ETIOLOGY AND EPIDEMIOLOGY OF ONION DOWNY MILDEW CAUSED BY *Peronospora destructor* (Berk.) Casp. IN KENYA

BY

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DECLARATION

a. I, George Willis Odhiambo, declare that this thesis is my original work and has not been presented for a degree in any other University.

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Date 22/7/86

b. This thesis has been submitted for examination with our approval as University supervisors.

Prof. D.M. Mukunya

Date 4/8/86

Dr. E.M. Gathuru

Date 5/8/86

DEDICATION

To my late mother.

ACKNOWLEDGEMENTS

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ABSTRACT

Etiology and epidemiology of onion downy mildew in Kenya caused by Peronospora destructor (Berk.) Casp. were investigated. The fungal spore dimensions and morphology were determined using a phase contrast microscope fitted with a calibrated eyepiece micrometer and a camera. Growth chamber and laboratory experiments were conducted to determine the best conditions for sporangial germination, penetration and longevity, while field and shed house experiments were conducted to determine the incubation period, disease gradient and increase. Experiments to test the survival of the pathogen were conducted in the field, laboratory and shed house using diseased onion debris and seeds harvested from a diseased onion field.

Morphological studies showed that sporangia were 45.1-70.7 x 20.4-35.9 µ while oospore were 18.3-36.6 µ in diameter. Field observations showed characteristic downy mildew symptoms. Sporangia germinated best in rain water, poorest in glucose solution and not at all in onion leaf juice extract. The optimum temperature range for germination and sporulation was 10-14°C at 90-100% Relative Humidity (RH), and free water was a necessary prerequisite for both sporulation and sporangial germination.

Sporangia attached to excised leaf tissues and kept at 90-100% RH and 10-14°C remained viable for about 7 days. At 35-45% RH and 20-24°C they remained

viable for about 2 days while exposure to direct sunlight killed them within one hour. Detached sporangia however remained viable for about 2 days at 90-100% RH and 10-14°C and about one day at 35-45% RH and 20-24°C. Sporangial germination was usually by one lateral germ tube which penetrated the host tissue only through stomata. The incubation period was estimated to be about 11-13 days and sporulating lesions continually produced sporangia for about 7 days before necrosis. Disease progress could be described by the compound interest equation and the epidemic could attain a high rate of 0.33 per unit per day. Temperature, rainfall and RH were important factors influencing disease increase in the field. Although oospores were observed, none of them could be induced to germinate at any of the conditions tested. Infection could not be obtained from onions planted in sterile soil mixed with diseased plant debris, neither could seed transmission be proved. Further work is recommended on the role of alternate hosts and survival spores on the life cycle of the pathogen in Kenya.

1. INTRODUCTION

Onion (*Allium cepa* L.) is a biennial vegetable grown as an annual and used for flavouring foods. It is also a rich source of vitamin C and calcium. Though there are many substitues to onion like garlic (*Allium sativum* L.), shallots (*Allium ascalonicum* L.) and leeks (*Allium porrum* L.), the production of the latter three is negligible compared to the former, because of the popularity of onion in making stews, soups and many other recipes. (Anonymous, 1982).

The most popular variety and the one used in this project is the 'Red Creole', whose smooth and round bulbous architecture and red colour has a distinct appeal. Indeed most of the onion sold in urban and semi-urban areas in Kenya is the 'Red Creole' variety though one may also find 'White Creole', leeks and the bulbless 'multiplier onions', the latter being a dominant feature of backyard gardens in most rural homes.

In Kenya onions are propagated through seeds which can be sown directly or planted in a seedbed before transplanting. The crop can take up to six months before maturity. (Anonymous, 1976).

Most commercially grown onions in Kenya are grown under irrigation by small scale farmers through the management of National Irrigation Board (N.I.B) and marketed through H.C.D.A. (Horticultural Crops Development Authority). According to the HCDA Annual Reports for July 1980 - June 1981, about 2.67 kilogrammes were marketed from H.C.D.A. Of this annual

production about 60% was produced in Loitokitok while the rest came from Perkerra Irrigation Scheme in Baringo District, Naivasha and other miscellaneous areas like Bungoma, Machakos, Nanyuki, Kisii, South Nyanza, Meru and Kirinyaga where rainfed onion crops were produced in small quantities.

Like other crops, onions are susceptible to various diseases and physiological disorders. One of the main economically important onion diseases is downy mildew caused by Peronospora destructor (Berk) Casp. (Yarwood, 1943). It is cosmopolitan in distribution and yield loss assessment records show that it can reduce yields by as much as 15-27% in bulbs and 46-100% in seed crops (Jones, 1939).

Onion downy mildew was first recorded in Kenya in 1958 at Koru in Western Kenya (Anonymous, 1959). Since then the disease had passed as economically less important because of its low incidence and severity. However, for the years 1975 - 1980, statistics at the Plant Pathology Advisory Laboratories, Nairobi, showed that the disease accounted for over half the number of onion samples received after diagnosis every year (Anonymous, 1975) and could with time be a major constraint in onion production. However, in Kenya very little is known about the pathogen and its biology and because such knowledge would be an important prerequisite for effective control of the disease, this project was undertaken, to study: the disease symptoms, morphology and signs of the pathogen; sporangial longevity and factors favouring

their germination; prepenetration and associated phenomena; disease progress and factors favouring it and the survival and source of primary inoculum.

2. LITERATURE REVIEW

2.1.. Etiology

Peronospora destructor (Berk.) Casp. the causal organism of onion downy mildew is an obligate parasite in the family Peronosporaceae under the order Peronosporales of the class Oomycetes (Alexopoulos, 1962). As other members of the class it reproduces sexually by oval shaped oospores measuring 15.8 - 43.2 μ which under favourable conditions germinate by germ tubes. Asexual reproduction is by lemon shaped sporangia measuring 53.4 - 61.7 x 24.2 μ (Jovicevic, 1964). Sporangia are produced on dichotomously branched and determinate sporangiophores. These sporangia always germinate by germ tubes (Alexopoulos, 1962).

Peronospora destructor parasitises onion tissues intercellularly deriving nutrients using haustoria which when mature curl and divide and measure 80 - 100 x 2.3 - 5 μ (Ikata & Yamauti, 1941). This absorption of nutrients can result into a green weight reduction of upto 55% per night, and this green weight reduction has a positive correlation with the reduction in yield mentioned earlier (Yarwood, 1941).

2.2 Symptoms and signs

The most characteristic feature of onion downy mildew is the development under humid conditions of a grey 'downy' growth on the leaves hence the name. This superficial growth which consists of sporangiophores and sporangia of the fungus arises from the substomatal

air spaces in the leaf tissue (Wheeler, 1972).

In the early part of the season, yellowing, paling, curling and narrowing of the leaves is typical, while in the later part of the season, large oval necrotic lesions on leaves and seed stalks are present (Yarwood, 1943). These lesions always start on the older leaves and develop in the presence of free moisture (Chupp and Sherf, 1960).

2.3 Epidemiology

2.3.1. Sporangial dissemination

Dissemination of sporangia of onion downy mildew is by wind. Viranyi (1975a) showed that a close correlation was frequently observed between the direction of the prevailing wind and the spread of the disease and local air convection to a height of at least 0.5 m was important in the dissemination of sporangia. Newhall (1938) observed that even though conditions were abnormally wet in New York State, onion downy mildew sporangia were caught in the air over diseased fields up to a height of 457.5 m and out of the 132 caught, 75% were viable and germinated. Yarwood (1943) had also showed that these sporangia were wind dispersed.

2.3.2. Sporulation

The effect of light, temperature, moisture and relative humidity (RH) on sporulation of the fungus has been extensively studied by various investigators.

Viranyi (1974), found that the incubation period was 11-14 days and previous exposure to light and at least 95% RH were essential for sporulation. The infection cycle took two humid nights with the optimum temperature at 11-13^{O}C.

Katterfeld (1926), found that the incubation period ranged from 10-15 days in the green house and 13-18 days in the field.

Cook (1932), from field observations, found that the development of the mildew is favoured by abundant moisture and relatively low temperatures. In the laboratory, he found that the fungus sporulated over a wide range of temperatures, free water on the leaves being a necessary condition. The incubation period was 11-15 days.

Yarwood (1937 b), also showed that sporulation took place at night, sporangia maturing in the early morning and being liberated throughout the day. He found the optimum relative humidity for sporulation to be about 100% and the minimum to be about 90% while the minimum temperature was 4-7^{O}C, maximum 22-25^{O}C and optimum 13^{O}C. At low RH, sporangiophores were shorter than at higher RH. From studies on the relation of light to the diurnal cycle of sporulation, Yarwood (1937 b) showed that onion downy mildew failed to sporulate when infected leaves were exposed to artificial light (170 ft-candles from a Mazda lamp). Most sporulation took place when infected leaves or plants were placed in darkened moist chambers in the late afternoon or evening. Poor sporulation or none at all occurred when the leaves or plants were

placed in these chambers in the early morning. Exposure for 12 hr or more to darkness at low humidity and different temperatures also inhibited sporulation. These findings were considered to indicate that during sporulation, light was unfavourable to the process and that the normal diurnal cycle of sporulation of the fungus in nature is in part an adaptation to the alternation of light and darkness in the normal day. The nocturnal sporulation was therefore thought to be directly dependent on the darkness and high humidity frequently coincident at night.

2.3.3. Sporangial germination

Like sporulation, sporangial germination has been extensively studied with different results. Viranyi (1974) showed that germination only occurred in free water at 6-27°C with optimum between 10-12°C. The first germ tubes developed in 2-4 hr under favourable conditions.

Katterfeld (1926) found that at temperatures ranging from 12.5-15°C, about 85% of viable sporangia germinated within 2½ hr and after 5 hr all sporangial had germinated. In all cases, germination was by germ tubes measuring up to 1700 x 5-10.5 μ and always penetrated the host through stomata.

Cook (1932) found that sporangia germinated best in lake water and within temperatures ranging from 3-27°C with the optimum at 11°C.

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Literature review * life cycle

Yarwood (1937, 1943) also showed that sporangia required free water for germination and the germ tubes always entered the host through stomata. He observed that inside the host, mycelia grew at the rate of 300 μ/hr.

2.3.4. Sporangial longevity

Yarwood (1943) showed that sporangia, when attached to living leaves, remained viable for about 3 days but when detached only remained viable for up to one day. He, however, did not indicate under what conditions these observations were made.

Viranyi (1974) found that sporangia exposed to direct sunlight lost their viability within a few hours but those kept in the shade germinated after 24 hr..

Katterfeld (1926) found that sporangia remained viable for up to 10 days in damp air but on exposure to sunshine lost their viability within 2 hr.

2.4. Perpetuation of Peronospora destructor

2.4.1. Resting mycelia

It is certain that resting mycelia in onion bulbs act as primary inoculum in agricultural systems where onions are propagated through bulbs (McKay, 1957). The perpetuation of the fungus in those systems where onions are propagated through seeds however appears to be uncertain.

Murphy (1921) concluded that it was erroneous to conclude that since onion downy mildew produced oospores on the host, it was perpetuated by oospores yearly. He found the non-septate mycelium in the bulbs to be capable of a perennial existence in the bulbs and the shoots produced there from developed systemic infection. It was however impossible to induce the fungus to fruit on the infected bulb scales. Infection from bulb scales also failed to be transmitted into healthy tissue or produce mycelia on artificial media. The whole mycelial structure was easily visible without staining. The hyphae were stout and differentiated from the host into which some of the large convoluted haustoria extended. It was possible to trace the mycelium from the bulbs to the apical portions of the leaf on which sporangiophores were being produced (Murphy, 1921). Im one badly mildewed plot almost 66% of small sized onions contained non-septate mycelia.

Although such infected bulbs sprouted prematurely, there was no sporulation in the greenhouse under winter conditions though the mycelia grew up with some of the shoots. These systemically infected plants, when placed under favourable conditions overnight, sporulated the next day.

Murphy and McKay (1926), showed that the perennial mycelium is quite common in Ireland compared to oospores, and evidently play a major role in perpetuating the organism.

Katterfeld (1926) also found similar results from his observations on the role of perennial mycelia in the perpetuation of the disease. He found mycelia in 42-45% of the cases he examined. These mycelia were more abundant towards the apex of the bulbs and were differentiated from the summer ones by their thicker walls and presence of numerous large oil drops. These mycelia remained dormant during winter. Microscopic examination of infected bulbs at two monthly intervals showed no increase in length. On planting under favourable conditions of moisture and temparature (about 25°C), the mycelia immediately started to grow especially towards the apex of the leaf sporulating 9 days after planting the bulbs.

The survival of the pathogen as mycelia in bulbs was also reported by Hiura (1930), Iosifescu (1974), Viranyi (1969 b) and Tsupkova (1976).

2.4.2. Oospores

The longevity and viability of oospores has been studied by various investigators with very inconclusive results. McKay (1957) indicated that after separation from the host tissues, an after-ripening or maturation period of 2-3 years was necessary before the oospores could germinate. Although they could remain viable up to 24 years, the exact maximum viability time was unknown. Even then, these oospores on incoulation on onion plants caused no infection. This prompted him to infer that

some stimulus was necessary to break the long dormancy. Addition of 0.01-0.02% potassium permanganate to water containing 5-6 year old oospores weathered out of doors resulted into 60-85% germination within 48 hr at 15-20°C, compared to 0.05-1% in the controls.

