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ON THE PARASITISM OF *BREMIA LACTUCAE* REGEL ON LETTUCE[1]

Met een samenvatting: Het parasitisme van Bremia lactucae *Regel in sla*

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1. INTRODUCTION

In recent years head lettuce, *Lactuca sativa* L. var. *capitata* L., has been grown in the Netherlands on an ever increasing scale. This is due partly to larger demands from the side of the consumers, and partly to the rapid expansion of the practice of growing vegetables in greenhouses. Growers of tomatoes became aware of the fact that often good financial results could be obtained when in the greenhouses, no matter whether they were heated or not, as a first crop lettuce was grown. When heated greenhouses are available, the growers do their best to harvest as much tomatoes as possible in the period between April and June, because in these months tomatoes usually fetch a high price. In order to obtain a crop at this time of the year, the tomato seedlings have to be planted out in January or in February. This means that at that time the last lettuce must have been removed, and to make this possible the planting of this vegetable has to be started in September. This, therefore, is one of the reasons why so much lettuce is grown in autumn and in winter. Another reason is to be found in the high price which at this time of the year lettuce fetches at the auctions. As appears from table 1, the amounts of greenhouse lettuce that was brought to the auctions in the autumn months, has, since 1950, continually increased; the table also shows that the supply is now more regularly distributed over the season.

TABLE 1. Amounts of lettuce grown under glass which from November up to June are sent to the auctions in the Netherlands; × 1.000 heads.
Veilingaanvoer van glassla in Nederland van november tot en met mei; × 1.000 stuks.

Period *Periode*	November *november*	December *december*	January *januari*	February *februari*	March *maart*	April *april*	May *mei*	Total *Totaal*
1949–'50	2.500	1.100	125	215	15.400	52.500	71.600	134.480
1952–'53	4.100	3.200	1.200	900	7.400	64.600	32.100	113.500
1955–'56	9.500	8.500	2.800	1.900	20.000	65.000	54.600	162.300
1958–'59	15.000	18.600	13.700	10.500	49.700	102.900	9.100	219.500

Growing lettuce in the autumn appeared to offer some difficulties. In that season temperature, day-length and light intensity decrease, whereas the humidity of the air shows an increase. Under these circumstances a disease which the growers call "het wit" (downy mildew), and which is caused by *Bremia lactucae* Regel, comes more to the fore than it does in other seasons. The name by which this disease is known to the growers, is derived from its most common and at the same time most prominent symptom, viz. the patches of white fungal growth by which the diseased parts of the leaf are covered; the leaf spots themselves show a lightgreen to yellow discoloration. The disease causes a deterioration of the crop, because the plants grow out irregularly and because the head does not develop well. A decrease of up to 40 per cent is not exceptional. It furthermore appeared that all lettuce varieties that so far have been grown, are susceptible to the attacks of *Bremia lactucae*.

The aim of the present study was to find out:

1. what conditions must prevail in the environment in order that *Bremia lactucae* may complete its life cycle;
2. whether by means of special methods of cultivation the fungus may be prevented from obtaining a hold on the plant and from spreading in it;
3. whether the fungus can be combated by means of fungicides; and
4. whether resistant *Lactuca* species, or varieties exist which might serve as a starting point for the development of a commercial breed.

2. DIFFERENT WAYS OF LETTUCE GROWING

"*Autumn lettuce*". The name "autumn lettuce" is generally applied to the crop which in the autumn is grown under glass, and which is harvested from October to the beginning of January. This lettuce is sown from the 20th of August on, the date depending on the aim the grower has in view, i.e. whether he wants to obtain an early or a late autumn lettuce. Sowing is done either under Dutch lights or in a greenhouse, and the young plants are transferred to the bed in which they are to reach their full development, either directly or indirectly. If the last-mentioned way is chosen, they are first transplanted into soil-blocks; this is done when the cotyledons have reached their full length. In the soil-blocks they are usually left until they have developed two leaves, but the condition of the root system is also taken into account. By the aid of this method the growers expect to obtain, among other things, a regular growth. The other way is less often followed; in this case no soil-blocks are used, and the young plants are usually left in the seed beds until they are provided with one or two leaves; at this stage they are transplanted.

