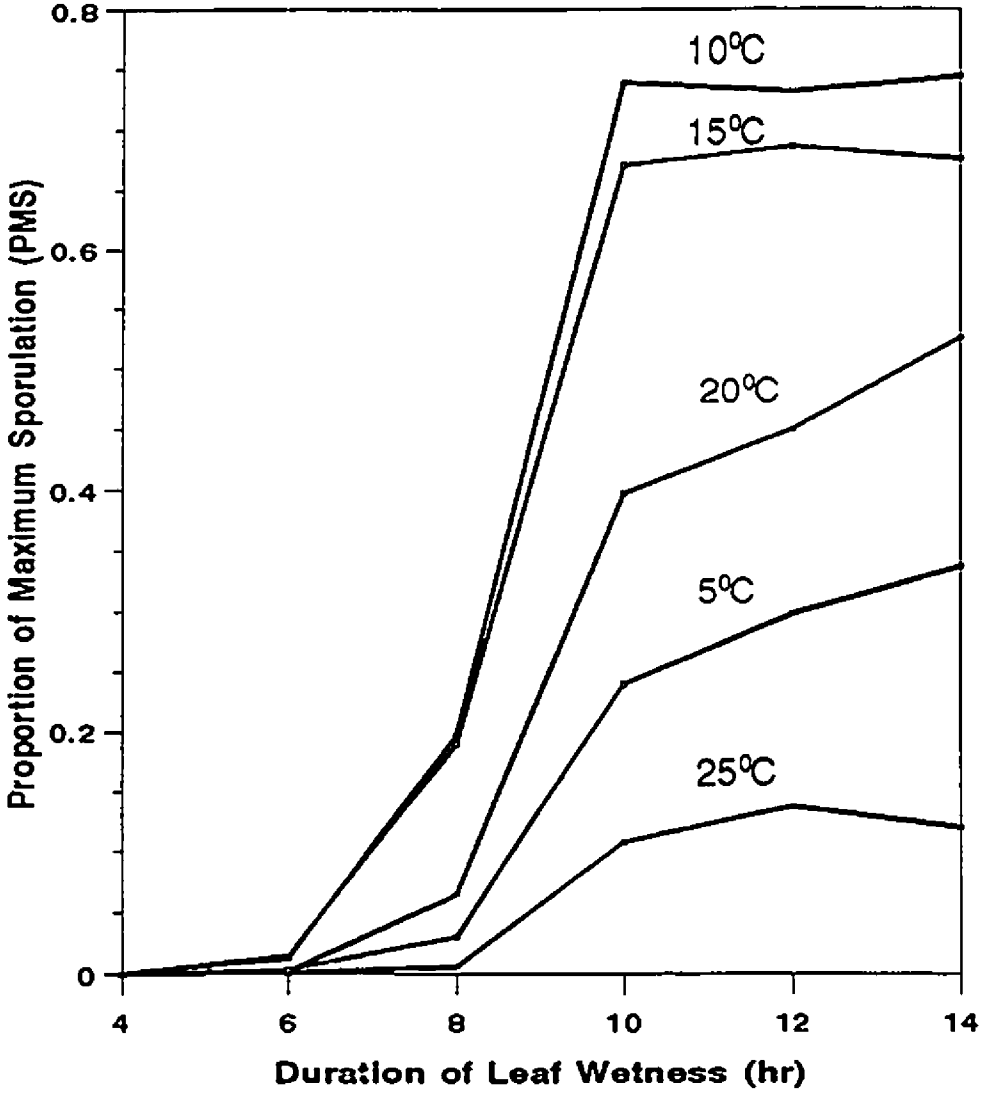


Fig. 1. Observed proportion of maximum sporulation of *B. lactucae* on lettuce seedlings at different durations of leaf wetness and temperatures. Each point is an average of two experimental replicates.

model (eq. 1) are given in Table 2. The W_0 (initial leaf wetness) was estimated from the Gompertz model by giving the value of 0.0001 to the y_0 (initial parameter). It varied for different temperatures from 0.12 at 25 C to 2.03 at 20 C The initial value for all temperatures was established at 1.50.

Table 2. Estimates of parameters (b_0 and b_1) for Gompretz model (eq. 2) to describe proportion of maximum sporulation as a function of duration of leaf wetness for each temperature and on the average over all temperatures.

Model	DF Error	SS Error	R^2	F P-value	Estimate/P-value b_0	b_1
5 C	8	0.68	0.85	<0.001	-2.962	0.222
					<0.001	<0.001
10 C	8	2.99	0.77	<0.001	-3.222	0.355
					<0.002	<0.001
15 C	8	2.55	0.76	<0.001	-3.026	0.319
					<0.002	<0.001
20 C	8	1.23	0.85	<0.001	-3.386	0.296
					<0.001	<0.001
25 C	7	0.61	0.69	<0.006	-2.609	0.147
					<0.003	0.006
Pooled	50	26.43	0.55	<0.0001	-3.016	0.262
					<0.0001	<0.0001

The best possible fit for the m parameter at all temperatures was obtained by comparing the coefficient of determination (R^2), residuals and the fit of the data. It was estimated at m=2.1, with a best fit for 10 and 15 C (R^2=97) and R^2=0.92 and 0.81 at 5 C and 25 C, respectively.

The rate parameters estimated from eq. 3 produced a

bell shape curve with its maximum at 15 C (r=2.17) and its minimum at 5 C (r=1.90). Up to four polynomials were tried but the increasing order of the polynomials did not seem to change significantly the coefficient of determination. The second order polynomial explained 61 % of the variation in the rate parameter (Table 3).

Table 3. Estimation of the rate (r) parameter and upper asymptote (k) parameter from the Richards model, using equation 3 and 4 respectively, to describe the effect of temperature and duration of leaf wetness on sporulation of *B. lactucae* in lettuce.

Model	DF Error	SS Error	R^2	F P-value	Estimete/P-value b_0	b_1	b_2
Upper asymptote (k)							
Rep 1	2	0.017	0.93	0.07	0.9311 <0.0001	-0.0000447 <0.0001	-15.68851 <0.05
Rep 2	2	0.002	0.99	0.007	1.00015 <0.0012	-00005650 <0.0035	-15.460754 <0.0071
Pooled	7	0.03	0.95	0.0001	0.966 <0.0001	-0.00005062 <0.0001	-15.575 <0.0001
Rate parameter (r)	2	0.026	0.61	0.4	1.6888 <0.02	0.06131 <0.24	-0.002117 <0.22

The reduction in the coefficient of determination was due to the poor fit at 25 C. This was proved by trying the

model without 25 C, which increased the coefficient of determination to 0.99. The rates predicted by the model were overestimated at 20 C and slightly underestimated at 10 and 25 C. The predicted values for the rate parameter reached the maximum at 14 C (Fig 2). Although the coefficient of determination for the rate parameter was not very high it did not seem to significantly influence the regression coefficients of the final fit of the Richards model for all temperatures taken as a whole.

Different polynomials were also tried by regressing the upper asymptote parameter (k) to the different temperatures. Although we achieved high R^2 (0.96), the predicted values by the model for 10 C were significantly different from the observed ones. A good fit was provided using the model in equation 1. It explained 95% of the variation in the k parameter (Table 3). The model predicted maximum PMS at 12 C (0.8) and minimum at 25 C (0.2) which was close to the data observed in the growth chamber experiment (Fig. 3).

The sporulation model explained 88% of the variation in PMS as a function of temperature and DLW, for the data pooled from both experiments (Fig. 4). The model predicted that high sporulation should be expected after 8 h of high humidity at 10 and 15 C and low amount of spores at 5 and 25 C. This agree with the observed data.