McKay (1939) from other observations also showed that while in some seasons oospores were produced in enormous numbers, as many as 90% of the leaves containing up to 1176 oospores, germination was first observed when they were 4 years old. After 4 years and 5 months, it reached 1% at room temperature and 2% after 5 years. After 7 years it reached 5% after 48 hr at 20°C. In all cases germination was with a germ tube measuring up to 960 x 6-9 μ.

Takahashi (1958) also showed that a stimulus was needed to accelerate oospore germination. They showed that germination occured above 17°C in water, soil and seedling leaves at 20-30°C but not below 12°C or above 40°C and was accelerated by pretreatment with potassium permanganate.

2.4.3. Alternate hosts

Very little is documented on the role of alternate hosts in the perpetuation of onion downy mildew. Murphy and McKay (1926) postulated that in Ireland, autumn sown seed could carry inoculum through winter and that potato onions (*Allium cepa* var. *multiplicans*), shallots, onions grown for seed, Egyptian onions and possibly other

Allium spp. may be carriers of inoculum. Their observations indicated that the fungus from A. cepa readily attacked A. fistulosum, A. scorodoprasum and A. cepa bulbelliferum but under the same conditions, A. porrum, A. fistulosum, A. scorodoprasum, A. sativum, and A. choenoprasum did not contract the disease.

2.4.4. Seed infection

The role of seed as a source of primary inoculum is also not well covered in literature. Murphy and McKay (1926) from their observations found no evidence of seed transmission. Hiura (1930), although he observed fungal mycelia on flower parts like ovary, stamens and pistils, no infection was observed from plants sown from such seeds.

3. MATERIALS AND METHODS

3.1. Morphology and identification of Peronospora destructor

Onion leaves presumed to be infected with P. destructor were collected from five field in the following areas: National Agricultural Laboratories and Jamhuri Park Showgrounds in Nairobi, Limuru in Kiambu District, Nanyuki in Laikipia District and Loitokitok in Kajiado District.

Sporulation was induced on the infected leaves by placing them in a moist dish. The dish was covered and placed in a growth chamber at 95 ± 5% relative humidity (RH) and 14 ± 2°C for 48 hr. Infection by P. destructor was confirmed by microscopic examination of sporangiophores and sporangia harvested from the leaves, and mounted in water on a slide. The length and width of sporangia were measured using a phase contrast microscope fitted with a calibrated eyepiece micrometer and camera. Their photographs were also taken. For each location 100 sporangia were measured.

The presence of oospores was determined in dried diseased leaves. Twenty leaves were randomly collected from each field and 1cm^2 pieces randomly cut from each leaf. Each piece was divided into four equal parts each of which was cut into very thin sections and teased in tap water on a slide. The slides were examined under the microscope for the presence of oospores. Once located their diameters were measured and photographs taken. Twenty oospores from each location were measured.

3.2. Field symptoms and signs

A field of onions 10 x 10m in sixe was established at National Agricultural Laboratories, Kabete. Five diseased onion plants obtained from a farmer's field in Limuru in Kiambu District were planted in an alternating manner with healthy plants in the middle of the plot just transplanted. Daily observations were made to determine the incubation period (time when spores land on the infection court to the time when symptoms first appear) and subsequent disease development. Photographs were taken during the course of the epidemic to show different stages of symptom development. In addition, visits were made to farmers' fields to note any symptoms and signs that were different from those observed at National Agricultural Laboratories. These observations were made for the whole growing period of six months till harvesting.

3.3. Factors influencing Sporangial germination

3.3.1. Temperature

Sporangia harvested from fresh-sporulating detached onion leaves incubated 48 hr earlier in a moist dish kept in a growth chamber at 14 ± 2°C and 95 ± 5% RH, were mounted in tap water and the slides kept in a moist dish. The dish was covered and kept in the growth chamber at 4 ± 2°C and 95 ± 5% RH. Twenty hours later the slides were withdrawn from the chamber and examined

under a light microscope. For every slide, ten microscope fields chosen randomly were examined for sporangial germination.

Germination was deemed to have occured when the length of the germ tube was more than half the length of the sporangium. This procedure was repeated for every interval of 2°C in an ascending order up to 24 ± 2°C. For every treatment eight slides were similarly prepared.

3.3.2. Media

The following six treatments were tested for the best growth medium: Onion leaf juice extract (OLJE) prepared by grinding fresh onion leaves in a mortar and filtering through cheese cloth), rain water, 1 molar glucose solution, tap water, distilled water and sterile distilled tap water as a control.

Sporangia obtained from leaves as outlined in 3.3.1. were mounted in each of the six solutions on a microscope slide and placed in a moist dish in a growth chamber at 12 ± 2°C and 95±5% RH. Eight slides were prepared per treatment. Twenty hours later the slides were withdrawn from the chamber and examined for sporangial germination.

3.3.3. Optimum germination time

Sporangia obtained from detached sporulating leaves were mounted in rain water on each of 6 slides. Rain

water was used because it had given the highest germination in experiment 3.3.2. The slides were kept in a moist dish which was in turn placed in a growth chamber at 12 ± 2° and 95±5% RH. At one hourly intervals for 5 hr the slides were withdrawn and examined for sporangial germination. Exposure time was minimal so the effect of temperature and relative Humidity was insignificant. The examination was repeated after 12, 20, 24, 30 and 40 hr when germination seemed to be constant.

3.3.4. Free water

Sporangia were harvested from fresh sporulating onion leaves incubated for 48 hr in the growth chamber. Some sporangia were kept on a dry microscope slide, while others were mounted in rain water on a slide. The slides were kept in a covered moist dish and kept in a growth chamber at 12±2° and 95±5% RH. Twenty four hours later the slides were withdrawn from the chamber. The sporangia on the dry slide were mounted in rain water. Two sets of eight slides for both groups were examined for germination.

3.3.5. pH

Phosphate buffer solutions made by dissolving pH tablets in sterile distilled water were prepared with the following pH: 2, 4, 6, 8 and 14 with pH 7 as a control.

For every pH, eight slides were prepared and kept in a moist dish. The dish was kept in a growth chamber at 12±2°C and 95±5% RH for 24 hr before examination for sporangial germination.

3.3.6. Humidity

Eight cavity slides with sporangia mounted in rain water were placed in a dessicator in which was kept about 500 ml of water instead of the normal silica gel. A hygrometer was placed in the chamber. The dessicator was placed in a growth chamber at 12±2°C (the hygrometer reading was about 100% RH). Twenty four hours later these slides were withdrawn and examined to determine germination percentage. This process was repeated but water was replaced with silica gel. (The hygrometer reading was about 75±5% RH). The dessicator was placed in a growth chamber at 12±2°C, and left for 24 hr before examination.

3.4. Sporangial longevity

3.4.1. Longevity in situ

Detached diseased leaves were kept in a moist dish and the dish placed in a growth chamber at 95±5% RH and 12±2°C. Twelve hours later, sporangia were harvested and mounted in rain water on a set of eight slides. The slides were kept in a moist dish in the growth chamber at 95±5% RH and 12±2°C for 24 hr before examination for sporangial germination. This procedure was repeated

after 24, 42, 48, 66, 96, 120, 144, 168, 192 and 216 hr when germination had stopped.

After forty eight hours in the chamber at 95±5% RH and 12±2°C, some sporangia were harvested and mounted in rain water on slides for twenty four hours before examination for sporangial germination. This time was marked-Hour 0. The leaves were at this time put quickly in a dessicator sealed 24 hr earlier in which the hygrometer reading was 40±5% RH. The purpose of the dessicator was to suoject the sporangia to a less humid environment. The dessicator was left at room temperature (22±2°C).

After every 24 hr, sporangia were harvested and mounted in rain water on slides for 24 hr for germination tests. At hour 0, some of the leaves were exposed in direct midday sun and sampled at one hourly intervals for three hours for sporangial germination.

3.4.2. Longevity of detached sporangia

At hour 0, while other sporangia were mounted in water for germination tests, other sporangia were detached and placed on a microscope slide. The slide was kept in a moist dish which was kept in the growth chamber at 95±5% RH and 12±2°C. After every 24 hr, these sporangia were sampled for germination. Some of the detached sporangia on the slide were kept in a dessicator left at room temperature (22±2°C) with the

hygrometer reading showing 40±5% RH. These sporangia were sampled for germination tests after every 24 hr till no more germination was recorded.

3.5. Pre-penetration, penetration and associated phenomena

3.5.1. Laboratory experiments using excised leaves

Detached onion leaves wiped with dry cotton wool to remove the waxy coating, were inoculated with a sporangial suspension using an atomiser and kept in a moist dish in a growth chamber at 12±2°C and 95±5% RH. Six, 18, 24 and 48 hr after inoculation, several leaves were cut into small 'cylinders' about 1cm long and dropped into a vial containing Farmer's Fluid (2 parts of absolute alcohol to one part glacial acetic acid). Twenty four hours after leaf material had been added to the final vial containing Farmer's fluid, the material was transferred to each of the 4 vials containing Fuchsin stain. After ascertaining satisfactory staining by periodically examining the pieces, the samples were transferred into vials containing pure lactophenol briefly to rinse excess stain. The samples were then mounted in lactophenol on glass slides for microscopic examination.

3.5.2. Shed house experiments using potted plants

Leaves from 10 weeks old potted onion plants were wiped with dry cotton wool and inoculated with a fresh sporangial suspension using an atomiser at 6.30 p.m.

Twenty plants, ten in each pot were inoculated. Five plants in one pot were atomised with sterile distilled water to act as control. The plants were covered with plastic paper bags and left on a shed house bench. The paper bags were removed the next morning and thereafter daily observations for the symptoms were made.

3.6. Disease progress and increase

Disease progress was followed in seven onion plots A to G, each measuring 17.5 x 1.2m and planted 1m from each other along a straight line at increasing distances from an infected plot. The infected plot was established by planting diseased seedlings obtained from an infected field. Plot A was nearest to the inoculum source. Immediately after the appearance of the disease in the plots, records of infected plants per plot were taken at weekly intervals for one month.

3.6.1. Effect of ambient weather conditions on disease increase

The following field weather data were recorded daily during the duration of the experiment.

i. Dry and wet bulb thermometer readings taken at 9 a.m. and 3 p.m.

ii. The minimum and maximum temperatures

iii. Rainfall.

After completion of the experiment, the RH was calculated from the dry and wet bulb thermometer readings

using a humidity slide rule. The average daily RH was then calculated using the morining and afternoon readings for all the days during the experiment and therefrom the average weekly RH for the weeks disease assessment was made. The average daily temperatures were calculated from the minimum and maximum temperatures and from these the average weekly temperatures. Likewise calculations of the weekly minimum temperatures and weekly rainfall readings were ootained using the average daily records.

3.7. Survival of the pathogen and source of inoculum

3.7.1. Cospore germination tests

Seven treatments at 3 different temperatures (16, 18 and 20°C) were used to test cospore viability. These were: sterile distilled water as control, onion root exudate (water in which onion seedlings had been grown for 2 weeks), sterilised root exudate, rain water, sterilised rain water, onion leaf juice extract and distilled water into which was added potassium permangate. Thin sections were cut from dry diseased leaves, teased and mounted in each of the seven media, and examined for the presence of oospores under a phase contrast microscope. After locating the oospores the slides were kept in a moist dish placed in a growth chamber at 16±2°C and 95±5% RH. They were withdrawn after every 24 hr for 96 hr and examined for oospore germination. This was repeated for temperatures, 18±2°C and 20±2°C.

Some of the dry diseased leaves were separated into 3 parts: one lot was kept in a refrigerator, the other was left at room temperature while the third was taken to the field into a 1.5ft^2 box filled with soil. This third lot was also divided into two parts: one was burried in a pot inside the box while the other was left on the soil surface. After 6 months, the leaves were sampled for oospore germination.

3.7.2. Debris-soil inoculation tests

Certified onion seeds were planted in twenty five 6cm pots filled with macerated onion leaf debris plus sterilised soil. The debris were collected from a severely, diseased onion plot. Each pot had 10 plants. The pots were left on a shed house bench under observations for 4 months for any symptom development. For controls 25 similar pots were planted with certified seeds in sterilised soil and kept in a separate shed house. Two pots were planted like controls but inoculated 2 months later and kept separately.

3.7. Seed transmission tests

Seeds harvested from a former severely diseased onion plot were planted in sterile soil in two 1m^2 boxes. After thinning, 50 seedlings were left per box. The boxes were left on a shed house bench for 4 months under close observations for any symptom development. For controls, certified seeds were similarly planted but

plants in one box were inoculated with viable sporangia of P. destructor after two months and kept in a separate shed house.