In the case of the "autumn lettuce" the external circumstances become gradually more unfavourable to the development of the plants. As appears from table 2, in this period a decrease in temperature and in the number of sunshine hours as well as a shortening of the days are accompanied by an increase of the air humidity. It is true that these observations were made in the open, but the conditions prevailing under glass may be expected to be similar. With this method of cultivation much airing is practised, and only late in autumn the temperature is artificially raised to some extent, which, of course, is accompanied by a decrease in the humidity of the air. It should be realized that the most

TABLE 2. Mean day-length and the averages of temperature, humidity and hours of sunshine measured at Naaldwijk between 1953 and 1959.
Gemiddelde daglengte en het gemiddelde van de temperatuur, de relatieve luchtvochtigheid en het aantal zonuren gemeten te Naaldwijk van 1954 tot 1959.

Month *Maand*	Day-length in hours *Daglengte in uren*	Temperature in °C *Temperatuur in °C*	Relative humidity in % *Relatieve luchtvochtigheid in %*	Hours of sunshine *Aantal zonuren*
January/*januari*	8	5.1	86.5	54.2
February/*februari*	10	1.6	85.3	76.6
March/*maart*	12	5.9	79.8	137.0
April/*april*	14	8.7	75.1	186.0
May/*mei*	15.5	12.9	70.8	242.1
June/*juni*	16.5	15.8	71.0	220.0
July/*juli*	15.5	17.6	76.0	198.6
August/*augustus*	14	17.6	77.0	187.4
September/*september*	12	15.9	79.6	131.7
October/*oktober*	11	11.9	84.2	87.2
November/*november*	9	7.2	84.8	66.5
December/*december*	8	5.6	88.7	39.6

favourable temperature for the development of the head lies approximately between 11 °C and 17 °C, and that the day-length must be at least 10 hours. With this method of cultivation the grower has therefore during the earlier stages of development of his plants a temperature and a day-length that are favourable to a rapid growth, but in the subsequent stages these factors become unfavourable, and this is the more regrettable as in these stages the plants are apparently in a more or less labile condition, with the result that unfavourable conditions may easily lead to physiogenic deviations.

"*Hot-bed lettuce*". When the grower wishes to force his lettuce, he usually sows between the 10th and 15th October, and transplants in December under Dutch lights, but when soil-blocks are used, the final transplantation may be postponed. The beds in which they will reach their final development, consist of soil resting on a layer of manure or of some other forcing material. By the fermentation processes that are going on in this layer, the temperature is raised, and this rise of temperature also affects the soil above it. In this way the roots of the lettuce plants may continue their activities even in times of frost. During the earlier stages of development the grower tries to retard the growth of his plants by airing repeatedly, but during the last weeks, i.e. in the forcing period, airing is reduced as much as possible, in this way the temperature is raised, and a very high degree of air humidity is reached; as a result the growth of the plants is considerably accelerated, and so towards the end of March or at the beginning of April lettuce of first-rate quality can be harvested. When the shelter is heated artificially, the plants can even be harvested at the end of February or at the beginning of March.

Factors that are not changed, are the day-length and the number of hours of sunshine. However, as appears from table 2, these factors are in the later stages favourable to a good development of the head. During the earlier stages they are less favourable. In the later stages the air round the plants reaches, on account of the infrequent airing, a very high degree of humidity.

"*Winter lettuce*". This is the name used for the crop that is harvested in the period between January and the second half of March. To obtain such a crop the lettuce should be sown in the last part of September or in the beginning of October. The seedlings are usually twice transplanted, i.e. they are first transferred to soil-blocks. The final transplantation takes place in the second half of October. During the period of cultivation the temperature may be raised artificially; just as in the case of the "hot-bed lettuce" day-length and the number of sunshine hours must be accepted as they are. However, it appears from table 2 that these factors are unfavourable only when the plants are harvested early in the season; they are no longer so when the lettuce is harvested later.

"*Spring lettuce*". This crop is harvested in the period which begins in the middle of March and extends to the middle of May. The lettuce is grown in the unheated greenhouse, but the method of cultivation practised in the region immediately behind the dunes differs from that which is applied in some places in the central districts. In the first-mentioned area the lettuce is sown in the middle of October, and is transplanted but once, viz. in the middle of November. During the winter months the plants grow but slowly, and only when the

winter is past, and the external conditions become favourable to growth as well as to the development of the head, they begin to grow better. As the greenhouse is aired but sparingly, its temperature is always a few degrees above that of the air outside; records of the latter temperature are to be found in table 2.