4. Effect of sporulation temperature and DLW on spore

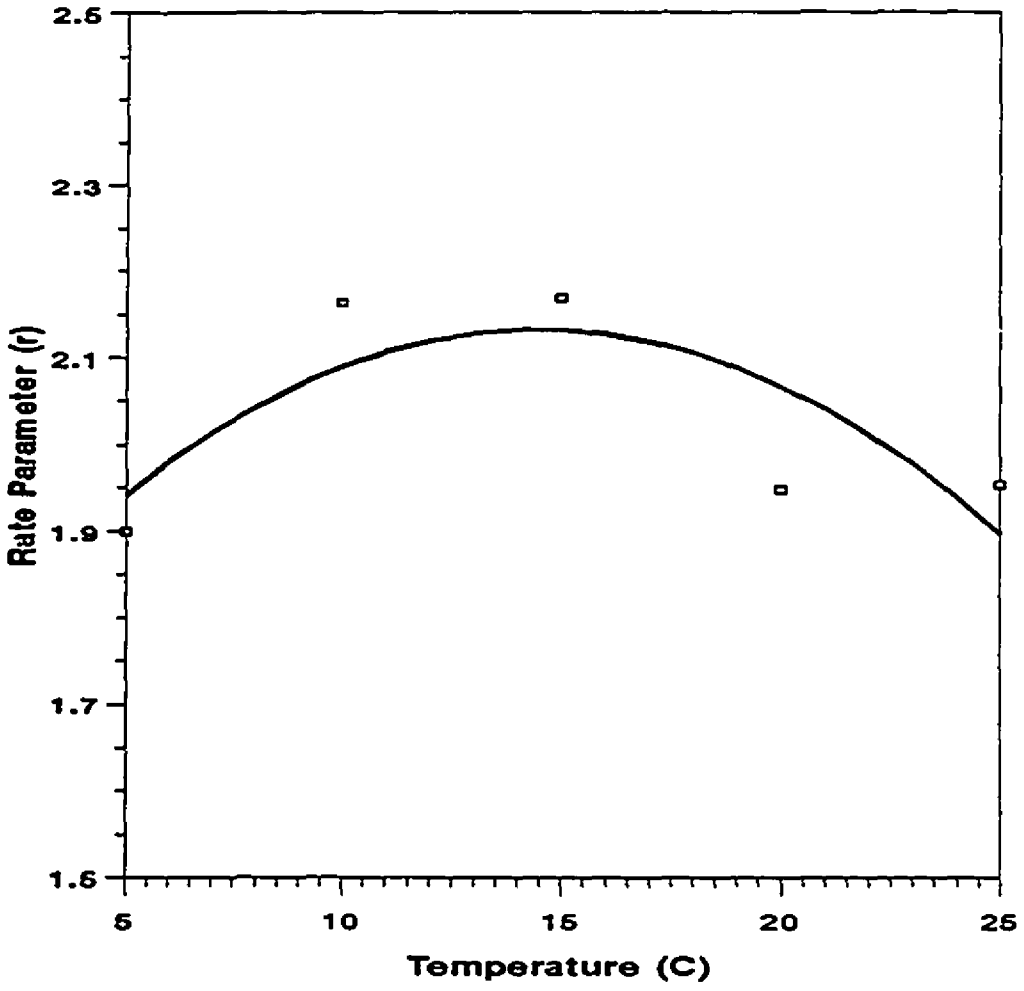


Fig. 2. Relationship between the rate parameter (r) from the Richards function and the temperature for the pooled data of both experimental replicates. The ■ corresponds to the values calculated from eq. 1. The solid line is the predicted value of r calculated by using eq. 4 (see text).

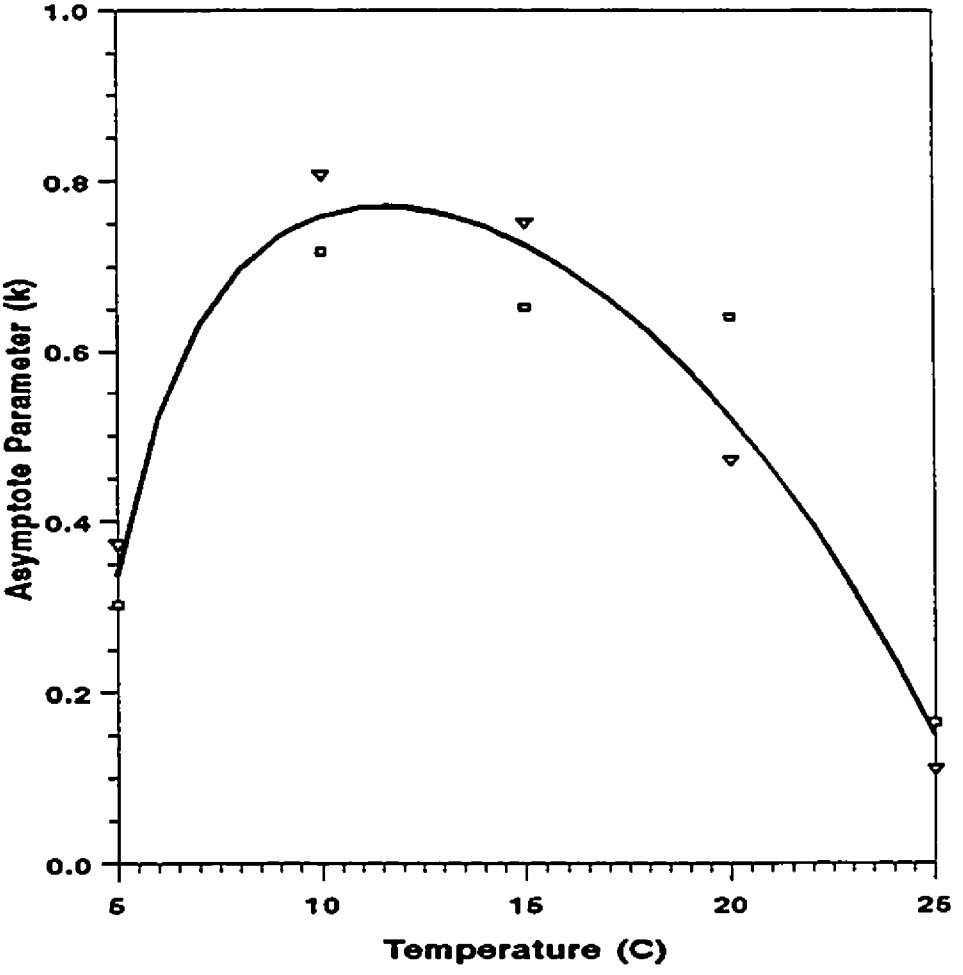


Fig. 3. Relationship between the asymptote parameter (k) and the temperature. Each point corresponds to the observed data for the first (▸) and the second (■) experimental replicate.The solid line is the value of k calculated by using eq. 3 (see text).