4. EXPERIMENTAL RESULTS

4.1. Morphology and identification of Peronospora destructor

Lemon shaped sporangia whose measurements were 45.1 - 70.7 x 20.4 - 35.9 μ, were produced on dichotomously branched and determinate sporangiosphores with acute angles of tertiary branches (Figure 1). Oval shaped oospores which were light brown to orange brown in colour were 18.3 - 36 μ in diameter. The oospores had wall thicknesses which ranged from 3.2 - 3.6 μ (Figure 2). These oospores were recovered on any part of diseased leaf and on some leaves, 1 cm^2 yielded up to 10 oospores. There was no location effect in morphology from the five different samples.

4.2. Field symptoms and signs

The signs first appeared as brown specks of sporangia on any leaf part. These spread to form a dark brown downy growth of superficial coating on the leaf. Underlying the coating in the presence of rain or dew were formed large oval necrotic lesions. In the absence of moisture, however, there was no apparent lesion development.

Initially the leaf turned yellowish, started to wither and later senesced into brown and finally black debris (Figure 3). A few plants had pale yellowish and curled leaves which produced sporangial

blooms under favourable conditions. Leaf tip infection led to dieback while basal infection led to premature senescence of green leaves. All leaves were attacked but older ones were more susceptible. Very early and severe infection at the seedling stage invariably led to premature senescence, seed set failure and consequently poor yield.

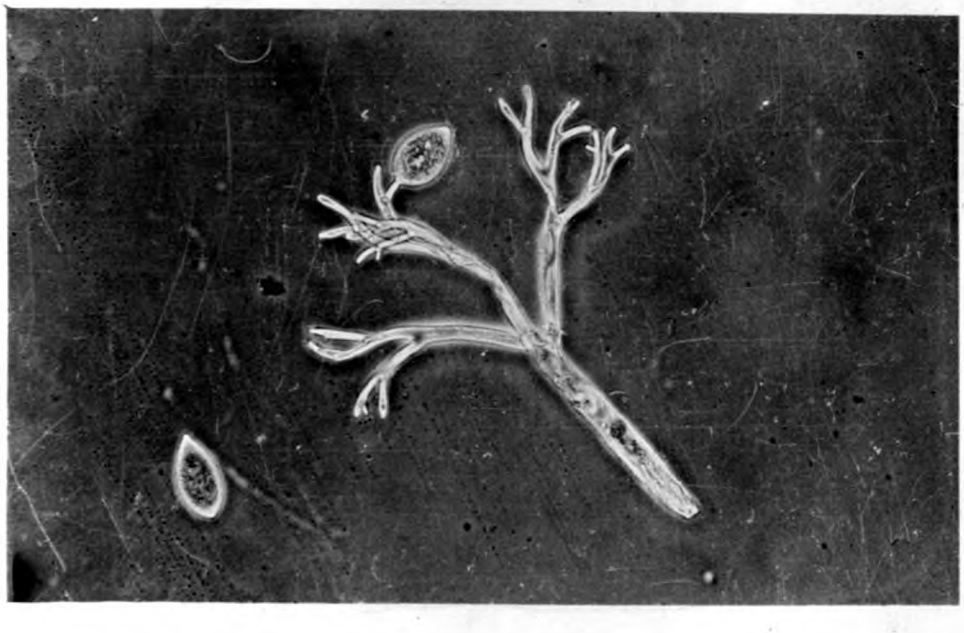


Figure 1. Sporangiophore and sporangia of Peronospora destructor (x200).

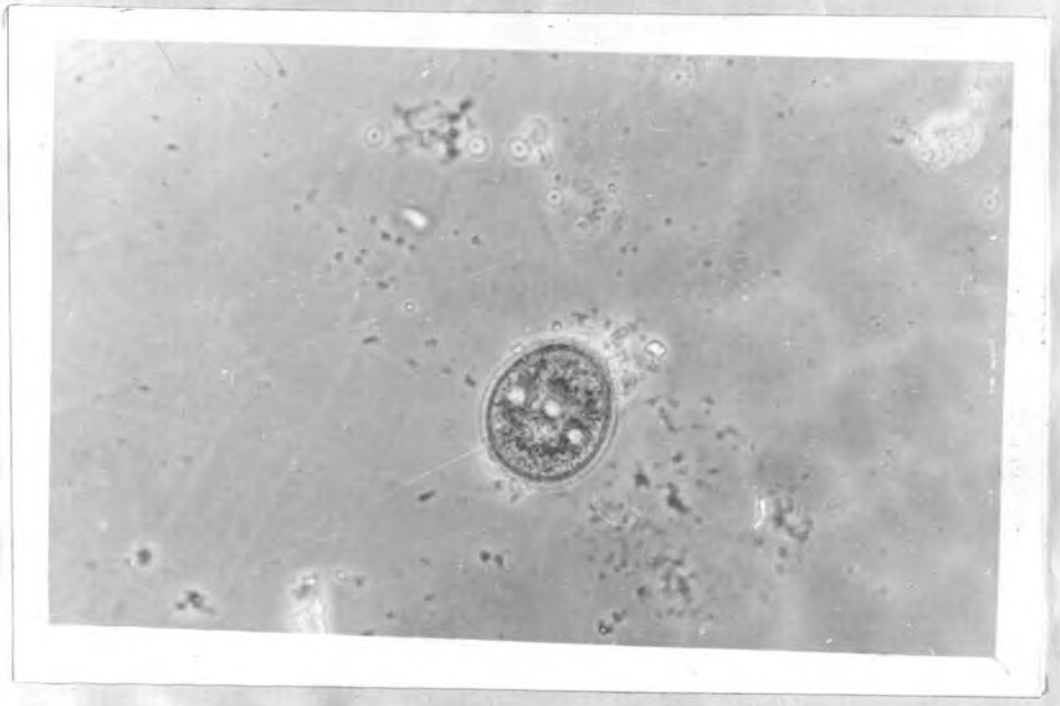


Figure 2. An oospore of Peronospora destructor (x200).

Figure 3. Developmental stages of symptoms of onion downy mildew caused by Peronospora destructor.

4.3. Factors influencing sporangial germination

Sporangia always germinated by germ tubes which measured 6.25 - 12.5 µ in width and grew at a rate of 0.72 - 6.09 µ per hour under optimum conditions.

4.3.1. Temperature

All the temperatures tested gave moderate germination (Figure 4). The highest germination percentage was recorded at 12 ± 2°C with 49% and the next highest was at 14 ± 2°C with 42.3% and the lowest was at 24 ± 2°C with 17.7%. There were significant differences among the treatments ($p = 0.5$). Temperatures 12 ± 2°C and 14 ± 2°C were not significantly different from each other but were significantly different from the rest. The lowest temperature (4 ± 2°C) and highest (24 ± 2°C) were also not significantly different from each other (Table 1). The lower and upper limits for sporangial germination were not determined.

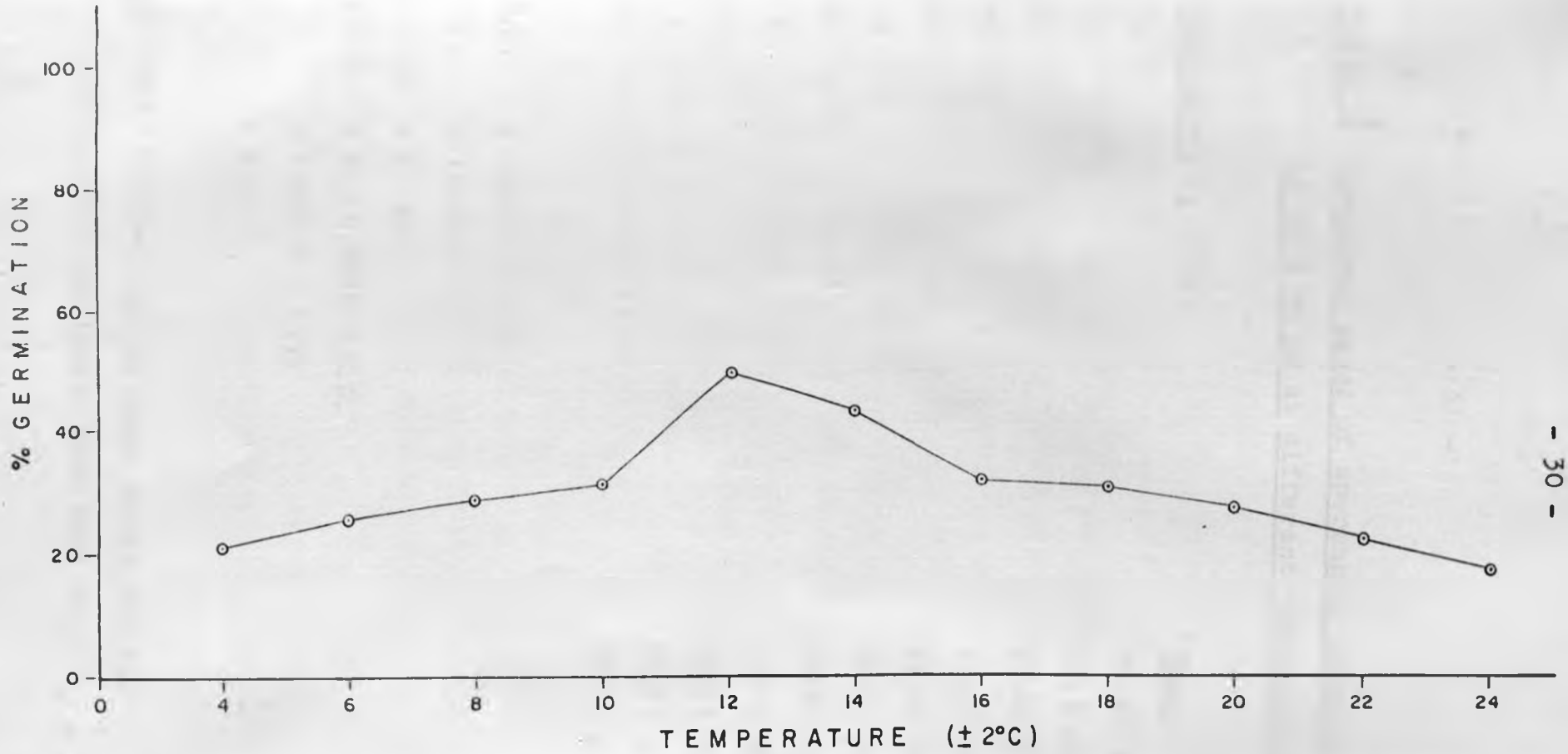


Figure 1: Sporangial germination of Peronospora destructor at 95 ± 5% RH at different temperatures

Table 1. Treatment means of sporangial germination at 95 ± 5% RH at different temperatures

Treatments (± 2°C)	Means
12	44.20 h
14	40.7 gh
16	33.87 f
18	33.57 ef
10	33.03 def
8	32.07 cdef
20	31.50 bcdef
6	29.98 abcdef
22	28.73 abc
4	27.11 a
24	25.75 a

S.E. = 1.51

C.V. = 13.05%

S.E.D. = 2.1389

L.S.D. = $t_{70}(0.95)$ x S.E.D.

= 1.997 x 2.1389

= 4.27

NB Means followed by the same letter are not significantly different from each other (p = 0.05)

4.3.2. Media

Among the six media tested, maximum germination was recorded in rain water (69.1%) followed by sterile distilled water (63.1%), non sterile distilled water (52.3%) and tap water (48.2%). Poorest germination was recorded in glucose solution (17.1%) and no germination at all in the onion leaf juice extract (Figure 5). There were significant differences among the treatments ($p = 0.05$). Both rain water and sterile distilled water were not significantly different from each other. The other treatments (sterile distilled water, distilled water and tap water) were not significantly different from each other, while glucose solution was significantly different from the rest (Table 2).

4.3.3. Optimum germination time

Results obtained indicated that sporangial germination did not start before the first hour after incubation and progressed slowly during the initial hours, but increased steeply between 12 and 20 hr. Maximum germination was obtained after 24 hr and total germination of viable spores ended within 40 hr (Figure 6).