With the method of cultivation that is applied in the central part of the country, the lettuce is sown at the beginning of December. Towards the end of this month the seedlings are transferred to soil-blocks, and only when the period of frost is past, i.e. at the end of February or in the beginning of March, the young plants are transferred to the beds in which they are to complete their growth. In this way is obtained that the most important phase of their development takes place in a period in which the external conditions are specially favourable.

"*Summer lettuce*". "Summer lettuce" is grown in the open. The cultivation starts in April and lasts to the end of October, and varieties are used that can stand rather high temperatures. In this period the external circumstances are, on the whole, favourable; large amounts of precipitation, however, may act as a limiting factor.

3. THE FUNGUS *BREMIA LACTUCAE* REGEL

3.1. TAXONOMIC POSITION AND MORPHOLOGY

Bremia lactucae belongs to the Peronosporales, an order of the Phycomycetes, and was first found and described by REGEL (1843). DE BARY (1863) mentioned it under the name *Peronospora gangliformis* (BERK.) DE BARY. The genera *Peronospora* Corda and *Bremia* REGEL are doubtless very nearly related, the difference being confined to the structure of the conidiophores. In *Peronospora* the latter are several times dichotomously branched, the ultimate branches each bearing a conidium. In *Bremia* the conidiophores also branch out dichotomously, each branch finally terminating in a flattened expansion like the upturned palm of the hand. The digits representing the 3 to 5 fine sterigmata which each bear a conidium. *Bremia lactucae* has been found on various Compositae.

In the literature (e.g. in BUTLER & JONES, 1955) descriptions are to be found of the oospores. The latter occur in leaf tissue that has been killed by the fungus; they are light brown and spherical, and measure 26–35 μ in diameter. They have, to our knowledge, not yet been found in lettuce leaves.

The coenocytic hyphae of the intercellular mycelium have a diameter which depends to some extent upon the space that is available in the intercellular passages, and which varies approximately between 5 and 12 μ. The haustoria of the parasite are saccate excrescences of the hyphae which penetrate into the cells of the host. They measure, as a rule, approximately 15.5 μ in length and 9.5 μ in diameter. The mycelium in the substomatal cavities produces 1 to 3, but usually 2 conidiophores, which project beyond the stomata. At the height of the guard cells they are slightly constricted. At the leaf surface they are circ. 11 μ in diam.; but they become gradually thinner; at the first ramification they are still 9 μ in diam.. Their total length varies approximately between 200 and 1500 μ, and they are three to six times, but usually three to four times dichotomously branched; the clavate swelling at the end of the ultimate branches is provided with 3 to 5, usually 4, thin, digitiform sterigmata, each crowned by a single conidium. The hyaline conidia are ovoid-ellipsoidal to spherical, and are provided with a smooth, rather thick wall. The place where they were originally attached to the conidiophore, is recognizable in the form of a small papilla. They measure on the average 20.5 μ $\times$ 18.7 μ, the average quotient between length and diameter being 1.1.

3.2. SYMPTOMS OF THE DISEASE

The most common and at the same time the most easily recognizable symptom of the disease is the presence of light green to yellow leaf spots, which, especially in the older leaves, often end abruptly against some of the thicker veins (Plate I, A). At the lower side of the leaf these spots are often covered by white tufts consisting of conidiophores with conidia. However, when the plants grow very near to each other, the conidiophores may be present on the upper side of the leaves too. In that case the latter are still green when the conidiophores

become visible, and the yellow discoloration appears some days later. In case the cotyledons are attacked, the conidiophores and the conidia are often to be found on both sides. Here too the yellow discoloration shows itself a few days after the conidiophores have become visible. The infection of the cotyledons is never confined to definite spots, and soon after the discoloration becomes visible, they die.

In the older leaves the leaf spots may contain centres of necrosis; the latter are in the middle almost completely transparent. Conidiophores have never been found in such places.

In young plants sometimes leaves are found in which the yellow discoloration has spread over the whole surface or in which only the margin is still green. Although the typical yellow-green leaf spots are absent, and although no conidiophores are as yet visible, this discoloration too must be ascribed to an attack by *Bremia lactucae*, as the conidiophores appear afterwards.