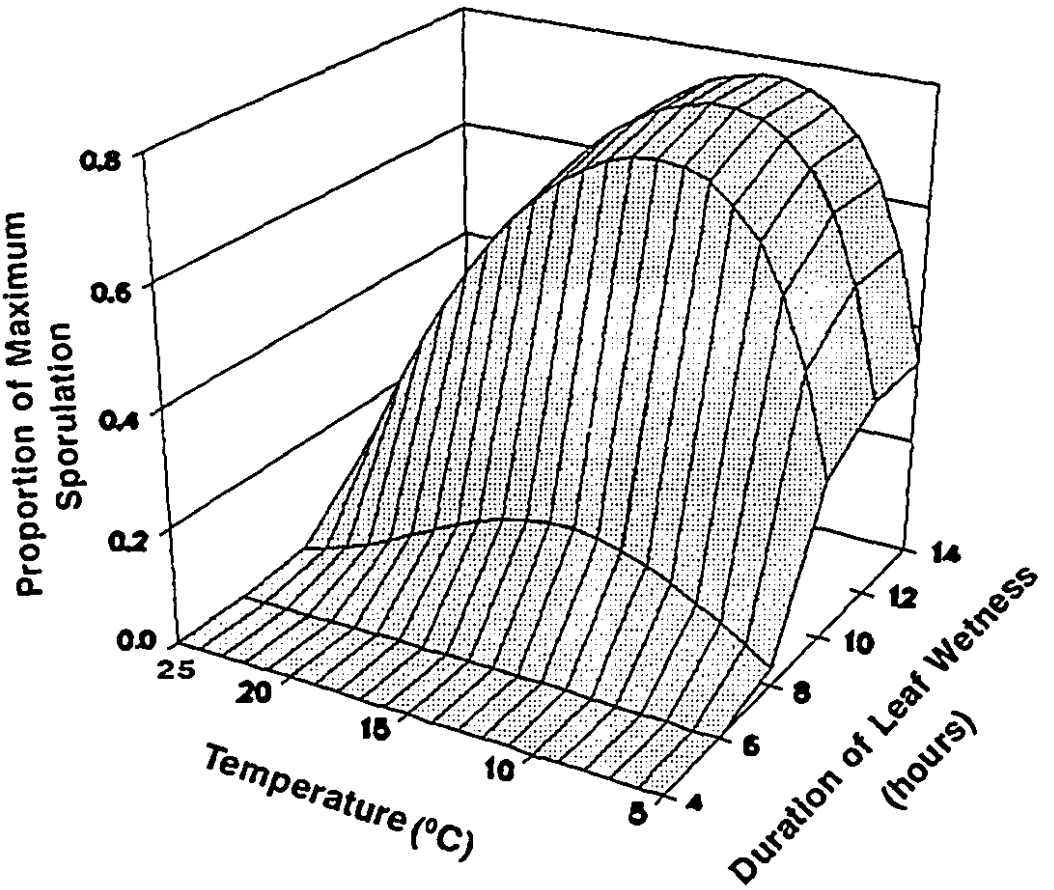
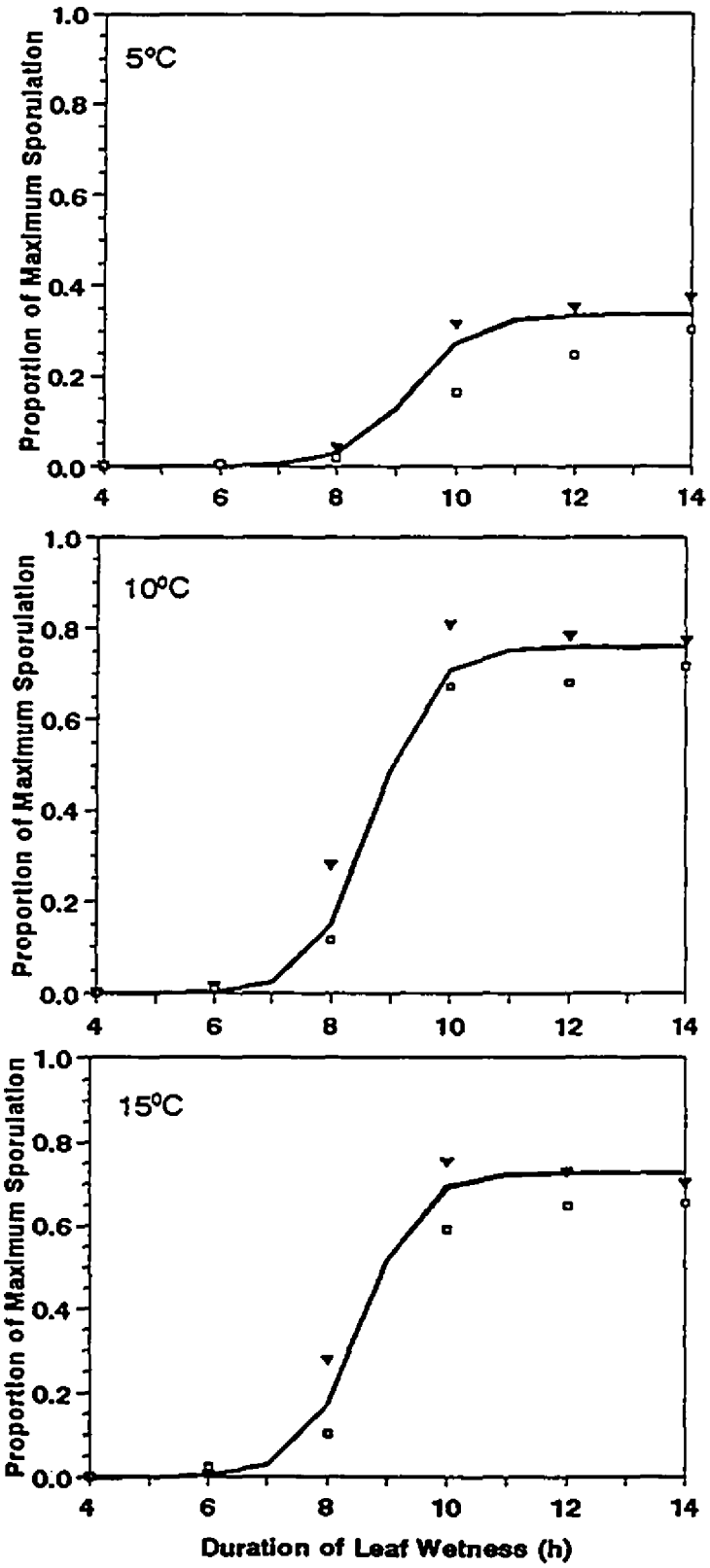
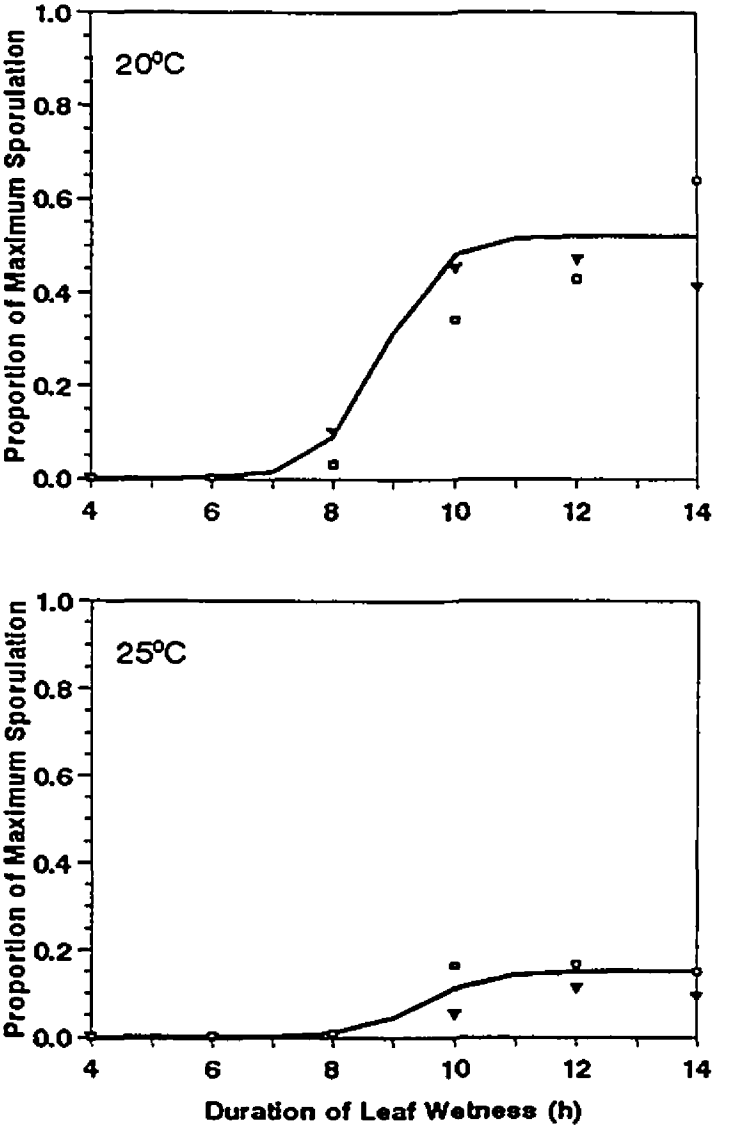


Fig. 4. Three-dimensional representation of the proportion of maximum sporulation of B. lactucae predicted by the Richards model (eq. 1) as a function of temperature and duration of leaf wetness. The response surface model was calculated by using the pooled data from both experimental replicates, on the average values from three experimental units for each temperature and duration of leaf wetness.

Fig. 5. The proportion of maximum sporulation (PMS) predicted by the Richards model (eq. 1) and observed values at different temperatures and duration of leaf wetness. The solid line represents the PMS predicted by the model and the ▼ and ■ represent the data for the first and second experimental replicate, respectively.


5°C
10°C
15°C
Proportion of Maximum Sporulation
Duration of Leaf Wetness (h)
0.0
0.2
0.4
0.6
0.8
1.0
4
6
8
10
12
14


20°C
25°C
Proportion of Maximum Sporulation
1.0
0.8
0.6
0.4
0.2
0.0
4
6
8
10
12
14
Duration of Leaf Wetness (h)

viability.

Increase in the duration of leaf wetness significantly increased spore viability (Fig. 6). At 6 h of DLW not only fewer spores were produced at all temperatures (except 25C), but their viability was 0. The temperature seemed to influence the spore viability only in the beginning of the sporulation period or before eight hours of leaf wetness where most of the viable spores were observed at 10 and 15 C. After ten hours of DLW during sporulation the amount of viable spores reached a near maximum at all temperatures.

The logistic model provided a good fit to the pooled data from both experimental trials. It described 95% of the variation in spore viability (Fig. 6). The increase in the duration of leaf wetness resulted in high percent viable spores predicted by the model. This model predicted maximum percent viable spores (89%) for 14 h of DLW and almost zero spore viability (0.43%) for 6 h of DLW. The intercept parameter (x_0) was estimated close to zero, the maximum asymptote ($\dot{k}$) was estimated by the model at 0.89 and the rate parameter (r) at 1.83.