Table 2. Treatment means of sporangial germination at 95 ± 5% RH and 12 ± 2°C in different media

Treatments	Means
Rain water	57.5 e
Sterile distilled water	52.15 cde
Distilled water	45.84 c
Tap water	44.47 bc
Glucose solution	23.12 a

S.E. = 2.93

S.E.D. = 4.1440

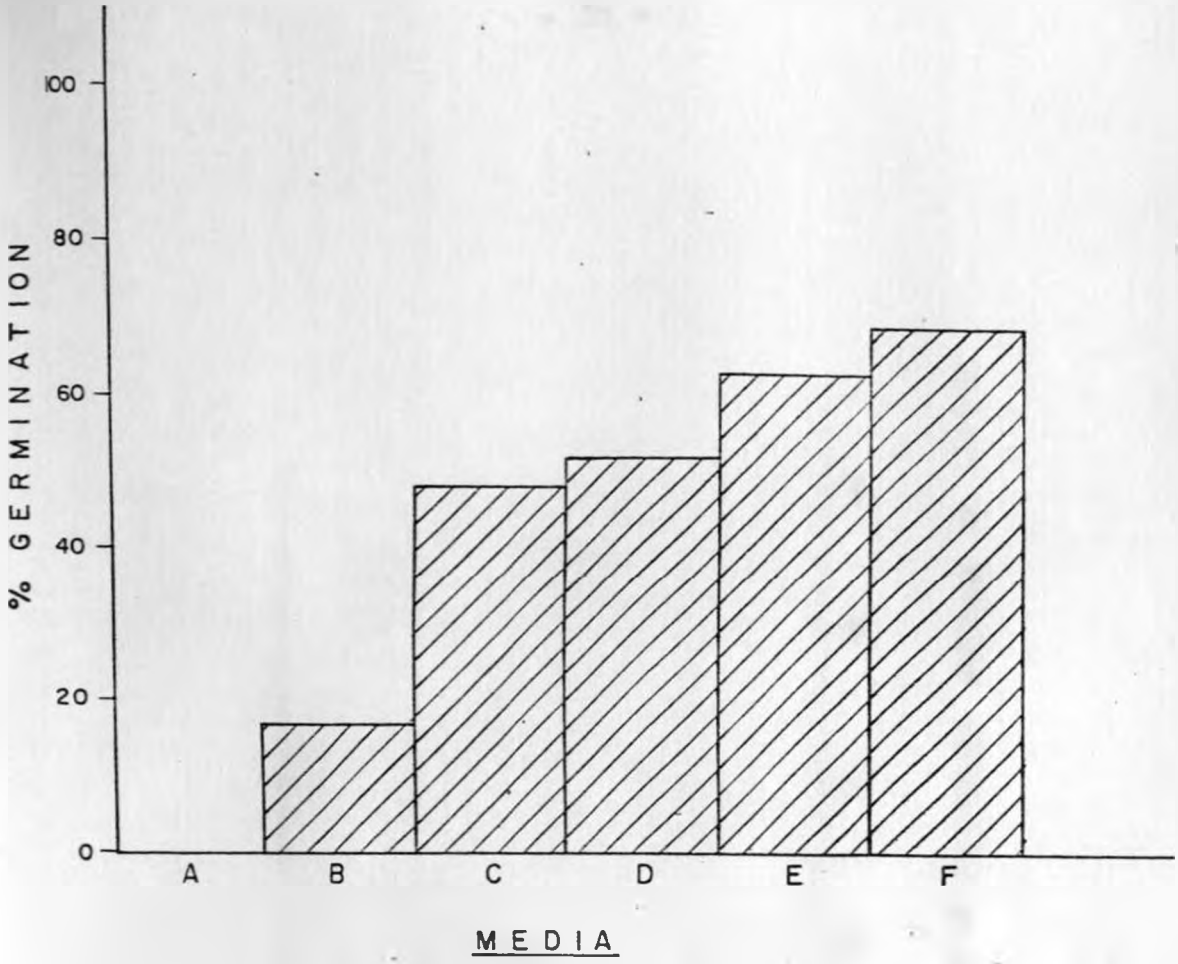
C.V. = 18.6%

L.S.D. = 4.1440 x t_{28} (0.95)

= 4.1440 x 2.048

= 8.49

NB Means followed by the same letter are not significantly different from each other (p = 0.05)



KEY

A — Onion Leaf Juice Extract
B — 1 Molar glucose solution
C — Tap water
D — Distilled water
E — Sterile distilled water
F — Rain water

Figure 5: Sporangial germination of Peronospora destructor at 12± 2°C and 95±5% in different media.

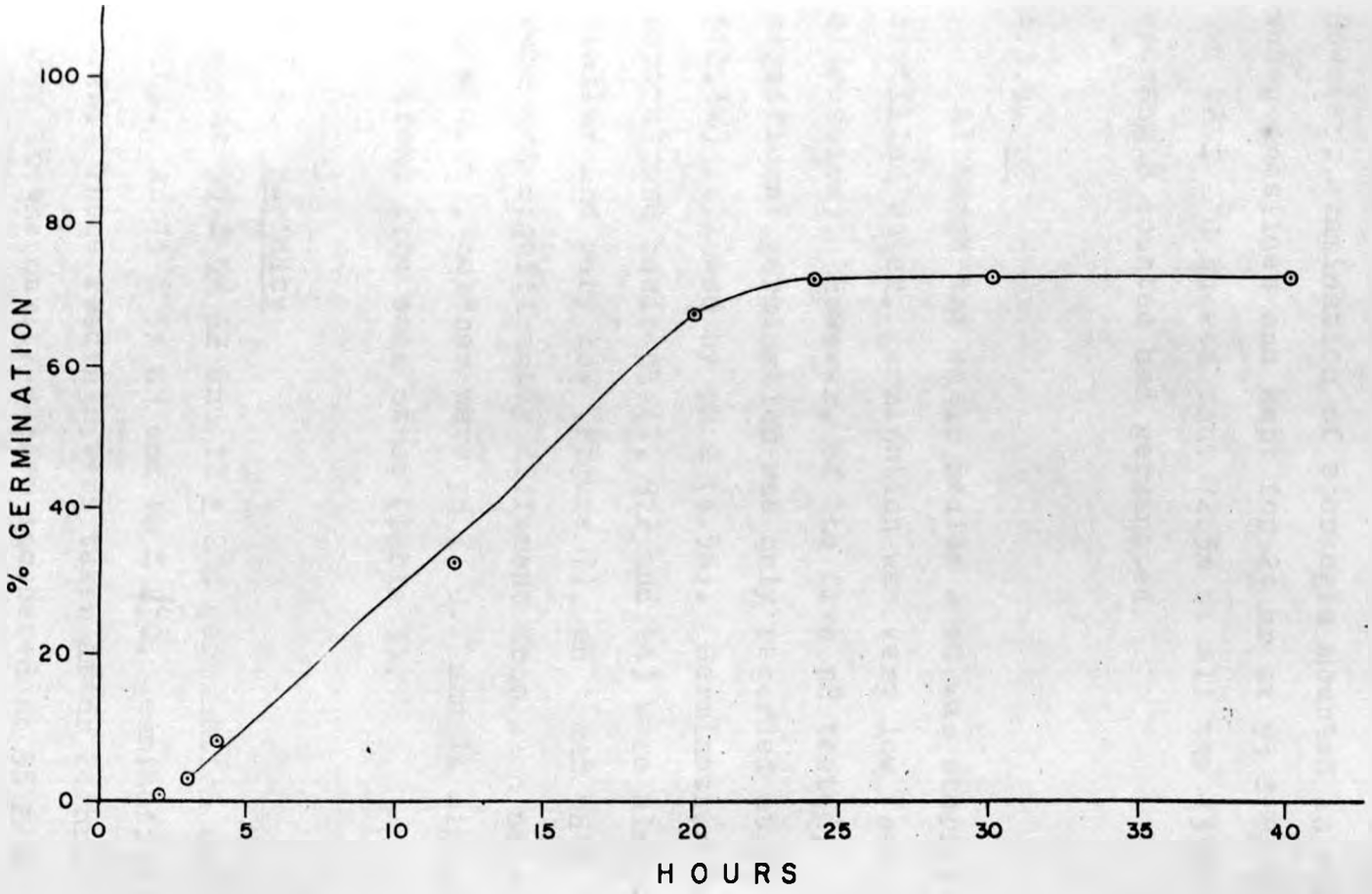


Figure 6: Optimum time for sporangial germination of Peronospora destructor at 12 ± 2°C and 95 ± 5% RH.

4.3.4. Free water

Examination of sporangia kept on a dry microscope slide at 95 ± 5% and 12 ± 2°C for 24 hr showed that no germination had taken place. However, examination of sporangia mounted in rain water on slides and kept for 24 hr at 95 ± 5% RH and 12 ± 2°C showed that 72.3% of all the 1336 sporangia counted had germinated.

4.3.5. pH

Although the basic medium used was sterile distilled water, germination was very low (see discussion). However, of the five pH tested, significant germination was only recorded at pH 4 (10.3%) followed by pH 2 (4.5%). Germination at neutral and basic pH (7, 9.2 and 14) were almost similar and very low (Figure 7), pH 4 and pH 2 were not significantly different from each other ($p = 0.05$), neither were pH 7, 9.2 and 14 significantly different from each other (Table 3).

4.3.6. Humidity

At 95 ± 5% RH and 12 ± 2°C germination was 67.6%. At 75 ± 5% RH and 12 ± 2°C, germination was 50.9%. These readings were taken after 24 hr. While water in the cavity slides incubated at 95 ± 5% RH was still present after 24 hr, that on slides incubated at 75 ± 5% RH evaporated within 18 hr.

Table 3. Treatment of sporangial germination at 95 ± 5% RH and 12 ± 2°C in different pH

Treatments (pH)	Means
4	17.65 b
2	12.22 ab
9.2	7.96 a
7	7.57 a
14	7.00 a

S.E. = 1.92

S.E.D. = 2.7176

C.V. = 51.9%

L.S.D. = $2.7176 \times t_{28}\ (0.95)$

$= 2.7176 \times 2.048$

$= 5.57$

NB Means followed by the same letter are not significantly different from each other ($p = 0.05$)

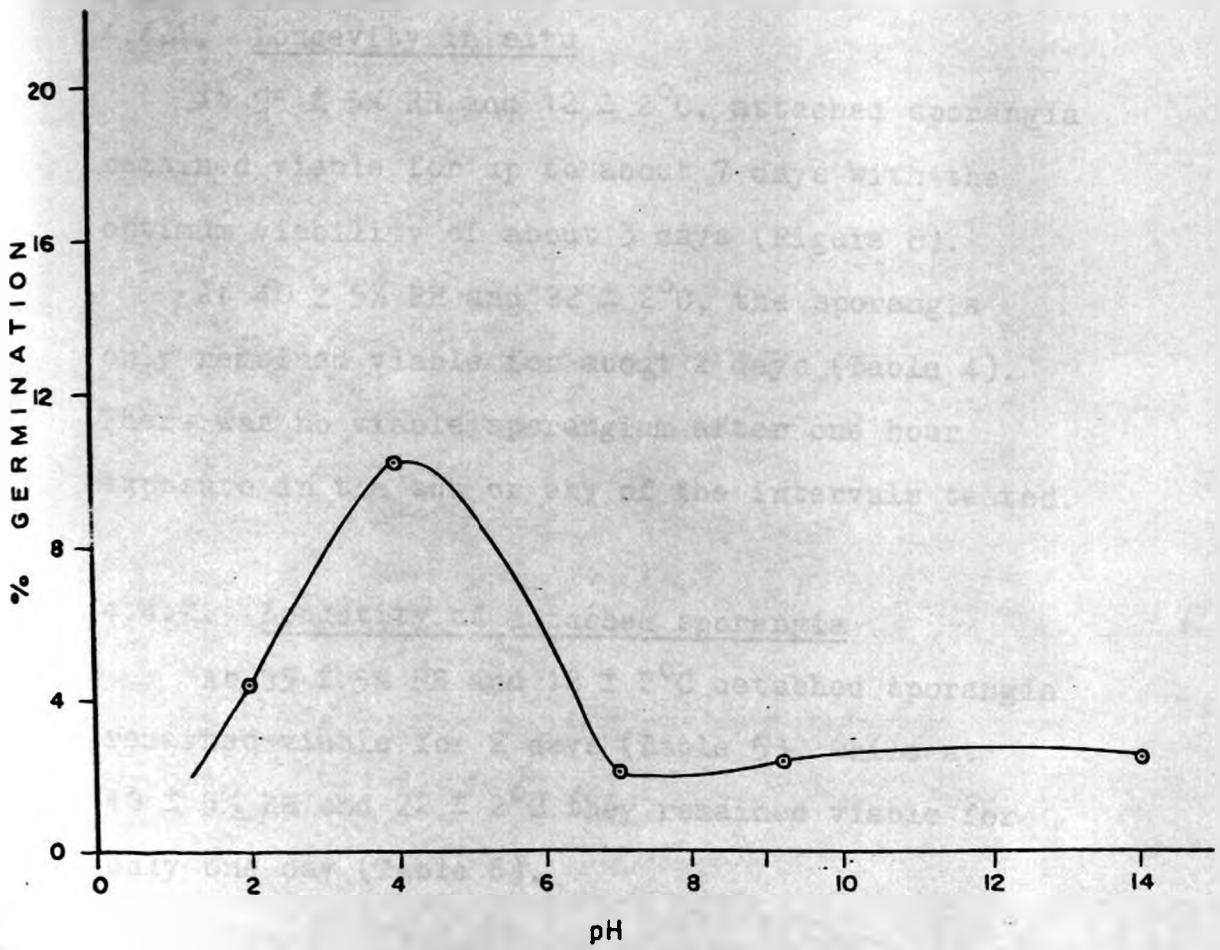


Figure 7:- Sporangial germination of Peronospora destructor at 12±2°C and 95± 5% RH at different pH.

4.4. Sporangial longevity

4.4.1. Longevity in situ

At 95 ± 5% RH and 12 ± 2°C, attached sporangia remained viable for up to about 7 days with the optimum viability of about 3 days (Figure 8).

At 40 ± 5% RH and 22 ± 2°C, the sporangia only remained viable for about 2 days (Table 4). There was no viable sporangium after one hour exposure in the sun or any of the intervals tested.

4.4.2. Longevity of detached sporangia

At 95 ± 5% RH and 12 ± 2°C detached sporangia remained viable for 2 days (Table 5), while at 40 ± 5% RH and 22 ± 2°C they remained viable for only one day (Table 6).