The presence of light green to yellow leaf spots and that of centres of necrosis in the latter, has also been mentioned by earlier authors, e.g. by ERWIN (1921), MILBRATH (1923), WEBER & FOSTER (1928), SCHULTZ & RÖDER (1938), MÜLLER (1939), WILD (1948), WINGRAVE (1952), POWLESLAND & BROWN (1954), and LOUVET & DUMAS (1958). The presence of leaves that are entirely yellow, has not yet been reported.

Symptoms like those described for lettuce attacked by *Bremia lactucae* are also observed in vine leaves that are attacked by *Plasmopara viticola* BERL. et DE TONI. Here too, light coloured spots appear on the leaves, and here too, on the lower side of the latter sporangiophores are present. Such spots may become glassy, and in that case they are called "oil spots". The discoloration of the leaves does not take place when from the beginning of the attack the humidity of the air remains high; under such circumstances the sporangiophores with the sporangia appear on entirely green leaves, and only at a later stage the discoloration sets in (ISTVANFFI & PALINKAS, 1912; GREGORY, 1914; MÜLLER & SLEUMER, 1934; GAUDINEAU, 1954; and PEREIRA CONTINHO, 1954).

In tobacco leaves attacked by *Peronospora tabacina* ADAM not only chlorotic, but also necrotic spots may appear, and the latter are not necessarily preceded by the chlorotic ones. Here too no conidiophores have ever been found on the necrotic spots (PINCKARD & SHAW, 1939).

4. THE FIRST SIGNS OF THE DISEASE; OBSERVATIONS MADE ON LETTUCE GROWN ON A COMMERCIAL SCALE

With each of the different ways of cultivating lettuce it is possible to indicate some periods in which very often the first signs of an attack by *Bremia lactucae* may be seen or in which the spreading of the parasite becomes manifest.

With the cultivation of "autumn lettuce" a first attack may take place already in the seed bed, especially when for the latter use is made of Dutch lights; sowing in a greenhouse appears to be more safe in this respect. On the seed bed the seedlings are so crowded that their cotyledons touch each other, with the result that water which may be present between them, evaporates but slowly, and that drops may remain attached to the cotyledons. As appeared later in this study, this circumstance favours the germination of the conidia of *Bremia lactucae* which eventually may be present. The result of the attack often remains hidden until the seedlings are transferred to the soil-blocks or to the beds.

Immediately after the seedlings have been transferred to the soil-blocks or, if no soil-blocks are used, immediately after they have been transferred to the beds, the first signs of the attack or even of a spreading of the disease to plants which had not been attacked in the seed bed, may become visible. The spreading of the disease in the soil-blocks may be due to the fact that the cotyledons of the transplanted seedlings sink down on the moist soil and are wetted in this way, with the result that eventually present conidia may be taken up by the water drops where they may germinate. When the young plants are transferred directly to their final place, it requires some time before they resume their growth, and during this time they often hang down, and in this way the young leaves come into contact with the wet soil, and are wetted themselves; as they remain in this condition for some time, they may easily be attacked by the fungus. It may, moreover, be desirable to water the young plants one or more times, with the result that the young leaves remain wet for an even longer time.

A first attack as well as a marked expansion of the disease may take place when the seedlings are left too long in the soil-blocks, because in that case the young plants are too near to each other so that the evaporation is retarded, and drops of water may become attached to the leaves. Only after the final transplantation the presence of the disease becomes recognizable; it may be restricted to a small number of plants, but it is also possible that nearly all of them prove to be affected. A drawback of the transplantation is, that when the plants are too large, a considerable number of roots, which did grow out through the soil-block, may be damaged, with the result that the growth of these plants stagnates. During this period of stagnation the leaves often hang down, and for some hours they may even rest on the soil, which, as we have seen already, may have disastrous results.

In the greenhouse a first attack, but especially a strong spread of the disease, may be expected when the plants have become so large that they touch each other. In this case water condenses on the lower side of the leaves that are nearest to the soil surface, and these drops of water may remain there during the remaining part of the cultivation period. Moreover, between the leaves too

water may collect and stay there for a rather long time. However, if the crop had not been attacked previously, the damage is not severe.