6. Model validation.

During the period when the sporulation model was validated (mid August until mid September) the temperatures varied approximately between 9 and 18 C but the most important factor for sporulation was obviously the duration

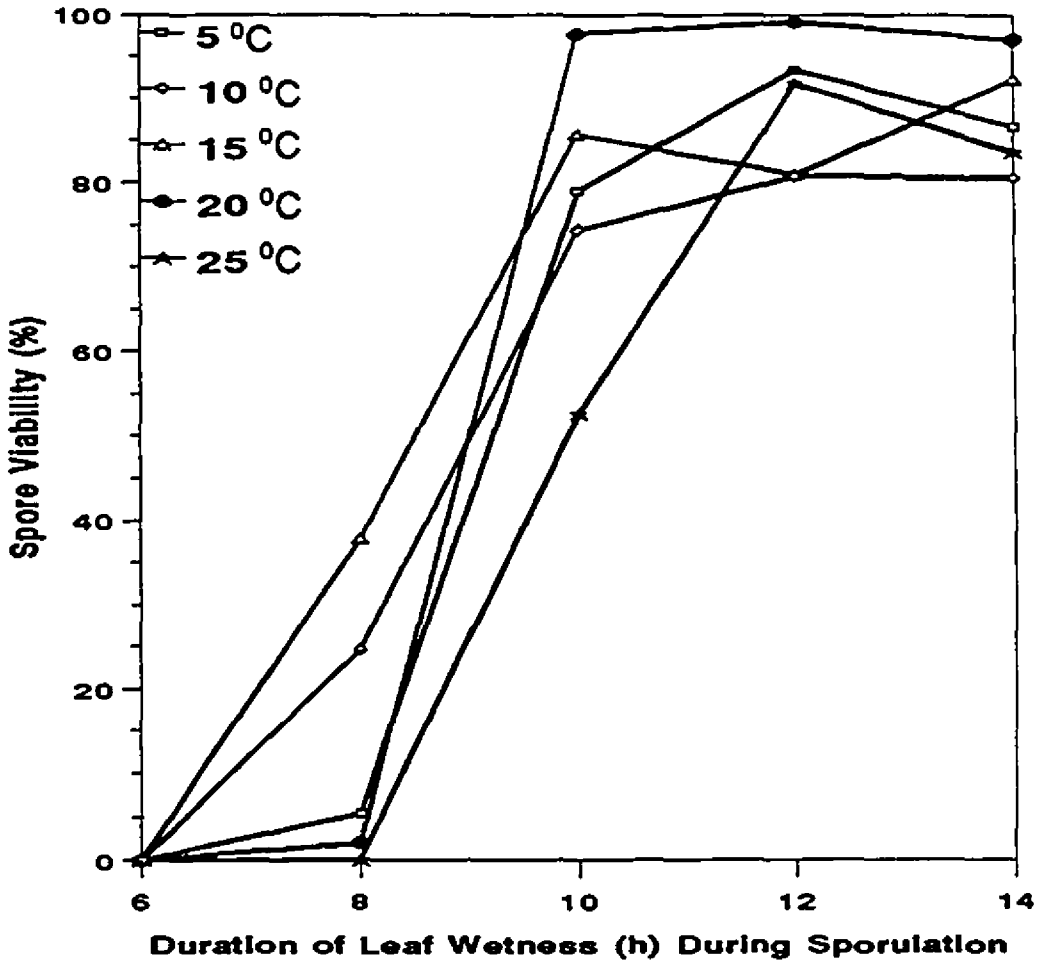


Fig. 6. Observed viability (% spore germination) of spores of B. lactucae produced at different durations of leaf wetness and temperatures. Each point is an average of two experimental replicates.

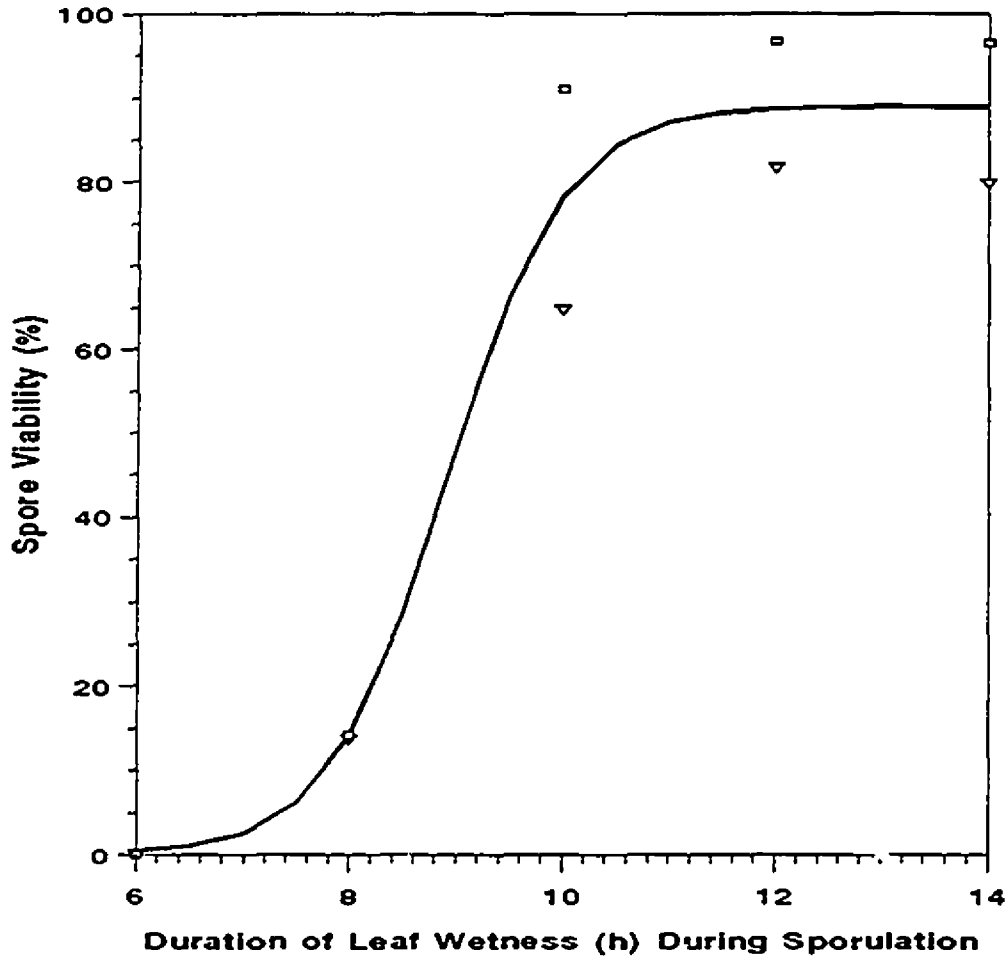


Fig. 7. Viability (% spore germination) of spores of *B. lactucae* produced at different durations of wetness (pooled for different temperatures-see Fig. 6). The solid line represents the values predicted by the logistic model (eq. 5) and the ■ and ▸ represents the observed values from the first and second experimental replicate, respectively. No spores were observed at 4 h of duration of leaf wetness and the spore viability at 6 h was zero at all temperatures.

of leaf wetness (Table 4). High sporulation was observed when DLW was at least 10 h or more.

The field validation test was conducted 11 times and out of this in four occasions no sporulation was observed. Very low amounts of sporulation were measured in tests 3, 7 and 10 and very high amounts in tests 1, 4 and 8 where the duration of the wet period was more than 10 h.

The amount of sporulation observed under field conditions was compared to the amount of sporulation predicted by the model for sporulation by substituting the values for temperature, leaf wetness duration and RH measured in the field. In general the model predicted eight out of eleven times no, low, medium or high sporulation. Two of the predicted values were overestimated and one was underestimated. By running a correlation analysis to compare the predicted to the observed values in the field we received 80% correlation. A difference was observed in test 9 where low sporulation was predicted by the model but no sporulation was observed in the field. In this case as well as in test 10, the wetness period was interrupted by a dry period. The dry period was not included in the total DLW if the relative humidity during this dry period was less than 88%.

When relative humidity was used to estimate the amount of sporulation in the field somewhat different results were obtained (Table 4). Here the model predicted 7 of the 11

observed values in the field where two were overpredicted and two were underpredicted.

Table 4. Observed sporulation in the lettuce field at Macdonald Horticulture Farm[a] and predicted sporulation by the model for the temperature, duration of leaf wetness (DLW) and relative humidity.

Field Validation Test	Average Temperature (C)	Duration Leaf Wetness (h)	RH≥88% (h)	Sporulation[c] Observed	Predicted (Wet period)	Predicted (RH≥88%)
1.	18.2	13	8	H (1.0)	H (0.61)	L (0.13)
2.	16.4	1	0	N (0)	N (0)	N (0)
3.	12.6	6	0	L (0.02)	L (0.01)	N (0)
4.	12.4	11.5	9	H (0.58)	H (0.77)	H (0.54)
5.	11.8	12	8	M (0.20)	H (0.77)	M (0.34)
6.	11.4	0	4	N (0)	N (0)	N (0)
7.	12.8	0	6.5	L (0.02)	N (0)	L (0.01)
8.	10.1	10.5	11	H (0.85)	H (0.74)	H (0.75)
9[b].	9.6	8	7.5	N (0)	L (0.14)	L (0.06)
10[b].	12.3	7.5	9	L (0.04)	L (0.08)	H (0.06)
11.	14.1	2.5	0	N (0)	N (0)	N (0)

[a] Lettuce plants were inoculated at the two leaf stage and incubated in a growth chamber for 7 days and were placed in the lettuce canopy in the evening. The following morning the plants were observed for sporulation.