TABLE 4. The effect of time on the germination of attached sporangia of Peronospora destructor at 40 ± 5% RH and 22 ± 2°C.

time (hr)	0	24	48	72
G	966*	39	46	0
NG	370	1063	1277	1066
TOTAL	1336	1102	1323	1066
%G	72.30	3.50	3.48	0

G - Germinated sporangia

NG - Non-germinated sporangia

* - Each reading is a total of 10 observations and 8 replications

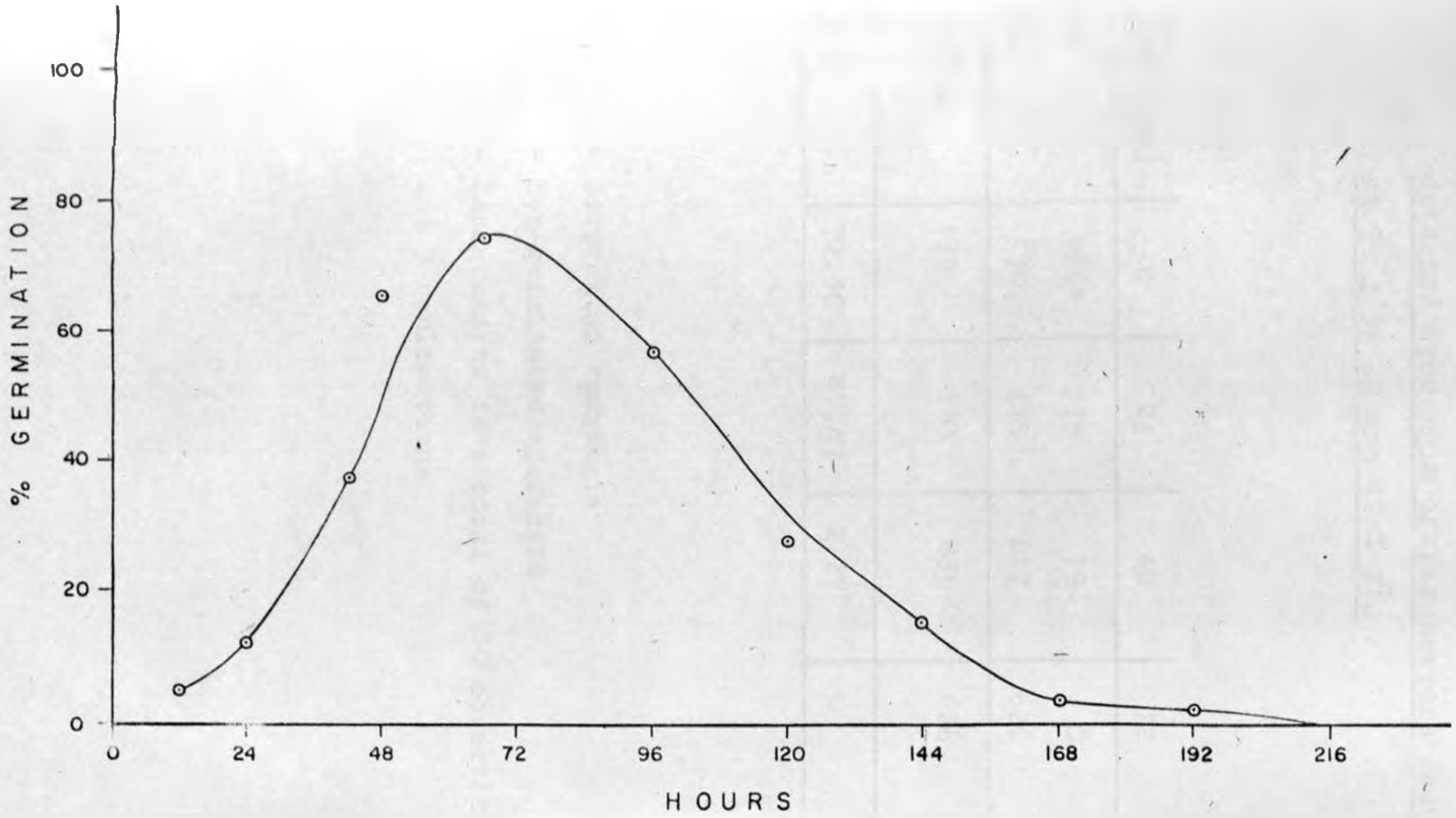


Figure 8: The effect of time on the germination of attached sporangia of Peronospora destructor at 95±5% RH and 12±2°C.

TABLE 5. The effect of time on the germination of detached sporangia of Peronospora destructor at 95 ± 5% RH and 12 ± 2°C

time (hr)	0	24	48	72
G	966*	378	19	0
NG	370	415	817	626
TOTAL	1336	793	836	626
% G	72.30	47.67	2.27	0

G - Germinated sporangia

NG - Non-germinated sporangia

* - Each reading is a total of 10 observations and 8 replications

TABLE 6. The effect of time on the germination of detached sporangia of Peronospora destructor at 40 ± 5% RH and 22 ± 2°C

time (hr)	0	24	48
G	966*	33	0
NG	370	157	1394
TOTAL	1336	190	1394
%G	72.3	2.05	0

G - Germinated sporangia

NG - Non-germinated sporangia

* - Each reading is a total of 10 observations and 8 replications

4.5. Pre-penetration, penetration and associated phenomena

4.5.1. Laboratory experiments using excised leaves

Most sporangia germinated within 6 hr after inoculation. Germination was usually by one, rarely two lateral germ tubes (Figure 9). It was rarely polar and was sometimes branched (Figure 10). The germ tube formed a simple appressorium in less than 6 hr and germination seemed to have been complete 24 hr after inoculation. Penetration occured through stomata and not through the epidermal walls (Figure 11) and appressoria (Figure 12) were observed on some germ tubes.

4.5.2. Shed house experiments using potted plants

Daily observations of inoculated shed house plants showed the incubation period to be between 11 to 13 days when one of the twenty plants inoculated showed a grey downy bloom on one of its leaves.

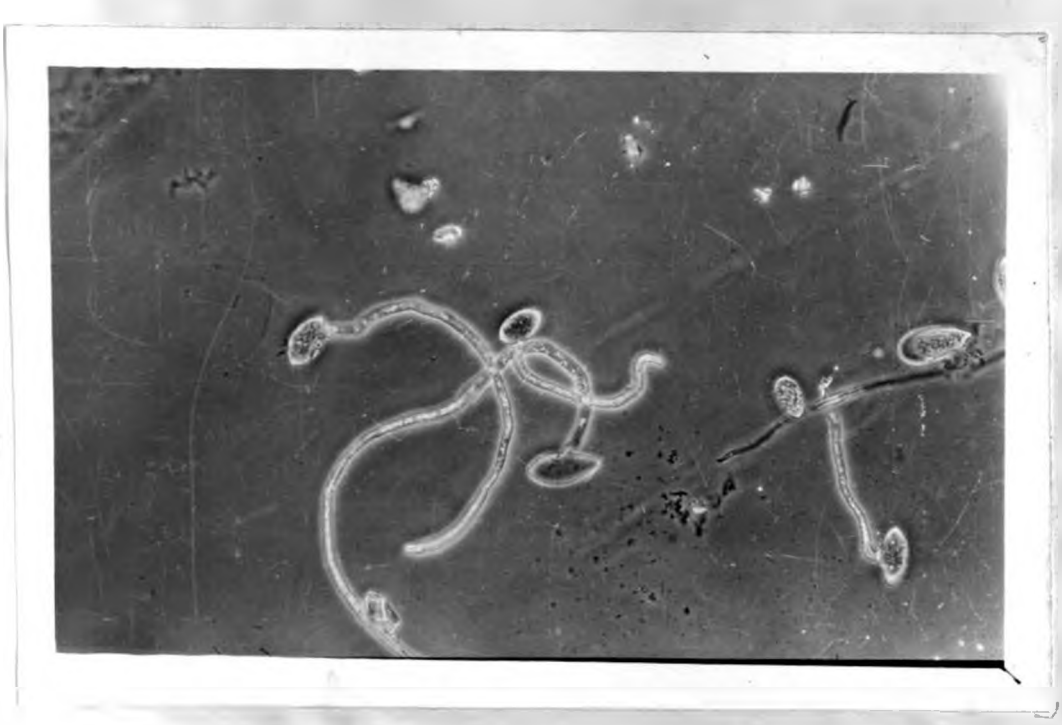


Figure 9. Sporangia of Peronospora destructor (x 200) showing germination by germ tubes.

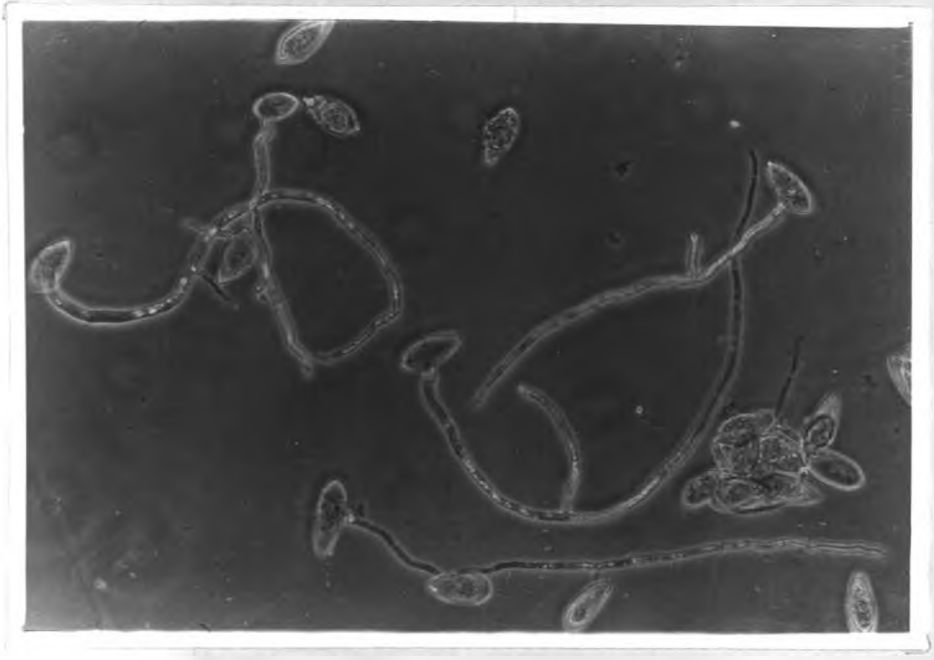


Figure 10. Germinating sporangia of Peronospora destructor (x 200) showing branched germ tubes

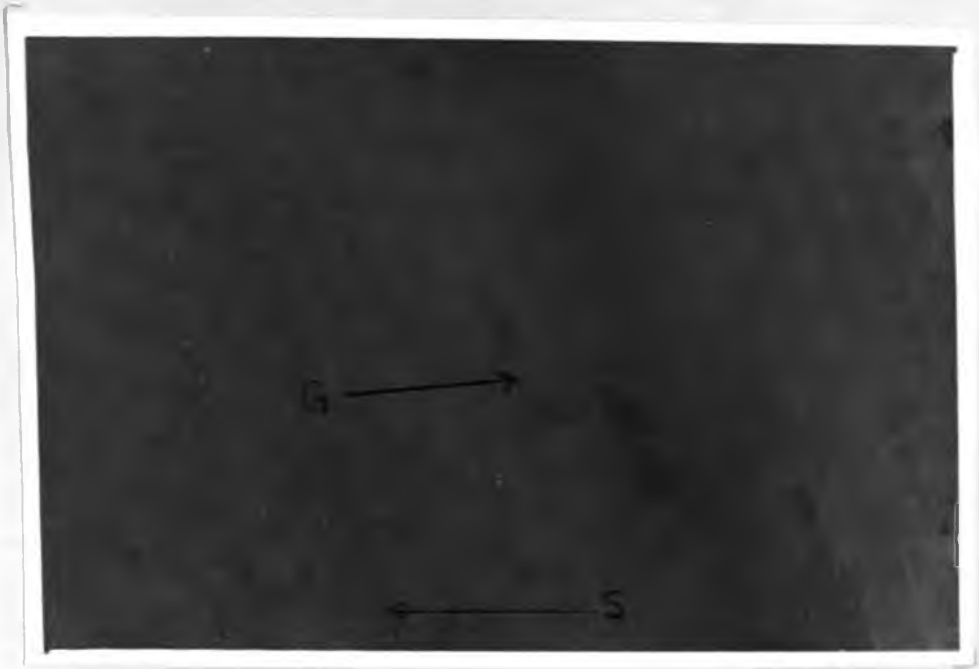


Figure 11. Germinating sporangia of Peronospora destructor (x 200) showing penetration of onion leaf tissue through stomata (S - stoma, G - germ tube).