With the other methods of lettuce growing described in chapter 2, the first attack by *Bremia lactucae* as well as the spreading of the infection may take place in the same stages of development and for the same reason, but here, apart from the degree of humidity, the external circumstances appear to be less favourable to the parasite. At lower temperatures the young plants grow less rapidly; they remain more compact, and are of a darker green. In this condition they suffer less from a transplantation; their leaves, for instance, do not hang down. Moreover, the surface of their leaves is in the earlier stages of development almost always dry, and infections with *Bremia lactucae* are therefore mainly confined to the larger plants. Sometimes special measures are taken by which the spreading of the disease is kept in check. With "hot-bed lettuce", for instance, a few weeks after the transplantation all young leaves which do not look healthy, are removed. In the case of "summer lettuce" infection with *Bremia lactucae* appeared to depend in a large measure upon weather conditions. After heavy rains followed by days with a high degree of cloudiness and little wind, the disease may spread considerably; this happened, for instance, in the later part of the summer of 1958. However, as the infection remains confined to the lower leaves, the damage is, as a rule, of no great importance.

The dependence of the disease upon the weather conditions, which comes so clearly to the fore in the case of the "summer lettuce", is also found with lettuce that is grown under glass. A short day-length or a small number of sunshine hours results in an increased elongation, especially of the leaves, which become long and flabby; they remain, moreover, light green. In this condition the plants are very susceptible to changes in the external circumstances. Immediately after they have been transplanted, they lose their turgescence and sink down; in contact with the soil the leaves become wet and are therefore easily attacked by the parasite. In dark, rainy weather the disease spreads more rapidly in these plants and affects them more seriously than it does in clear and dry weather.

In lettuce cultures often growth stagnations are noticed, and in that case a few days after the growth begins to stagnate, an infection by *Bremia lactucae* may become visible. Especially in young cultures this is a rather striking phenomenon (Plate II, B). The growers regard the growth stagnation as the cause of the infection by *Bremia*, but it might as well be the result of the latter (cf. p. 163).

In the parts of the plant that are infected by *Bremia lactucae*, very often another fungus begins to develop, viz. *Botrytis cinerea* PERS. Plants that are infected by *Bremia lactucae* alone, are mostly not killed, but plants that are secondarily infected by *Botrytis cinerea*, very often die. The leaves and the basal part of the stem begin to rot, and then the whole plant succumbs. In the literature too, this disastrous development has been ascribed to the intervention of *Botrytis*, e.g. by MILBRATH (1923) and by SMIETON & BROWN (1940).

5. PLANT MATERIAL AND INOCULATION METHODS

The lettuce plants used in our experiments belonged to the variety "Proeftuins Blackpool", which is very often grown either as an autumn or as a winter crop. This, however, was not the only reason for our choice; another reason was that it is more susceptible to infection by *Bremia lactucae* than most other varieties prove to be. Sowing was done in the usual way, and the further treatment too conformed to the usual practice. When the seedlings were transferred to the soil-blocks, care was taken that the cotyledons remained approximately 0.5–1.0 cm above the surface of the wet clod. In this way their surface remained dry, and there was therefore no danger that a too early infection would make them unsuitable for the experiments. Growth took place in a greenhouse; where in rainy weather the plants could be kept dry by a gentle heating. As a rule, the plants were used for the experiments when they were provided with two or three normal leaves, as at that stage they could easily be inoculated. Up to this stage they could be kept in the soil-blocks; transplantation in larger pots was necessary only when older or flowering plants were required. Cultures were made at all times of the year, and as in the variety "Proeftuins Blackpool" the critical day-length for the development of flowers is approximately 13 hours, there was a rapid shooting up of the heads in summer, with the result that at that time of the year flowering shoots and capitula were also available.

All inoculations were performed with conidia of *Bremia lactucae* that were obtained from artificially infected lettuce plants. The culture of the fungus was started with conidia obtained from diseased plants of the variety "Proeftuins Blackpool" grown for the sale.

Unless stated otherwise, the inoculations were performed by immersing the leaves of the young plants in a suspension of conidia. In order to obtain these suspensions, diseased leaves on which the conidiophores were discernible, were rinsed in water.

From May to the end of August, i.e. in the months with a rather high temperature, the inoculation was restricted to a part of the leaves, as it appeared that plants of which all leaves were inoculated, often succumbed in a few days. At lower temperatures this took more time.

The inoculation was sometimes performed in another way, viz. by spraying the plants with a suspension of conidia. With this method a larger number of test plants had to be used, as the infection was always less severe, and as often only a part of the plants became infected.