[b] Duration of leaf wetness interrupted for two hours or more and RH < 88%. In this case the dry period was not included in the total duration of leaf wetness.

[c] Sporulation is the predicted proportion of maximum sporulation (PMS) observed, and those predicted from the model using DLW or RH and temperature. N, L, M and H is respectively no, low, medium and high sporulation based on arbitrary estimation of the obtained values; N = 0; L = 0 - 0.2; M = 0.2 - 0.5; H ≥ 0.5. The actual PMS observed and predicted are given in the parenthesis.

V. DISCUSSION

The isolate of *B. lactucae* used in our study is apparently a new pathotype. It has a different virulence phenotype from isolates characterized in California (Schettini et al., 1991). California isolates of *B. lactucae* were classified into four main pathotype groups none of which was similar to the phenotype of our isolate. The main difference was the reaction of the pathogen to *Dm* gene 3 and *Dm* gene 5/8. The presence of these genes in the lettuce lines inhibited sporulation of our isolate while it did not seem to inhibit sporulation of most of the California isolates. Difference in the phenotype was observed also with the isolates of *B. lactucae* from Florida (Datnoff et al, 1994). However, we are not naming our isolate a different race or pathotype because of the great variability of this pathogen. At least 14 *Dm* genes exist by now and potentially 214 possible virulence phenotypes. A new race or pathotype should be designated if this particular virulence phenotype is detected repeatedly over a wide geographical area (Michelmore, personal communication, 1995).

Michelmore and Oswaldo (1995) observed oospores of the pathogen in commercial lettuce in California and reported the appearance of new isolates of *B. lactucae* that do not fit the established pathotype classification. Sexual

reproduction of *B. lactucae* was observed also in the region of New York (Yuen and Lorbeer, 1987) and both compatibility types were found in field-collected material. As Quebec is not far from the area of New York this suggests a probability that two mating types of *B. lactucae* could be found in the province and production of oospores is possible. This can create a variation in the pathosystem of the pathogen. No research has been performed at the moment in Quebec to determine how variable the pathogen is in this region. The identification of only one isolate of *B. lactucae* is not enough to establish that. To prove our observations and assumptions many more isolates should be characterized.

A model to predict sporulation of *B. lactucae* has not been previously developed. However, research has been done on the amount of sporangia produced at different temperatures and different relative humidities. Scherm and van Brugen (1991) reported that sporangia were produced abundantly at temperatures between 10 and 20 C and RH > 90% for 7 h or more, which agree with our results. However they reported also the beginning of spore formation after only 3 h of high relative humidity, while we did not observe any sporulation before 6 h. This could be explained by the fact that for their study they used cotyledons and not true leaves, the former are more susceptible to downy mildew. Our experiment was conducted with lettuce plants at the first

true leaf stage because the true leaves represent field infection better than cotyledons.

Our results indicated maximum sporulation at 10-15 C within a range of 5-25 C. At 30 C we did not observe any sporulation in our preliminary experiment. We did not test temperatures less than 5 C because the temperature in Quebec does not go that low during the growing seascn. Several authors have reported sporulation of *B. lactucae* in relation to temperature during the moist dark sporulation period. According to Powlesland (1954) the range for sporulation was 4-20 C with an optimum at 6-11 C while Verhoef (1960) observed sporulatien in the range of 5-24 C with an optimum of 20 C.

Sporulation of *B. lactucae* is a process greatly inhibited by light. The results from our preliminary experiment indicated no sporulation in conditions of continuous light with a light intensity of 250 μm (approx. 22 500 lux). Similarly, Raffray and Sequeira (1971) showed complete inhibition of sporulation for 24 h at a light intensity of about 21 700 lux. According to their results six hours of initial darkness were enough for maximum sporulation. Verhoeff (1960) also reported an inhibiting effect of light on the sporangiophore production although according to his results the process was not completely inhibited in a light intensity of 10 000 lux. In this study a continuous darkness of 14 h was considered, although these

conditions do not occur during the growing season of lettuce in Quebec. Our purpose was to create optimal conditions for the fungus to sporulate, until it reaches its maximum. Also, on a cloudy day at the lower leaf surface the light intensity, especially the UV range, may be low enough to support sporulation.

Although high sporulation was observed at 10 and 15 C, spore viability at these temperatures was not the highest. At 10 C after 10 h we observed 960 sporangia/mm^2 but only 73% of them germinated and the percentage of viable spores did not increase after 12 or 14 h. Conversely the amount of sporulation at 25 C was considerably low after 12 h (158 sporangia/mm^2) but their viability was 92%. However, the proportion of viable spores increased significantly after 10 h of leaf wetness for all temperatures. We assume that the lower spore viability at 8 h of DLW can be explained by a higher amount of immature spores which fail to germinate. Furthermore, to confirm our assumptions, spores that we obtained in 6 h had zero viability, so obviously 6 h of leaf wetness were not enough for the spores to mature. These spores were much smaller in size than the usual, normal spores. We harvested these spores during the procedure of the experiment, but in nature rain drops or water splashes may not cause the immature spores to liberate at that high rate. The amount of immature spores was high at 10 and 15 C because the level of sporulation was high as a whole with

more spores that failed to germinate immediately. Spore viability of 90-100% was often observed after 10 h of DLW at almost all temperatures which may indicate that about 10 h are enough for sporangia of *B. lactucae* to reach full maturity. Lalancette et al. (1988) reported a similar relationship between sporulation and spore viability with spores of *Plasmopara viticola* (downy mildew on grape). They observed almost maximum level of sporulation after about 12 h but only 50-70% of the spores were capable of germinating. Thus they suspected that at shorter duration, the majority of sporangia were too immature to germinate.

The Richards function gave a good fit to the pooled data from both experimental replications. However because high sporulation was observed at 10, 15 and 20 C and these temperatures are quite common during lettuce production in the field in Quebec, especially at night, we tried to provide a better fit of the model particulary for these three temperatures. The model slightly overestimated sporulation at 5 C. Venus and Causton (1979) received better results in closeness of fit when the Richards model was used, in comparison to polynomials for two sets of data. They concluded that regarding the statistical criteria there is no difference in choosing between the Richards function and polynomials but as far as the plant growth is concerned the trend of the curve derived from the Richards function are less artificial. By using the Richards function,

Lalancette et al. (1988) obtained a good fit to the data for three experimental replicates. However the absolute values of the asymptote and the rate parameters increased for each replicate so that the predicted values for the infection efficiency were lowest for replicate one and highest for the replicate three. The nonlinearized function of the Richards model provided accurate prediction of infection (Carisse and Kushalappa, 1990) and a significant relationship was observed between the asymptote parameter and temperature and between the rate parameter and temperature. They obtained a high coefficient of determination by fitting the Richards function to the data for all models, even though the model overestimated infection at 20 and 24 C and underestimated infection at 16 C.

The validation of the model in the field gave a good prediction of sporulation with a relatively high coefficient of correlation (80%) between the values predicted by the model and those observed in the field. However we met some difficulties when trying to estimate the duration of leaf wetness. In three of the tests the wetness period was interrupted by a dry period. We did not include the dry period in the total duration of leaf wetness if the relative humidity for these hours was less than 88%. Further research is necessary to establish exactly the effect of the interrupted wet period on the sporulation of *B. lactucae*. Also we received more precise prediction of sporulation in

the field when we used the data for leaf wetness instead of those for relative humidity. But since the growers are more familiar with the use of relative humidity this should be considered in further studies of downy mildew in the field.

According to Scherm and van Bruggen (1995) sporulation and infection can occur in one night. They observed that spore release usually begins early in the morning and during mornings with prolonged leaf wetness infection can also take place. Our study also proved that it is possible for spores to be produced at night and the plants to be infected on the same day if the wetness period continued in the morning. Only seven hours of DLW are necessary for sporulation to occur and to produce mature spores ready to germinate. Further more, only two hours of initial darkness are enough to initiate sporulation while the rest of the wetness period could be in light (Raffray and Sequeira, 1971). According to our field data during the summer of 1994, the period of high relative humidity usually starts after 2300 when it is already dark. These conditions can trigger sporulation and in seven hours spores ready for infection can be produced. If there is a prolonged leaf wetness in the morning, the infection process can also take place.