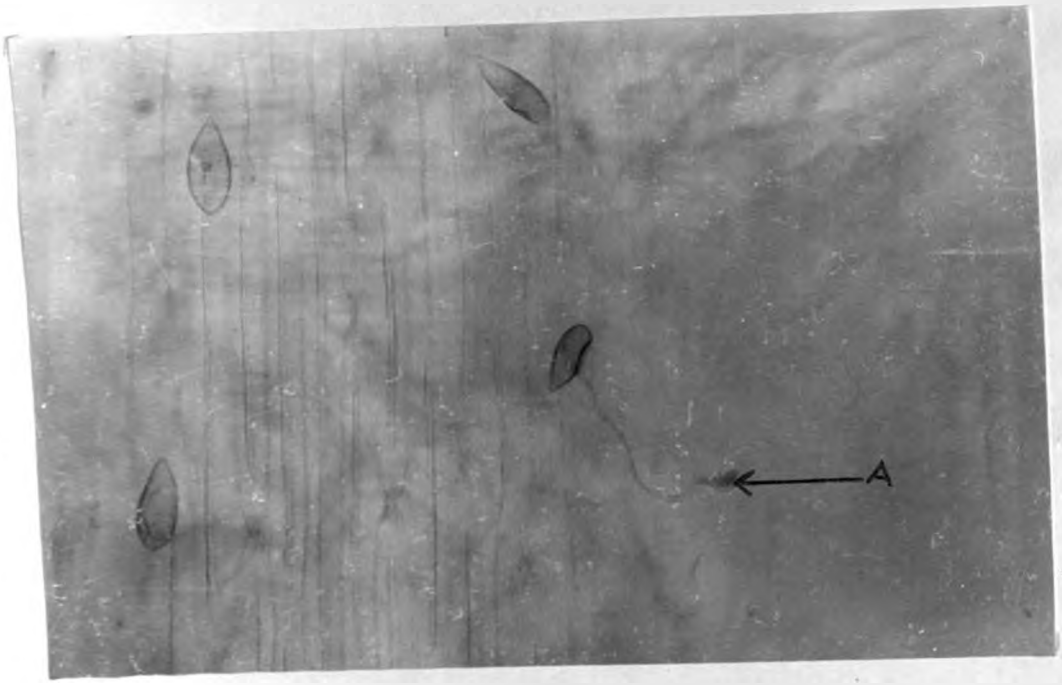


Figure 12. Germinating sporangium of Peronospora destructor (x 100) on onion leaf surface showing an appressorium (A - appressorium).

4.6. Disease progress and increase with time

After one month of abservations, the results (Appendix VII A) showed that there was an increase of disease incidence from the infected plot to the uninfected ones with time. In each plot, increase of proportion of diseased plants with time could be described by the compound interest equation. According to the equation, the proportion of disease at any particular time is a function of the initial inoculum (X_o) and the available infectible sites ($1 - X_o$), Van der Plank (1963)

i.e. $X_t = X_o e^{rt}$

Where X_t = proportion of disease at time 't'

X_o = initial inoculum (proportion of disease at time 0).

e = a constant ($Log_e = 2.7183$)

r = rate of epidemic

t = time

In the early stages of the epidemic therefore, the rate of the epidemic is only limited by the amount of inoculum available while in the later stages it is limited by the number of infectectible sites available. From the number of infected plants, the proportion of disease (x) which is the number of diseased plants divided by the total number of plants, was calculated for each of the seven plots at the four different dates (Figure 13). These in turn were transformed into logits ($Log_e \frac{x}{1-x}$)

using tables by van der Plank (1963) (Appendix VII C). This was because if $\log_e \left(\frac{x}{1-x}\right)$ is plotted against time, using standard statistical methods, a straight regression line with a regression coefficient (b) and an estimated standard error (e) is obtainable. Such a line fits the general regression equation,

$Y = \alpha + bx + e$

Where Y = logits (dependent variable)

α = intercept

b = regression co-efficient

x = time (indipendent variable)

e = error

Using a calculator the regression co-efficient and their estimated standard errors were obtained for all the seven regression lines corresponding to the seven plots A-G (table 7). These regression co-efficients are a measure of the disease gradient and therefore correspond to the rate of the epidemic (r) (van der Plank, 1963). The highest epidemic rate obtained was 0.33 per unit per day in plot D (Table 7).

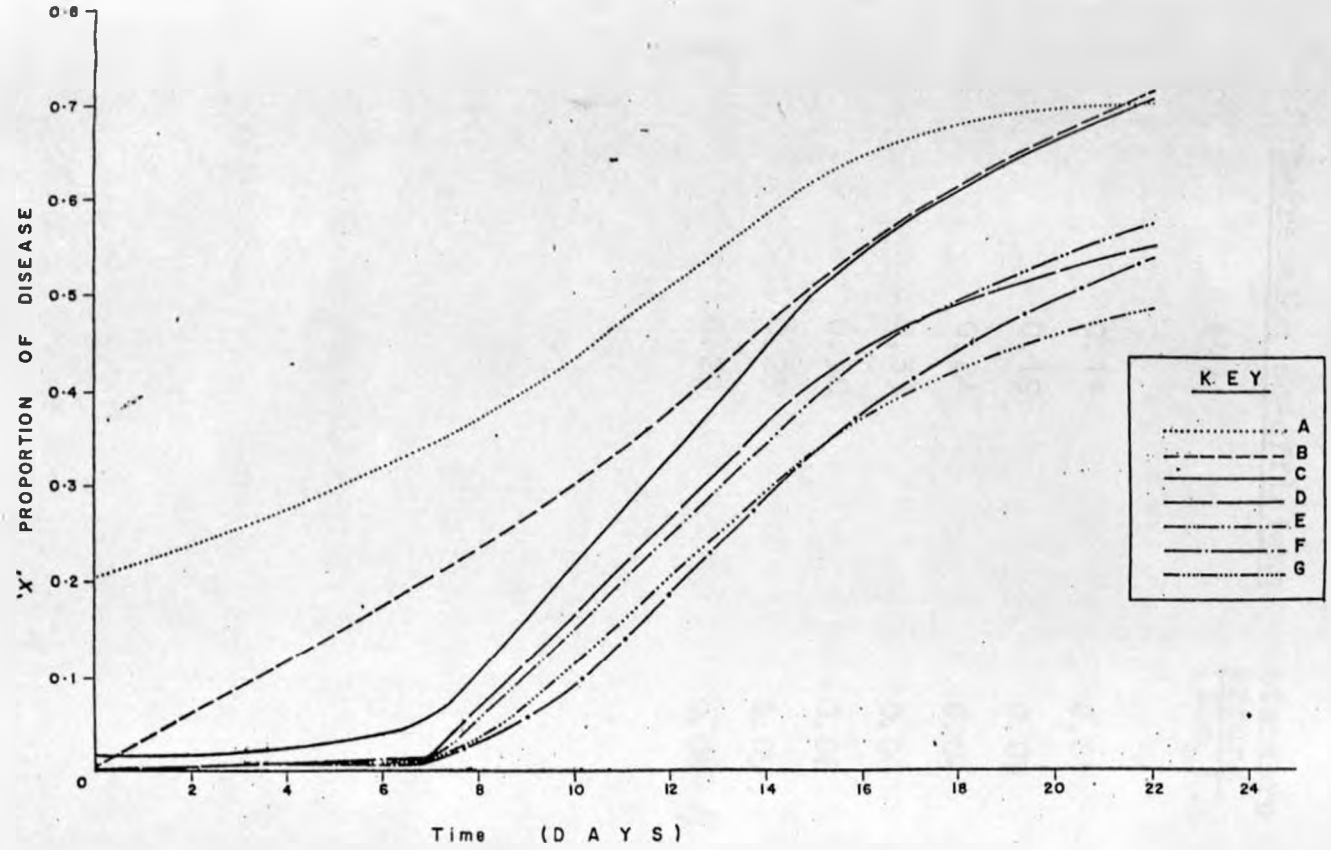


Figure 13 : PROGRESS OF ONION DOWNY MILDEW CAUSED BY PERONOSPORA DESTRUCTOR IN PLOTS A - G

ABLE 7: Calculated regression co-efficient and their standard errors corresponding to the epidemic rates of downy mildew of onion in seven plots A-G.

ot	Regression co-efficient (b)	Standard errors ($S.e_b$)
	0.11	0.01
	0.19	0.03
	0.24	0.04
	0.33	0.06
	0.30	0.06
	0.25	0.05
	0.29	0.06

4.6.1 Effect of ambient weather condition and disease increase

The multiple regression analysis of variance of disease increase and various weather parameters; temperature, rainfall and relative humidity (Appendix VII E), showed that there was a very strong multiple correlation between disease increase and the weather factors combined -R = 0.9395, (p=0.01). The proportion of the variation that could be attributed to regression as determined by R^2 was 0.8827 or 88.27%. This means that 88.27% of the variation found in disease increase could be accounted for by temperature, rainfall and relative humidity. The t-tests for the partial regression coefficients of the four weather parameters tested (Appendix VII E), showed all of them to be very significant (p=0.001). This means that temperature, rainfall and relative humidity combined were important weather factors that affected the increase of disease with time.

4.7. Survival of the pathogen and source of inoculum

4.7.1. Oospore germination tests

All the seven treatments at all the temperatures tested gave negative results even after 4 days of observations. After six months, no debris was recovered from the pot burried with debris earlier. Oospores were observed from surface debris which was recovered after six months but like other observed from debris left in the refrigerator and at room

temperature could not be induced to germinate under the conditions tested.

4.7.2 . Debris - soil inoculation tests

No sign of the pathogen was observed on any of the 250 plants after 4 months of observation.

Two of the 20 inoculated plants however showed symptoms 13 days after inoculation.

4.7.3. Seed transmission tests

After 4 months, all the 100 plants in the test boxes showed no symptoms of the disease. For the controls, no infection was observed in the uninoculated plants in the box, but one plant showed symptoms 12 days after inoculation among inoculated plants.

5. DISCUSSION

The results obtained from morphological studies did not vary much from observations made by Jovicevic (1964) on P. destructor. There were slight differences in dimensions which could be attributed to natural variation. The results confirmed that sporangia which are lemon shaped are produced on dichotomously branched sporangiophores with acute tertiary branches. The oospores produced later in the growing season are oval shaped and thick walled. These resting spores (oospores) could play a role in the perpetuation of the pathogen.

Field symptoms of the disease and signs of the pathogen conform to descriptions by Wheeler (1972), Yarwood (1943), Chupp and Sherf (1960). The presence of free water seems to have a profound effect on the development of symptoms in as far as necrotic lesions only developed in the presence of moisture. This is related to germination of sporangia discussed later. The presence of some symptoms like yellowing, curling and palling only during the early growth stages of the plant seems to indicate early infection at the seedling stage either through oospores or sporangia and thereafter suppression of the disease signs and symptoms during adverse conditions. The manifestation of these 'early stage symptoms' could thus be viewed as a survival mechanism. This is because these symptoms were never observed later in the growing season even though conditions were favourable for

disease development.

The susceptibility of older leaves could be explained more in terms of the dispersal mechanism of sporangia in a field and positioning of young leaves, other than any special features of these leaves. The probability of sporangia blown from an infected plant to an adjacent one in a field, of falling on a young leaf shielded by other older leaves is quite small compared to it falling on an older leaf. This is because once one leaf in a plant was infected, all the leaves became infected thereafter. Early infection leads to seed set failure most probably because this occurs before the formation of floral primordia.

Sporangia germination by germ tubes as opposed to zoospores has been confirmed by Viranyi (1974), Cook (1932), Yarwood (1937, 1943) and Katterfeld (1926). The latter found germ tubes to measure 5-10.5 μ in width while the results from this project were 6.25-12.5 μ. This ability to germinate by germ tubes like conidia is another distinguishing features of the genus Peronospora and is thought to be a adaptation of the higher fungi to terrestrial life (Alexopoulos, 1962). Sporangial germination occurred through a wide temperature range (2-26^{o}C) with an optimum between 10-14^{o}C. The optimum temperature could not be determined more precisely because temperatures within the incubator fluctuated by $\pm 2^{o}$C from the set temperature. Germination at the optimum temperature moderate (49%). This was because tap water was used as the medium for germination. It was later found that the

rain or sterile distilled water were the best media for germination. In these two media germination was 69.1% and 63.1% respectively.

Published work also shows that water obtained from natural sources like lake water proved to be a good medium for germination (Cook, 1932). However the results of this investigation also showed sterile distilled water to be a good medium for germination. The figure is also low because examination of sporangia was after 20 hr of incubation while maximum germination was later found to occur 24 hr after incubation. No published work on maximum time for germination was obtained which could be compared with these results. Similar results on temperature as a factor have also been obtained by Viranyi (1974) who found tbe range to be 6-27°C; Katterfeld (1926) who found the optimum range to be 12.5-15°C and Cook (1932) who found the range to be 3-27°C with an optimum at 11°C. The fact that no upper or lower limit of temperature requirement was established, showed that the temperature may not be a limiting factor in sporangial germination although it is an important factor.

Sporangial germination or indeed any other spore is directly dependent on moisture. In nature, the source of this is rain water. The high germination obtained in rain water could probably be explained in terms of rain water being the

natural source of moisture. On the other hand, the relatively high percentage obtained for sterile distilled and distilled water compared to glucose solution (glucose was dissolved in distilled water) may be due to the availability of the moisture in a free form to the spores. In sterile distilled and distilled water, the water molecule is in its free form while in glucose it is relatively unavailable depending on the saturation of the solution. In onion leaf juice extract, however, the zero germination obtained may be explained by inhibition of sporangial germination by phenolic compounds in the extract since it is known that these compounds constitute part of the chemical defence mechanism in plants (Agrios, 1978). Tap water on the other hand is chlorinated and the reduced germination could be attributed to this.