The immersion method took up more time, but it was more effective, as after some days all inoculated leaves proved to be covered with a large number of conidiophores.

Immediately after they had been inoculated, the plants were brought in an environment in which the air had a high degree of humidity. To this end glass boxes with a content of approximately 1 cubic meter were used, in which the air, as much as possible, was kept at saturation point. The plants were left in these boxes, which stood in the greenhouse, until the parasite had produced conidiophores and conidia. In order to obviate the development of too high

temperatures, the glass walls of the boxes and of the part of the greenhouse in which the latter stood, had been chalked. The temperature in the boxes was registered by means of a thermograph, and proved to fluctuate between 15 °C and 25 °C.

As on the average every other day approximately 100 plants were inoculated, fresh conidia were never wanting. However, when at a certain moment an unusually large number of conidia was required, e.g. for an experiment with fungicides, then some time previously a larger number of plants had to be inoculated. Twenty four hours after the inoculation these plants were taken out of the glass boxes and removed for some time to another part of the greenhouse; one day before the large amounts of conidia were needed, the plants were returned to the glass boxes, or else their leaves were cut off and put in pots with a little water; under these circumstances the next day every leaf was covered with a dense layer of conidiophores and conidia.

As the flowering shoots are covered with wax, with the result that they are difficult to wet, they were inoculated in a slightly different way. The flowering shoots were pushed into tubes with a slight U-shaped bent, which were fastened horizontally to a stand, and of which the bent part was filled with the suspension of conidia. The flowering shoots were left in these tubes for about 20 hours, and then the plants were placed in the glass boxes. The capitula were treated in the same way, but here results were also obtained by the ordinary method of immersion into the suspension.

6. PATHOLOGICAL ANATOMY

6.1. LITERATURE

Various authors (SCHWEIZER, 1919; SCHULTZ, 1937; SCHULTZ & RÖDER, 1938) have reported that the germ tubes penetrate into the leaves by way of the stomata. COHEN (1952) says that they pass into the interior of the leaf sometimes by way of the stomata and sometimes directly through the epidermis. POWLESLAND (1954), on the other hand, states that the latter way is the only one that is used by them, but that the points at which they penetrate into the interior of the leaf are found in the immediate vicinity of the stomata.

The non-septate hyphae grow out in the intercellular spaces of the host plant, and obtain their food, at least partly, by means of haustoria of rather variable shape. Except in the young leaves the latter are usually present in large numbers. The young leaves apparently contain nutrients which the parasite can absorb without the help of haustoria. The latter are produced in the somewhat older leaves at places where the hyphae come into contact with cells of the host. There the cell-wall becomes perforated, and then an outgrowth of the hypha passes through this opening into the interior of the cell, where it is subsequently enveloped by a thin membrane produced by the protoplast of the latter. As the haustorium is still expanding, this membrane is usually burst, and reduced to a ring surrounding the basal part of the haustorium. It seems that the protoplast of the host cell is not damaged by the haustorium, and that its semipermeability remains unchanged (FREYMOUTH, 1956).

6.2. METHODS OF INVESTIGATION

The way in which the germ tubes penetrate into the leaf, was studied: 1. in leaves which remained attached to the young plants and which were inoculated by immersing them in a suspension of conidia, 2. in detached leaves which after being immersed into such a suspension, were placed in a Petri dish lined with moist filter paper, and 3. in detached leaves lying in a Petri dish which were inoculated by means of drops of this suspension. Twenty four hours after the inoculation the leaves were cleared by boiling them cautiously in lactophenol-aethanol (10 gr. waterfree phenol, 10 ml. conc. lactic acid, 20 ml. glycerol and 20 ml. 96 per cent aethanol). Clearing may also be done in a mixture of glacial acetic acid and aethanol (1 volume part of glacial acetic acid to 4 volume parts of 96 per cent aethanol); when the leaves are left in this mixture for about 6 hours, they become fully transparent. The cleared leaves were stained for 16 to 24 hours with cotton blue dissolved in lactophenol-aethanol (20 mgr. cotton blue in 100 ml. lactophenol-aethanol). In the leaves that had been cleared with the mixture of glacial acetic acid and aethanol, the action of the stain remained confined to the parts of the fungus on the outside of the epidermis, as in this clearing agent the intercellular spaces remain almost completely filled with air, with the result that the stain can not well penetrate into them. In addition, a number of inoculated pieces of leaf were fixed about 48 hours after the inoculation by placing them for at least 24 hours in a mixture of formalin, propionic

acid and 50 per cent aethanol, the air above the fixing fluid being, as far as possible, sucked off; these fixed pieces of leaf were sectioned by means of a microtome, and the sections stained either with a solution of cotton blue in lactphenol-aethanol or in thionin-orange-G.