Research has shown that downy mildew spores cannot survive the high temperatures and low relative humidities during daytime that are often observed in the summer. However spore survival of *B. lacticae* in the field should be

tested to see if the spores formed during the previous nights are still able to cause infection.

Infection by *B. lactucae* can occur in the temperature range between 5-25 C, with very low infection at 25 and zero at 30 C (Scherm and van Brugen, 1993). Sporulation was observed also in the same temperature range. Thus temperature is not a limiting factor either for sporulation or for infection to occur. Conditions with relative humidity higher than 90% for about 12 h at night at temperature between 10 - 20 C could be considered favourable for the development of a *B. lactucae* epidemic.

A forecasting model was developed in California (Scherm and van Bruggen, 1995) based on environmental factors influencing infection. A fungicide spray was recommended if morning leaf wetness was 4 h or more. However this forecasting system did not include factors necessary for the sporulation process. They discovered that for the coastal area of California, with very high relative humidity at night, conditions for sporulation are always present and sporulation was not a limiting factor for development of the disease. According to the meteorological data for the region of Quebec, sometimes the duration of leaf wetness at night is only two or three hours, which is not enough for sporulation to take place. This was proved by our field experiment where sporulation was not observed in four out of eleven tests and in three of them the duration of wet period

was less than 3 h. Thus sporulation of *B. lactucae* plays a major role in the development of downy mildew epidemics in Quebec.

We believe that our model for sporulation together with the infection model could be successfully applied to the development of a forecasting system suitable for the conditions of Quebec.

LITERATURE CITED

Berger, R. D. 1988. The analysis of effects of control measures on the development of epidemics, pp. 137-156. In: Experimental techniques in plant disease epidemiology. J. Kranz and J. Rotem, Ed. Springer-Verlag, New York. 299 pp.

Berger, R. D. 1981. Comparison of the Gompertz and logistic equation to describe plant disease progress. Phytopathology 71:716-719.

Berger, R. D. 1977. Application of epidemiological principles to achieve plant disease control. Ann. Rev. Phytopathology 15:165-183.

Campbell, C. L., and Madden, L. V. 1990. Introduction to plant disease epidemiology. John Wiley & Sons, New York. 532 pp.

Carisse, O., Kushalappa, A. C., and Cloutier, D. C. 1992. Influence of temperature, leaf wetness and high relative humidity duration on sporulation of *Cercospora carotae* on carrot leaves. Phytopathology 80:1233-1238.

Carisse, O., and Kushalappa, A. C. 1990. Development of an infection model for *Cercospora carotae* on carrot based on temperature and leaf wetness duration. Phytopathology 80:1233-1238.

Cohen, Y., and Rotem, J. 1971a. Dispersal and viability of sporangia of *Pseudoperonospora cubensis*. Trans. Br. Mycol. Soc. 57:67-74.

Cohen, Y., and Rotem, J. 1971b. The effect of darkness and

moisture on sporulation of *Pseudoperonospora cubensis* in cucumbers. Phytopathology 61:265-268.

Cornford, C. A., and Pitt, D. 1985. The nature of virulence in isolates of *Bremia lactucae* from south Devon. Plant pathology 34:38-40.

CPVQ. 1987. Légumes. AGDEX 250-605.Consei des Productions Vegetals du Quebec. Ministère de l'Agriculture, des Pecheries et de l'Alimentation du Quebec pp. 48.

CPVQ. 1980. Légumes de serre. Agdex 290. Consei des Productions Vegetals du Quebec. Ministère de l'Agriculture, des Pecheries et de l'Alimentation du Quebec pp. 71.

CPVQ. 1978 Légumes. Agdex 250/20. Consei des Productions Vegetals du Quebec. Ministère de l'Agriculture, des Pecheries et de l'Alimentation du Quebec. pp. 85.

Crute, I. R. 1987. The occurrence, characteristics, distribution, genetics, and control of a metalaxyl-resistant pathotype of *Bremia lactucae* in the United Kingdom. Plant disease 763-767.

Crute, I. R. 1984. The integrated use of genetic and chemical methods for control of lettuce downy mildew (*Bremia lactucae* Regel). Crop protection 3:223-41.

Crute, I. R., and Dixon, G. R. 1981. Downy mildew diseases caused by the genus Bremia Regel, pp. 423-459. In: Downy mildews. Ed. D. M. Spenser, Academic Press, London.

Crute, I. R., and Norwood, J. M. 1981. The identification and characteristics of field resistance to lettuce downy mildew (*Bremia lactucae* Regel). Euphytica 30:707-717.

Crute, I. R., and Dickinson, C. H. 1976. The behaviour of *Bremia lactucae* on cultivars of *Lactuca sativa* and on other composites. Ann. appl. Biol. 82: 433-450.

Datnoff, L. E., Nagata, R. T., and Raid, R. N. 1994. Pathotyping of *Bremia lactucae* in Florida. Plant Disease 78:854-857.

Dickinson, C. H., and Crute, I. R. 1974. The influence of seedling age and development on the infection of lettuce by *Bremia lactucae*. Annals of Applied Biology 76:49-61.

Eenink, A. H. 1981. Partial resistance in lettuce to downy mildew (*Bremia lactucae*). Search for partially resistant genotypes and the influence of certain plant characters and environments on the resistance level. Euphytica 30:619-628.

Eisensmith, S. P., and Jones, A. L., 1981. A model for detecting infection periods of *Coccomyces hiemalies* on sour cherry. Phytopathology 71: 728-732.

Fry, W. E. 1982. Principles of plant disease management. Academic Press, New York. 378 pp.

Gaudreau, L., Charbonneau, J., Vezina, L., and Gosselin, A. 1994. Photoperiod and photosynthetic photon flux influence growth and quality of greenhouse-grown lettuce. HortScience 29:1285-1289.

George, R. A. T. 1985. Vegetable seed production. Longman. London and New York. pp. 320.

Gustafsson, M., Liljeroth, E., and Gustafsson, I. 1985. Theor. Appl. Genet. 70:643-649.

Hau., B. 1988. Modelling epidemics of polycyclic foliar diseases and development of simulators, pp. 267-278. In: Experimental techniques in plant disease epidemiology. J. Kranz and J. Rotem, Ed. Springer-Verlag, New York. pp. 299.

Hildebrand, P. D., and Sutton, J. C. 1984. Interactive effects of the dark period, temperature and light on sporulation of Peronospora destructor. Phytopathology 74:1444-1449.

Ilott, T. W., Hulbert, S. H., and Michelmore, R. W. 1989. Genetic analysis of the gene-for gene interaction between lettuce (*Lactuca sativa*) and *Bremia lactucae*. Phytopathology 79:888-897.

Ilott, T. W., Durgan, M. E., and Michelmore, R. W. 1987. Genetics of virulence in California populations of Bremia lactucae (lettuce downy mildew). Phytopathology 77:1381-1386.

Jesperson, G. D., and Sutton, J. C. 1987. Evaluation of a forecaster for downy mildew of onion (*Allium cepa* L.). Crop protection 6:95-103.

Johnson, A. G., Laxton, S. A., Crute, I. R., Gordon, P.L., and Norwood, J. M. 1978. Further work on the genetics of race specific resistance in lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). Ann. appl. Biol. 89:257-264.

Lalancette, N., Ellis, M. A., and Madden, L. V. 1988. Development of an infection efficiency model for *Plasmopara viticola* on American grape based on temperature and duration of leaf wetness. Phytopathology 78:794-800.

Lalancette, N., Madden, L. V., and Ellis, M. A. 1988. A

quantitative model for describing the sporulation of Plasmopara viticola on grape leaves. Phytopathology 78:1316-1321.

Lebeda, A., and Reinink, K. 1991. Variation in the early development of Bremia lactucae on lettuce with different levels of field resistance. Plant Pathology 40:232-237.

Lovelock, Y. 1972. The vegetable book. An Unnatural history. St. Martin's press, New York. pp 124.

Madden, L. V., and Ellis, M. A. 1988. How to develop plant disease forecasters, pp. 191-208. In: Experimental techniques in plant disease epidemiology. J. Kranz and J. Rotem, Ed. Springer-Verlag, New York. 299 pp.

Magarey, P. A., Wchtel, M. F., Weir, P. C., and Seem, R. C. 1991. A computer-based simulator for rational management of grapevine downy mildew (*Plasmopara viticola*). Plant protection quarterly 6:136-141.

Magboul, A. M., Geng, S., Gilchrist, D. G., and Jackson, L. F. 1992. Environmental influence on the infection of wheat by Mycosphaerella graminicola. Phytopathology 82:1407-1413.

Marlatt, R. T., McKittrick, and Lewis, R. W. 1966. Colonization and survival of *Bremia lactucae* in lettuce. Phytopathology. 56:856-857.