Results obtained from experiments to determine the role of free water as a factor in sporangial germination are much more explicit and confirmed the above inference. While sporangia kept on a dry glass slide at optimum conditions did not germinate, those mounted in water attained 72.3% germination. Viranyi (1974) and Yarwood (1937 and 1943) have also showed that germination only occurred in free water.

The inability to regulate the Relative Humidity precisely was limiting such that only two levels of RH were compared to give a non-statistical rough

comparison. From the results, RH perse did not seem to affect sporangial germination. At higher RH (90 - 100%), the air is quite saturated with moisture, this leads to condensation and hence availability of free water which is essential for sporangial germination. This means that germination would proceed as long as this water was available and once the water is depleted, germination stops. This is precisely what happened in the experiment. At 70 - 80% RH, water in the cavity slides evaporated within 18 hr by which time 50.9% of the sporangia had germinated while at 90 - 100% RH there was no evaporation and 67.6% of sporangia had germinated after 24 hr. This means that in a system where there is enough free water, temperature as opposed to RH would be a limiting factor in sporangial germination. Media, free water and RH are therefore factors that are intricately related as factors in sporangial germination.

Like germination, the fungal sporulation is directly dependent on free moisture and these processes seem to have similar environmental requirements in terms of temperature and RH. Luxuriant sporulation was always observed at 10 -14^{o}C and at 90 - 100% RH. These were incidentally the optimum temperature and RH for sporangial germination. Yarwood (1943) found the optimum temperature for both

sporulation and germination to be 13°C, maximum temperature was 22 - 25°C, while minimum was 4 - 7°C and the minimum RH was 90% and optimum 100%. Cook (1932) found similar results. He found that the fungus sporulated over a wide range of temperature and free water on the leaves was a necessary precondition for sporulation. In nature these conditions are normally attained at night which prompted Yarwood (1937 b) to infer that the nocturnal sporulation of the fungus is directly dependent on the darkness and high humidity frequently coincident at night. This ability of sporangia to germinate at the same conditions at which the fungus sporulates could be a measure to ensure speedy and successful infection especially within a field.

The effect of pH on the germination of sporangia is not clear from the results obtained. The germination obtained was too low despite the fact that the basic medium used was sterile distilled water which on its own gave 63.1% germination. It is therefore difficult to give definite conclusions based on these results.

The optimum germination time has no significance as a factor in sporangial germination although the experiment could be used to determine the quiescent period of the sporangia and the approximate time for examination of sporangia in germination tests as in viability tests. In brief, of the factors tested

for sporangial germination, free water (or RH) and temperature could be singled out to be of direct bearing on the process.

The longevity of sporangia shows some very interesting results. At 90 - 100% RH and 10 - 14°C, the sporangia in situ remained viable for about 7 days, while when detached they only remained viable for about 2 days. In fact the 7 days with an optimum of about 3 days represent the longevity of a population of sporangia. This is because any sample of sporangia harvested for any germination tests is a heterogenous population of sporangia with various ages. The 7 days are also a measure of how long a sporulating zone continually produces new sporangia. Katterfeld (1926) however, found the longevity to be 10 days in damp air, while Yarwood (1943) found 3 days although he did not indicate under what conditions these observations were made. The viability curve obtained is therefore a typical viability curve of a population. This, however, is not the case with detached sporangia which are about nearly similar in age. Their longevity is an important factor in the dispersal of the fungus, as seen below.

At 35 - 45% RH and 20 - 24°C, detached sporangia remained viable for about one day while at 90 - 100% RH and 10 - 14°C they remained viable for about two days. However, on exposure in the sun, sporangia took less than one hr before losing their viability.

Viranyi (1974) and Katterfeld (1926) found similar results. This means that dispersed sporangia must reach the host within 2 days at 90-100% RH and 10-14°C. On the other hand at 35 - 45% and 20 - 24°C they must reach the host within one day. This is assuming there is no sunshine, otherwise they must do so within one hour. These restrictions would have severe consequences on the spread and hence survival of the pathogen if there were no counteracting mechanisms. Three such mechanisms could be sighted from observations and results emanating from this project.

i. The pathogen is prolific. This means that large numbers of sporangia are produced to ensure that a high probability of reaching the host is enhanced even if most sporangia do not reach the host.

ii. The sporulating lesion produces sporangia for about 7 days continually under optimum conditions. This continuous supply of inocula ensures chances of infection.

iii. High epidemic rate. As mentioned earlier the disease could attain a very high rate of spread of about 0.33 per unit per day.

This fast speed could thus be regarded as a measure which ensures successful infection. These observations show that sporangial longevity is an important process in the epidemiology of the disease.

Pathogenicity studies showed that sporangial germination on and inside onion leaf tissue was faster than that in artificial media like rain water used in this project. Yarwood (1943) showed that inside the host the germ tube grew at a rate of about 300 µ per hour. Results obtained from this project, however, only showed a growth rate of about 6.09 µ per hour, in rain water. This could explain why most sporangial germination seemed to have been complete 24 hr after inoculation. This is in contrast to what was observed in 4.3.3 where germination was complete after about 40 hr in rain water. Cook (1932) also showed that after 24 hr in lake water, germination was not complete. These observations however, vary with those of Katterfeld (1926). He found that all sporangia germinated within 5 hr but there is no indication in what medium this was observed.

Penetration through stomata has been observed by all investigators who have studied the process: Viranyi (1974), Yarwood (1943), and Katterfeld (1926). The incubation period obtained was within the limits of those obtained by various authors. Viranyi (1974) found 11 - 14 days, Katterfeld (1926) found 10 - 15 days in the green house and 13 - 18 in the field and Cook (1932) found 11 - 15 days. In this project the incubation period was estimated at 11 - 13 days.

The influence of temperature, rainfall and relative humidity on the increase of the disease was very clearly shown from the multiple regression analysis. It in effect confirmed the laboratory results and partly the well known epidemiological facts that disease increase and spread with time depends on environmental, host and pathogen factors all acting together, a relationship sometimes referred to as 'the disease triangle'. And although not all environmental factors were tested, the very high R^2(88.27%) obtained seems to show that temperature, rainfall and relative humidity are very important environmental factors affecting the epidemics of onions downy mildew.

As was noted in the review, the survival of P. destructor in agricultural systems like Kenya where onions are propagated through seeds is still a matter of debate. This is much so in light of the results obtained in this project. Even though seeds were harvested from a former severely infected plot, these on planting showed no symptoms. Seed as a medium of survival therefore seems unimportant. These observations have been confirmed by Katterfeld, Murphy and McKay (1926). Cook and Hiura (1930) although they observed fungal mycelia on flower parts like ovary, stamens and pistils, they observed no infection from plants sown from such seeds. Debris-soil inoculation tests showed negative results and no burried debris was recovered after six months because of microbial

action (the six months sampling period was for convenience considering the project duration). The significance of this in relation to the survival of the pathogen is not clear since it is not known through what avenues (root or shoot) oospores if they infect, enter the host. It is because of this uncertainty that various media like onion root exudate and onion leaf juice extract were used in oospore germination tests. McKay (1957) before using potassium permanganate in the germination of oospores, thought that a stimulus was required to break their dormancy. He was probably right because he obtained 60 - 85% germination within 48 hr at 15 - 20°C after adding 0.01 - 0.02% potassium permanganate to water containing 5 - 6 years old oospores. It is, however, not known in what form this stimulus occurs in nature. The temperatures: 16, 18 and 20°C used however are the average soil temperature ranges at National Agricultural Laboratories. McKay (1957) used a temperature of 15 - 20°C to get the above germination percentage. Despite these combinations, however, there was no germination of oospores in this investigation.

So how does the pathogen survive from year to year or what is the source of the primary inoculum? From the results of oospore counts it was found that the probability of obtaining oospores when 0.25 cm^2 of dry diseased leaf tissue was macerated and teased in water is 0.8175 or 81.75% (Appendix I). For the

100 cm^2 length of tissue examined, 982 oospores were counted. That is approximately 10^3 oospores per 100 cm^2 length.

Consider an acre of onion with about 50,000 plants. Assume each plant has at least 5, six square centimetre diseased leaf tissues or 30 cm^2 length of tissue per plant. There will therefore be $(3 \times 10^2 \times 50{,}000) = 15 \times 10^6$ oospores per acre of onions.

The importance of such a high figure cannot therefore be ignored in the life cycle of the pathogen, taking into account the fact that only one oospores is needed to initiate infection (The fungus is prolific and the disease progress with time logarithmic).

The probability therefore of finding such a single spore or a few others is quite small considering the population of oospores. This is more so when one considers that oospores are survival spores. One distinguishing feature about such spores is their longevity. McKay (1957) has shown that these oospores can remain viable for about 25 years and the exact maximum period is not yet known. Their tendency would then be to have different maturity periods so that if one group germinated, another would remain dormant till a later date so that the pathogen would not perish incase all spores germinated and then conditions became so adverse that all the progeny were destroyed. The pathogen cannot therefore become extinct.

Another factor is the minimum maturity period before oospores germinate. McKay (1957) found that after separation from the host tissues, a maturation period of 2 - 3 years is needed before germination can occur. From other observations he found that although in some seasons oospores were produced in enormous numbers, germination was first observed when they were 4 years old. After 4 years 5 months, it reached 1% at room temperature and 2% after 5 years and after 7 years it reached 5% in 48 hr at 20°C. It is however noteworthy that McKay was working with onions that were propagated through bulbs. The role of oospores might therefore have been reduced due to the ability of the fungus to perpetuate itself as mycelia in the bulbs (see 2.4.1).

The other possible source of primary inoculum could be the bulbless 'multiplier onions'. In the introduction, it was noted that these are a dominant feature of backyard gardens in most rural homes in Kenya. Murphy and McKay (1926), have postulated that this onion variety could be a carrier of inoculum, but since no work was done on the incidence and survival of downy mildew on this variety, it is difficult to prove this claim. Therefore, in the absence of any concrete evidence on the role of oospores in the perpetuation of P. destructor, it would be speculative to pinpoint any sources as carriers of primary inoculum until further investigation is undertaken on the survival of the pathogen in Kenya.

6. CONCLUSION

The results obtained in the project are a useful guide for further research on the biology of *Peronospora destructor* in Kenya. The following are some of the salient points.

Sporangial germination occurred over a wide temperature range with an optimum between 10-14^{o}C at 90-100% RH. For both sporulation and sporangial germination, free water was a prerequisite. In the field disease increase was influenced by temperature, rainfall and relative humidity. Sporangial longevity took about seven days under optimum conditions and sporangial germination was by germ tubes which penetrated the host tissues only through stomata. The incubation period was estimated at 11-13 days, and sporulating lesions continously produced spores for about 7 days before necrosis. The epidemic could be described by the compound interest equation, and could attain a high rate of 0.33 per unit per day. Oospores were observed but none of them could be induced to germinate at any of the conditions tested. Any role that they may play in the perpetuation of the pathogen is therefore still uncertain. It is in light of these observations that further research is necessary on the role of alternate hosts and survival spores on the life cycle of the pathogen.

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8. APPENDICES

1A. Dictribution of oospores from freshly collected diseased onion leaves infected with Peronospora destructor

	1cm length pieces										
10cm pieces	1	2	3	4	5	6	7	8	9	10	Total
I	3*	3	0	3	3	4	1	2	4	3	26
II	12	14	11	7	16	4	8	11	9	10	102
III	6	8	8	9	12	10	5	10	10	14	92
IV	15	3	7	7	9	9	8	11	10	5	84
V	2	7	8	8	6	14	9	9	11	12	86
VI	6	9	7	6	8	7	9	5	5	10	72
VII	23	17	16	9	19	13	6	13	14	7	137
VIII	22	24	14	18	16	9	14	10	12	12	151
IX	14	11	12	10	11	16	13	17	15	17	136
X	8	8	14	10	9	6	16	8	7	10	96
											982

* Each reading is a total of 4 observations.

IB. χ^2 distribution of sample derived from Appendix 1A

x	f	fx	Probability	Calculated frequency	χ^2
u	1 } 3	0	0.0011	0.1 } 2.1	0.3857
1	2 }	2	0.0198	2.0 }	
2	13	26	0.1332	13.3	0.0069
3	37	111	0.3984	39.8	0.1970
4	47	188	0.4470	44.8	0.1080
	100	327		100	0.6976

df = 2

μ = 3.27

p = 0.8175

Mean number of slides with oospores is 327/100 = 3.27 i.e 3.27 out of 4 or 0.08175. Therefore the probability of obtaining oospores when 0.25 cm^2 tissue is macerated is 81.75%.