The growth of the mycelium in the interior of the leaf, the production of haustoria, and the various stages in the development of the conidiophores, were studied in totally infected leaves that had been cleared in lactophenol-aethanol and that were subsequently stained either in cotton blue dissolved in lactophenol-aethanol or in a mixture of this dye with safranine dissolved in the same liquid (100 mgr. safranine, 20 mgr. cotton blue, 100 ml. lactophenol-aethanol). In addition, microtome sections through infected leaves were used which were obtained in the same way as those that were used for the study of the way in which the parasite penetrates into the leaf.

6.3. Results and discussion

On most of the infected leaves conidiophores and conidia are found. The latter are easily dropped; one has to touch the conidiophore or even the leaf but very slightly, and they are immediately detached. If they arrive on a wet leaf of a lettuce plant, they may produce a germ tube, and this may penetrate into the leaf. Only once a germ tube was found to have entered by way of a stoma; in all other cases they found their way into the interior directly through the epidermis, and on the lower side of the leaf as well as on the upper side. At the place where the germ tube enters an epidermal cell, it forms a slight swelling, a so-called appressorium. The production of these appressoria rests apparently on a contact stimulus, for in vitro too swellings are produced by the germ tubes, viz. when they come in contact with other germ tubes or with conidia. The distance between the conidium and the appressorium is often very small.

From the appressorium a thin infection hypha emerges, which traverses the cell-wall. When this hypha arrives in the lumen of the cell, it often grows out into a kind of sac which may fill a large part of the latter (fig. 1). In the mean-

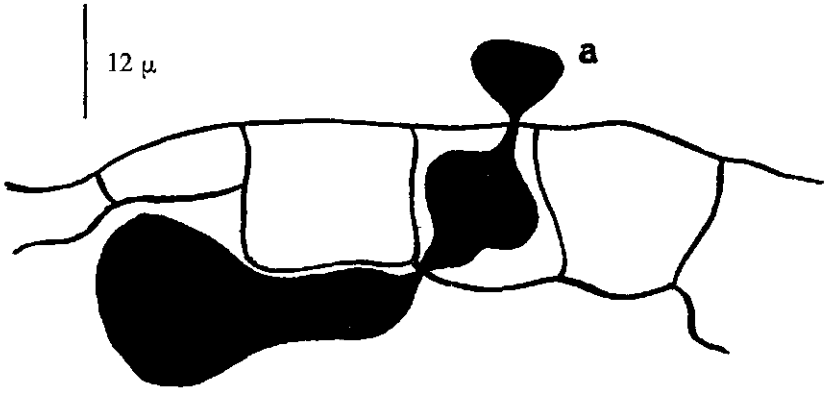


Fig. 1. Cross section through an infection spot; part of the appressorium (a), thickening of the infection hypha within an epidermal cell, and first development of the intercellular mycelium.

Dwarsdoorsnede door een plaats, waar de schimmel het blad is binnengedrongen. Gedeelte van het appressorium (a), verbreding van de infectie hyfe in de epidermis-cel en eerste ontwikkeling van intercellulair mycelium.

time the protoplasm withdraws from the conidium. Out of the vesicular swelling one to three hyphae emerge, which in their turn traverse the cell-wall, and enter into the intercellular spaces (Plate III, A). When no vesicular swelling is produced in the epidermal cell, the infection hypha immediately continues its way towards the intercellular spaces. The contents of an uninfected epidermal cell stain, as a rule, slightly with cotton blue, but the protoplasm of the cells into which the parasite has found its way, is after an interval of circ. 30 hours no longer stainable with this dye; the cell contents are then yellow to colourless, whereas the contents of the surrounding epidermal cells show the usual light blue colour. This difference in stainability exhibited by infected and non-infected cells is especially clear when the infection took place at a temperature of more than 15°C.