Michelmore, R. W., and Oswaldo, E. 1995. Lettuce breeding. Iceberg lettuce advisory board, pp. 35-59. In: Annual report, April 1, 1994, to March 31, 1995, Davis, California.

Michelmore, R. W., Ilot, T., Hulbert, S. H., and Farrara, B. 1988. The downy mildews.Advances in plant pathology 6:53-79.

Michelmore, R. W, and Crute, I. R. 1982. A method for determining the virulence phenotype of isolates of *Bremia lactucae*. Trans. Br. Mycol. Soc. 79:542-546.

Michelmore, R. W., and Ingram, D. S. 1982. Secondary homothallism in *Bremia lactucae*. Trans. Br. mycol. Soc. 78:1-9.

Milbrath, D. G., 1923. Downy mildew of lettuce in California. Journal of Agricultural Research 23:989-994.

Montesinos, E., Moragrega, C., Llorente, I., Vilardell, P., Bonaterra, A., Ponti, I., Bugiani, R., Cavanni, P., and Brunelli, A. 1995. Development and evaluation of an infection model for *Stemphylium vesicarium* on pear based on temperature and wetness duration. Phytopatology. 85:586-592.

Morgan, W. M. 1984. Integration of environmental and fungicidal control of Bremia lactucae in a glasshouse lettuce crop. Crop protection 3:349-361.

Mudita, I. W., and Kushalappa, A. C. 1991. Effect of media and temperature on sporulation of *Septoria apiicola*, and of inoculum density on septoria blight severity in celery. Phytoprotection 72:97-103.

Norwood, J. M., Michelmore, R. W., Crute, I. R., and Ingram, D. S. 1993. The inheritance of specific virulence in *Bremia lactucae* (downy mildew) to match resistance factors 1, 2, 4, 6 and 11 in *Lactucae sativa* (lettuce). Plant pathology. 32:177-186.

Palti, J., and Rotem., J. 1981. Control of downy mildews by cultural practices, pp. 289-304. In: Downy mildews, Ed. D. M. Spenser, Academic Press, London.

Paterson, C. L., Grogan, R. G., and Campbell, R. N. 1986. Economocally important diseases on lettuce. Plant disease 70:982-987.

Pegg, G. F., and Mence, M. J. 1972. The biology of *Peronospora viciae* on pea: the development of local and systemic infection and their effect on vining yield. Ann. Appl. Biol. 71:19-31.

Powlesland, R. 1954. On the biology of *Bremia lactucae*. Trans. Br. Mycol. Soc. 3:362-371.

Raffray, J. B., and Sequeira, L. 1971. Dark induction of sporulation in *Bremia lactucae*. Can. J. Bot. 49:237-239.

Rotem, J. 198[illegible]. Quantitative assessment of inoculum production, dispersal, deposition, survival and infectiousness in airborne diseases, pp. 69-84. In: Experimental techniques in plant disease epidemiology. J. Kranz and J. Rotem, Ed. Springer-Verlag, New York. 299 pp.

Rotem, J., Blickle, W., and Kranz, J. 1988. Effect of environment and host on sporulation of *Alternaria macrospora* in cotton. Phytopathology 79:263-266.

Royle, D. J. 1976. Downy mildews and weather. Annals of applied Biology 81:437-444.

SAS Institute Inc. 1987. SAS/STAT Guide for Personal Computers. Version 6 edition. SAS Institute Inc., Cary, NC. 108 pp.

Sauriol, P. 1990. La laitue est bien malade. Le producteur agricole. Novembre p.38-40.

Scherm, H., and van Brugen, A. H. C. 1995. Concurrent spore release and infection of lettuce by *Bremia lactucae* during mornings with prolonged leaf wetness. Phytopathology 85:552-555.

Scherm, H., Koike, S. T., Laemmlen, F. F., and van Bruggen, A. H. C. 1995. Field evaluation of fungicide spray advisories against lettuce downy mildew (*Bremia lactucae*) based on measured or forecast morning leaf wetness. Plant disease. 79:511-516.

Scherm, H., and van Bruggen, A. H. C. 1994a. Effects of fluctuating temperatures on the latent period of lettuce downy mildew (*Bremia lactucae*). Phytopathology 84:853-859.

Scherm, H., and van Bruggen, A. H. C. 1994b. Weather variables associated with infection of lettuce downy mildew. Phytopathology 84:860-865.

Scherm, H., and van Bruggen, A. H. C. 1993. Responsesurface models for germination and infection of *Bremia lactucae*, the fungus causing downy mildew of lettuce. Ecological Modelling 65:281-286.

Schettini, T. M., Legg, E. J., and Michelmore, R. W. 1991. Insensitivity to metalaxyl in California populations of *Bremia lactucae* and resistance of California lettuce cultivars to downy mildew. Phytopathology 81:64-70.

Schwinn F. J. 1981. Chemical control of downy mildews, pp. 305-320. In: The Downy mildews, Ed. D. M. Spenser, Academic press, New York.

Statistics Canada. 1995. Fruit and vegetable production. Catalogue 22-003 Seasonal p.24.

Sutton, J. C., James, T. D. W., and Rowell, P. M. 1986. Botcast: A forecasting system to time the initial fungicide spray for managing botrytis leaf blight of onions. Agriculture, Ecosystems and Environment, 18:123-143.

Thomas, C. S., Marois, J. J., and English, J. T. 1988. The effects of wind speed, temperature and relative humidity on development of aerial mycelium and conidia of Botrytis cinerea on grape. Phytopathology 78:260-265.

Trimboli, D. S., and Crute, I. R. 1983. The specific virulence characteristics of *Bremia lactucae* (lettuce downy mildew) in Australia. Aust. Pl. Pathology 12:58-60.

Van Der Plank, J. E. 1963. Plant diseases: Epidemics and control. Academic Press, New York. 349 pp.

Van Bruggen, A. H. C. 1991. Epidemiology and control of downy mildew on lettuce, pp. 72-80. In: Annual report April 1, 1990 through March 31, 1991, Davis, California.

Venus, J. C., and Causton, D. R. 1979. Plant growth analysis: the use of the Richards function as an alternative to polynomial exponential. Annals of Botany 43: 623-632.

Verhoeff, K., 1960. On the parasitism of Bremia lactucae Regel on lettuce. Tijdschr. Planteziekten, 66:133-203.

Vincelli, P. C., and Lorbeer, J. W. 1989. BLIGHT-ALERT:A weather-based predictive system for timing fungicide applications on onion before infection periods of *Botrytis squamosa*. Phytopathology 79:493-98.

Wicks, T. J., Hall, B., and Pezzaniti, P. 1993. Fungicidal control of downy mildew (*Bremia lactucae*) on lettuce.

Australian journal of experimental agriculture 33:381-384.

Wild, H., 1946. Downy mildew disease of the cultivated lettuce. Transaction British Mycological Society 31:112-125.

Yarwood, C. E. 1937. The relation of light to the diurnal cycle of sporulation of certain downy mildews. J. agric. Res. 54:365-373.