IIA. Sporangial germination of Peronospora destructor at 95 ± 5% RH at different temperatures

Temp. (±2°C)		Replications 1	2	3	4	5	6	7	8	Total	%G
4	G	9*	34	16	14	9	17	22	6	127	21.56
	NG	91	61	52	34	47	53	66	58	462	
6	G	15	48	20	26	18	15	21	10	173	25.82
	NG	61	87	46	72	75	47	54	55	497	
8	G	19	19	15	28	38	24	15	17	175	28.83
	NG	56	59	49	49	56	69	52	42	432	
10	G	42	42	37	29	28	22	15	30	245	30.36
	NG	90	79	80	70	65	40	64	74	562	
12	G	41	88	80	55	48	73	127	86	598	49.02
	NG	76	86	72	38	76	74	122	78	622	
14	G	49	42	53	60	60	66	59	47	436	42.33
	NG	73	63	96	103	64	73	68	54	594	
16	G	36	30	28	27	28	28	46	34	257	30.63
	NG	93	94	62	76	67	77	72	41	582	
18	G	28	40	33	27	42	29	30	20	249	30.04
	NG	63	77	80	76	100	50	92	41	579	
20	G	28	35	41	22	20	31	20	19	216	27.07
	NG	104	109	80	57	63	46	59	64	582	
22	G	16	23	15	16	20	20	15	24	149	22.82
	NG	47	93	44	75	56	70	59	60	653	
24	G	17	15	16	15	13	13	14	12	115	17.67
	NG	55	40	35	74	94	71	69	98	536	

* Each reading is a total of 10 observations

G - Germinated sporangia

NG - Non-germinated sporangia

II B. Arcsin transformation of per cent sporangial germination at 95 ± 5% RH at different temperatures derived from Appendix II A

Temp. (± 2°C)	Replications								
	1	2	3	4	5	6	7	8	$\bar{X}$
4	17.46	36.75	29	32.71	23.58	29.53	30	17.85	27.11
5	26.35	36.63	25.24	30.98	26.13	29.47	31.95	23.11	28.73
8	30.20	29.60	28.93	37.11	39.47	30.53	28.25	32.46	32.07
10	34.33	36.09	34.20	32.77	33.27	35.24	25.84	32.46	33.03
12	36.27	45.34	46.49	50.24	38.47	44.83	45.57	46.38	44.20
14	39.35	39.23	36.63	37.35	44.08	43.57	42.99	42.99	40.77
16	31.88	29.47	33.89	30.79	32.90	31.11	38.65	42.30	33.87
18	33.71	35.79	32.71	30.79	32.96	37.29	30.40	34.94	33.57
20	27.42	29.53	35.61	31.82	29.40	39.41	30.20	28.59	31.50
22	30.26	26.42	30.26	24.85	30.85	28.11	26.78	32.33	28.73
24	29.06	31.50	34.08	24.27	20.36	23.19	24.27	19.28	25.75

ANOVA

Source	df	ss	ms	f
Total	87	3764.40	43.27	
Treatments	10	2433.09	243.31	14.18
Replications	7	130.30	18.61	1.08
Error	70	1201.01	17.16	

III A. Sporangial germination of Peronospora destructor at 95 ± 5% RH and 12 ± 2°C in different media

Media		Replications								Total	%G
		1	2	3	4	5	6	7	8		
Tap water	G	60*	50	45	53	50	83	73	63	477	48.18
	NG	40	52	53	38	104	79	71	76	513	
Rain water	G	82	88	155	87	60	144	89	76	711	69.10
	NG	40	20	16	16	51	39	91	45	318	
Distilled water	G	48	54	43	43	104	68	137	54	551	52.28
	NG	58	46	88	47	105	65	27	67	503	
Sterile distilled water	G	90	77	78	85	55	41	46	45	517	63.05
	NG	29	40	47	25	41	46	40	44	303	
Glucose solution	G	26	27	28	36	21	3	3	2	146	16.95
	NG	64	57	74	66	110	113	106	125	715	
OLJE	G	0	0	0	0	0	0	0	0	0	0
	NG	67	53	49	85	37	42	58	38	449	

* - Each reading is a total of 10 observations

G - Germinated sporangia

NG - Non-germinated sporangia

OLJE - Onion leaf juice extract

IIIB. Arcsin transformation of per cent sporangial germination at 95±5% RH and 12±2°C in different media derived from Appendix IIIA

Media	1	2	3	4	5	6	7	8	$\bar{x}$
Tap water	50.77	44.43	42.65	49.72	34.76	45.69	45.4	42.3	44.47
Distilled water	42.3	47.29	34.94	43.94	44.85	45.63	66.03	41.90	45.86
Rain water	55.06	64.52	69.56	66.81	47.35	59.67	44.6	52.42	57.5
Sterile dist. water	60.4	54.21	52.18	61.53	54.03	42.48	47.01	45.34	52.15
Glucose soln.	32.52	34.51	31.63	36.45	23.66	9.63	9.63	7.27	23.16

ANOVA

Source	df	ss	ms	F
Total	39	8235.9544	211.1783	
Treatments	4	5491.033	1372.7583	19.9844***
Replications	7	821.5554	117.3651	2.5820
Error	28	1923.366	68.6916	

IV A. Sporangial germination of Peronospora destructor at 95 ± 5% RH and 12 ± 2°C in different pH

		Replications									
pH		1	2	3	4	5	6	7	8	Total	%G
2	G	10*	13	2	9	8	5	5	2	54	4.51
	NG	107	88	142	203	220	95	113	176	1144	
4	G	24	20	27	10	7	6	3	4	101	10.32
	NG	120	129	70	111	120	113	97	118	878	
7	G	2	1	2	1	8	2	0	6	22	2.14
	NG	114	135	127	109	179	99	128	177	1008	
9.2	G	2	2	3	10	6	2	0	1	26	2.39
	NG	109	131	84	115	212	121	148	142	1062	
14	G	15	1	1	1	3	0	5	0	26	2.5
	NG	116	25	123	120	110	151	124	143	1012	

* - Each reading is a total of 10 observations

G - Germinated sporangia

NG - Non-germinated sporangia

IV B. Arcsin transformation of per cent sporangial germination at 95 ± 5% RH and 12 ± 2°C in different pH derived from Appendix IV A

	Replications								
pH	1	2	3	4	5	6	7	8	$\bar{X}$
2	17.05	21.05	6.80	11.97	10.14	12.92	11.83	6.02	12.22
4	24.2	21.47	31.82	16.74	13.56	12.92	9.98	10.47	17.65
7	7.49	7.49	7.27	5.44	11.97	8.13	0	12.79	7.57
9.2	7.71	7.04	10.78	16.43	9.63	7.27	0	4.80	7.96
14	19.82	5.13	5.13	5.13	9.46	0	11.39	0	7.0

ANOVA

Source	df	ss	ms	F
Total	39	1796.9732		
Treatments	4	649.9868	162.4967	5.5006**
Replications	7	319.8238	45.6891	1.5466
Error	28	827.1626	29.5415	

V. The effect of time on the germination of attached sporangia of Peronospora destructor at 95 ± 5% RH and 12 ± 2°C.

Time (Hrs)		1	2	3	4	5	6	7	8	Total	%G
12	G	9*	7	14	11	61	6	10	9	72	5.6
	NG	110	72	164	164	187	169	183	155	1204	
24	G	9	21	22	20	20	24	17	25	158	12.6
	NG	125	145	132	112	129	152	157	143	1095	
42	G	33	26	45	70	40	53	22	28	317	37.4
	NG	91	136	70	62	35	44	50	42	530	
48	G	84	54	66	59	58	92	76	70	559	65.1
	NG	32	23	20	16	51	63	62	33	300	
66	G	183	179	134	76	87	92	94	146	991	74.2
	NG	70	62	27	30	30	40	42	44	345	
96	G	41	49	41	33	61	50	46	51	374	
	NG	38	34	56	27	41	37	36	23	292	
120	G	28	35	41	22	20	31	20	19	216	
	NG	102	107	78	54	61	44	57	61	564	
144	G	12	19	17	10	25	8	9	23	123	
	NG	81	118	64	62	63	102	107	53	650	
168	G	0	3	5	1	8	4	2	7	30	
	NG	68	97	81	106	124	83	84	100	743	
192	G	1	4	4	0	0	3	2	3	17	
	NG	77	129	103	61	87	81	63	69	670	
216	G	0	0	0	0	0	0	0	0	0	
	NG	67	107	99	87	63	43	74	64	604	

* Each reading is a total of 10 observations.

G - Germinated sporangia NG - Non-germinated sporangia

VI. Optimum time for sporangial germination of *Peronospora destructor* at 95 ± 5% RH and 12 ± 2°C.

Time (Hr)		Replications 1	2	3	4	Total	%G
1	G	0*	0	0	0	0	0
	NG	135	109	126	105	475	
2	G	2	2	1	2	7	1.4
	NG	127	132	105	125	489	
3	G	2	5	9	8	24	3.9
	NG	100	186	149	156	591	
4	G	7	19	5	8	39	9.1
	NG	110	119	60	102	391	
5	G	7	21	30	37	95	10.35
	NG	168	289	173	193	823	
12	G	30	32	25	17	104	32.6
	NG	64	53	54	44	215	
20	G	64	40	47	50	282	67.6
	NG	13	121	44	38	135	
24	G	121	141	87	74	423	71.4
	NG	36	48	53	32	169	
30	G	96	160	84	108	449	71.7
	NG	51	39	29	58	177	
40	G	20	56	23	28	127	71.8
	NG	8	16	7	19	50	

* Each reading is a total of 10 observations

G - Germinated sporangia

NG - Non-germinated sporangia

VII A. Number of plants infected with Peronospora destructor in seven plots at four dates at National Agricultural Laboratories

Plots	Plants/Plot	Assessment dates Day 0	Day 7	Day 15	Day 22
A*	546	112	188	335	377
B	472	26	53	238	332
C	461	10	27	229	321
D	457	1	8	187	248
E	527	2	8	205	299
F	526	5	9	175	251
G	561	2	7	188	297

* Plot A was nearest to the inoculum source.

VII B. $\text{Log}_e \left(\frac{x}{1-x}\right)$ transformation of porportion of diseased onions infected with downy mildew in seven plots A-G derived from Appendix VII A

Plots	Assessment dates			
	Day 0	Day 7	Day 15	Day 22
A	-1.36	-0.65	-0.47	-0.80
B	-2.84	-2.07	-0.02	-0.86
C	-3.79	-2.79	-0.01	-0.82
D	-6.21	-4.06	-0.37	+0.18
E	-5.52	-4.18	-0.45	+0.25
F	-4.70	-4.06	-0.69	-0.09
G	-5.52	-4.41	-0.69	+0.12

Appendix VII C. Disease increase in seven onion plots infected with onion downy mildew at National Agricultural Laboratories in relation to various weather parameters at four dates

Day	Disease increase	Average RH	Average Temp. (°C)	Average Rainfall (mm)	Average minimum temp. (°C)
0	0.205	66.85	19.21	1.70	14.57
	0.055	66.85	19.21	1.70	14.57
	0.022	66.85	19.21	1.70	14.57
	0.002	66.85	19.21	1.70	14.57
	0.004	66.85	19.21	1.70	14.57
	0.010	66.85	19.21	1.70	14.57
	0.004	66.85	19.21	1.70	14.57
7	0.139	72.09	17.91	3.08	13.35
	0.057	72.09	17.91	3.08	13.35
	0.037	72.09	17.91	3.08	13.35
	0.016	72.09	17.91	3.08	13.35
	0.011	72.09	17.91	3.08	13.35
	0.007	72.09	17.91	3.08	13.35
	0.008	72.09	17.91	3.08	13.35
15	0.27	76.50	18.12	10.34	13.75
	0.392	76.50	18.12	10.34	13.75
	0.438	76.50	18.12	10.34	13.75
	0.391	76.50	18.12	10.34	13.75
	0.374	76.50	18.12	10.34	13.75
	0.316	76.50	18.12	10.34	13.75
	0.323	76.50	18.12	10.34	13.75
22	0.076	72.27	18.74	3.30	15.43
	0.199	72.27	18.74	3.30	15.43
	0.199	72.27	18.74	3.30	15.43
	0.134	72.27	18.74	3.30	15.43
	0.178	72.27	18.74	3.30	15.43
	0.144	72.27	18.74	3.30	15.43
	0.194	72.27	18.74	3.30	15.43

Appendix VII D. Multiple regression analysis of data derived from appendix VII C, using the abbreviated Doolittle method from Steel & Torrie (1960)

ANOVA

Source	df	ss	ms	f
Total	27	1.1784		
Regression	4	1.0402	0.2601	43.39***
Error	23	0.1382	0.0060	

The multiple regression equation is:

$Y = -2778.9556 + 20.1987 X_1 + 106.6442 X_2 - 12.9620 X_3 - 43.6722 X_4$

Y = disease increase

X_1 = Relative humidity

X_2 = Temperature (^oC)

X_3 = Rainfall (mm)

X_4 = Minimum temperature (^oC)

The multiple correlation coefficient - $Ry_{1234} = 0.9395$***

The coefficient of multiple determination - $R^2 = 0.8827$

The standard errors for the partial regression coefficients and t-tests

Sb_1 = 2.069178, t = 9.7617 ***

Sb_2 = 11.130273, t = 9.7611 ***

Sb_3 = 1.332053, t = -9.7308 ***

Sb_4 = 4.479608, t = -9.7491***