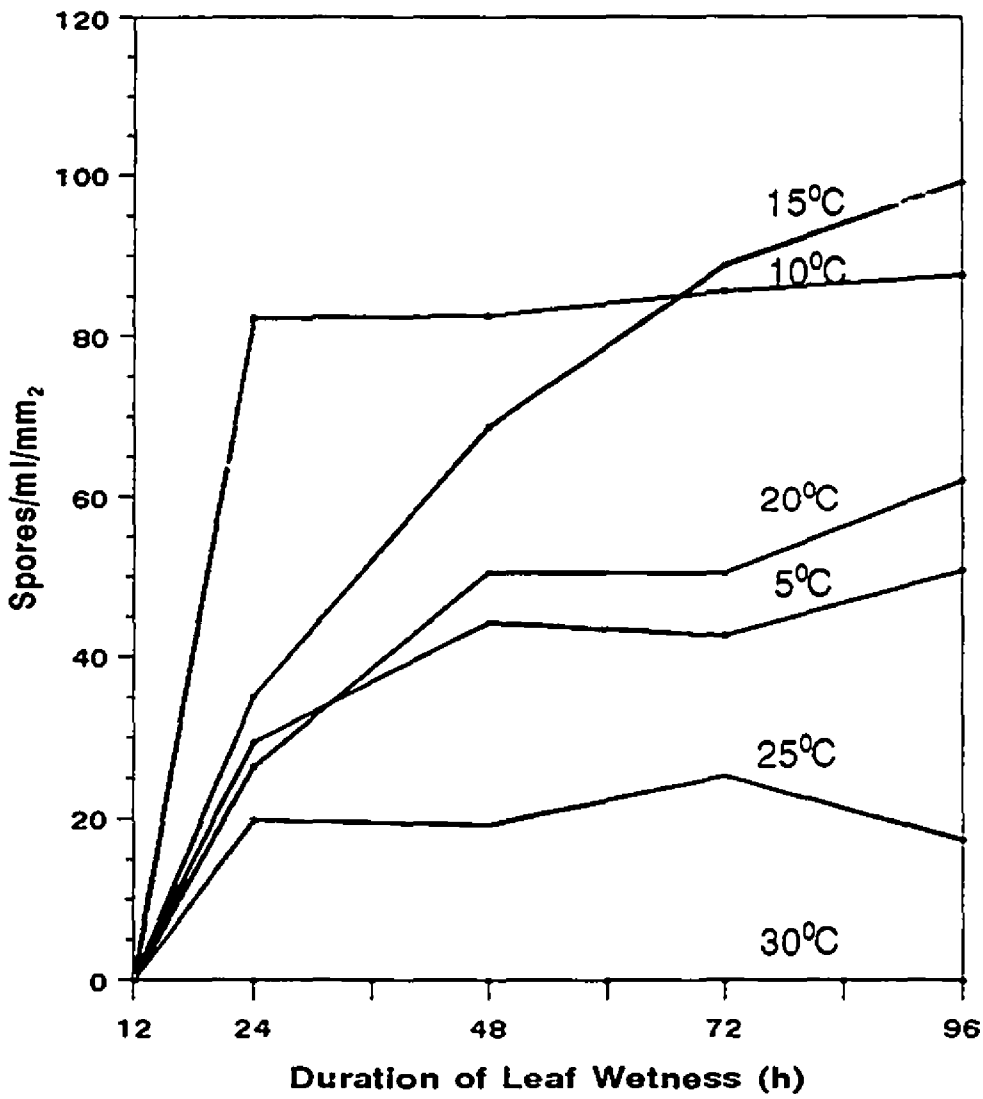
Yuen, J. E., and Lorbeer, J. W. 1987. Natural and experimental production of oospores of *Bremia lactucae* in lettuce in New York. Plant disease 71:63-64.

Yuen, J. E., and Lorbeer, J. W. 1984. Field resistance of crisphead lettuce to *Bremia lactucae*. Phytopathology 74:149-152.

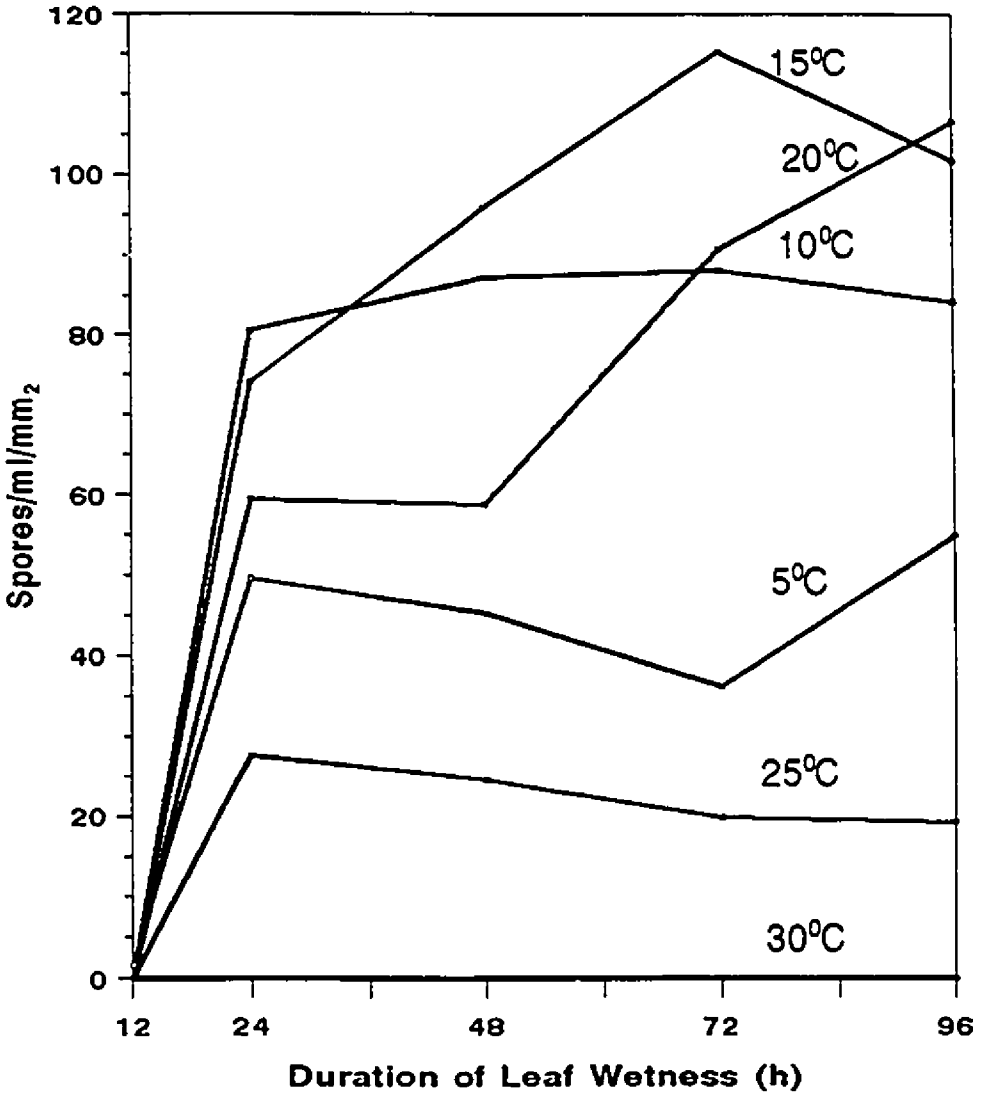
Yuen, J. E., and Lorbeer, J. W. 1983. Metalaxyl control of downy mildew and supplements for horizontal resistance to *Bremia lactucae* in lettuce grown on organic soil in New York. Plant disease 67:615-618.

Zadoks, J. C., and Schein, R. D. 1979. Epidemiology and plant disease management. Oxford University Press, New York. 428 pp.

APPENDICES



Appendix 1. Observed sporulation of *B. lactucae* on lettuce seedlings at five durations of leaf wetness (12-96 h) and six temperatures (5-30 C) for the first experimental replicate.



Appendix 2. Observed sporulation of *B. lactucae* on lettuce seedlings at five durations of leaf wetness (12-96 h) and six temperatures (5-30 C) for the second experimental replicate.

Appendix 3. Predicted proportion of maximum sporulation of *B. lactucae* using Richards function (equation 1) at different temperatures and duration of leaf wetness.

TEMP (C)	Duration of Leaf Wetness (h)									
	4	5	6	7	8	9	10	11	12	13	14
5	0	0	0	0.01	0.03	0.13	0.27	0.32	0.33	0.34	0.34
6	0	0	0	0.01	0.06	0.23	0.44	0.55	0.52	0.52	0.52
7	0	0	0	0.01	0.08	0.32	0.56	0.62	0.63	0.63	0.63
8	0	0	0	0.02	0.11	0.40	0.63	0.69	0.70	0.70	0.70
9	0	0	0	0.02	0.13	0.45	0.68	0.73	0.74	0.74	0.74
10	0	0	0	0.03	0.15	0.49	0.71	0.75	0.76	0.76	0.76
11	0	0	0	0.03	0.16	0.52	0.73	0.76	0.77	0.77	0.77
12	0	0	0	0.03	0.17	0.53	0.73	0.77	0.78	0.77	0.77
13	0	0	0	0.03	0.18	0.54	0.73	0.76	0.76	0.76	0.76
14	0	0	0	0.03	0.18	0.53	0.71	0.74	0.75	0.75	0.75
15	0	0	0	0.03	0.17	0.52	0.69	0.72	0.73	0.73	0.73
16	0	0	0	0.03	0.16	0.50	0.66	0.69	0.70	0.70	0.70
17	0	0	0	0.03	0.15	0.50	0.62	0.66	0.66	0.66	0.66
18	0	0	0	0.02	0.13	0.42	0.59	0.62	0.62	0.62	0.62
19	0	0	0	0.02	0.11	0.37	0.54	0.57	0.57	0.58	0.58
20	0	0	0	0.02	0.09	0.32	0.48	0.52	0.52	0.52	0.52
21	0	0	0	0.01	0.07	0.26	0.42	0.46	0.46	0.46	0.46
22	0	0	0	0.01	0.05	0.20	0.35	0.39	0.39	0.39	0.39
23	0	0	0	0.01	0.04	0.14	0.27	0.31	0.32	0.32	0.32
24	0	0	0	0	0.02	0.09	0.19	0.23	0.24	0.24	0.24
25	0	0	0	0	0.01	0.05	0.11	0.14	0.15	0.15	0.15