However, it is not necessary that the way along which the germ tube penetrates into the interior of the leaf, leads through an epidermal cell; the germ tube may force its way also through the wall between two epidermal cells. In that case the appressorium is but small. In the single instance in which the entrance via a stoma was observed, there was no appressorium at all.

Our finding that the germ tube does not, as a rule, penetrate into the leaf by way of a stoma, does not correspond with what SCHWEIZER (1919), SCHULTZ (1937) and SCHULTZ & RÖDER (1938) have stated, but it agrees better with the observations of COHEN (1952) and of POWLESLAND (1954). According to our own observations the way in which *Bremia lactucae* enters into the leaf, is similar to that found in various species of *Peronospora*, e.g. in *P. spinaciae* (MONT.) DE BARY, in *P. brassicae* GÄUMANN and in *P. tabacina*; these species too were found to penetrate into the interior of the leaves of their hosts by way of the epidermal cells (CHU, 1935; RICHARDS, 1939; HENDERSON, 1937; HILL, 1957). Especially HENDERSON's description of the way in which the germ tubes of *P. tabacina* penetrate into the leaves, is in this respect noteworthy, as it agrees completely with that given above for *Bremia lactucae*, whereas in older publications (DARNELL-SMITH, 1929; WOLF et al., 1934) it had been reported that the germ tubes of *P. tabacina* enter by way of the stomata. Indications of a degeneration of the protoplasm, as described above for the cells that are infected by *Bremia lactucae*, have not been reported. *Phytophthora infestans* (MONT.) DE BARY, on the other hand, enters the potato leaves not only by way of the epidermal cells but also through the stomata (CROSIER, 1934; PRISTOU & GALLEGLY, 1954), whereas *Plasmopara viticola* penetrates the leaves of its host, the vine, only by way of the stomata (cf. e.g. GREGORY, 1914; MÜLLER-THURGAU, 1915; BOUBALS, 1957).

The intercellular spaces in the lettuce leaves become almost completely filled with the non-septate hyphae of the parasite (Plate III, B). In many places utriculiform haustoria are produced within the surrounding cells. This happens in ordinary leaves as well as in cotyledons (Plate III, C). The first haustoria are formed when the hyphae are removed but three to four cell lengths from the cell through which they forced their entrance. As a rule, but a single haustorium is produced per cell, although occasionally up to five of them may be present. They are often surrounded by a thin lamella consisting of a differently coloured material, and in one instance a ring of a similar substance was found round the basal part of a haustorium. FREYMOUTH (1956) had reported that neither in the cotyledons nor in the young leaves haustoria are produced, but in our material

they proved to be present in large numbers in the cotyledons as well as in the young leaves.

As the tissue in the interior of the lettuce leaves consists almost completely of spongy parenchyma, there is nothing which prevents a spreading of the mycelium to all parts of the leaf. It may even spread into the tissue which fills the wings of the petiole. One week after the parasite entered the leaf, it had already reached every part of the latter with the exception of the parenchyma round the thicker veins and of that in the centre of the petiole. As the intercellular spaces in lettuce plants that are grown under glass, vary but slightly in width, the diameter of the hyphae too differs but slightly in the various parts of the leaf.

Although the age of the leaves does not influence the degree of infection or the development of the parasite, the latter may be influenced by other factors. In this respect two groups of plants might be distinguished, one group consisting of plants that are grown either at a low temperature or in strong sunlight, or at a low temperature as well as in strong sunlight; the second group consisting of plants that are grown either at a high temperature or that receive but little sunshine or that are grown at a high temperature and which at the same time receive but little sunshine. In these two groups of plants the anatomical structure of the leaves appears to be different. In the first-mentioned group the parenchyma cells, and also the intercellular spaces too, are smaller. In this group the hyphae appear to be smaller and to form a less dense network. Moreover, the separation of the infected parts by the thicker veins is in this group more marked. It is apparently not easy for the fungus to pass the thicker veins, especially in plants belonging to the first group. It may be that there is a barrier of a physiological nature, e.g. a shortage of nutrients in the tissue round the veins, but it is also conceivable that the barrier is of an anatomical nature; the development of haustoria might, for instance, be impeded by the thickness of the cell-walls. Another fungus belonging to the Peronosporales seems to experience a similar difficulty in its attempts to spread in the parenchyma of its host. In the leaves of the vine the hyphae of *Plasmopara viticola* were seen to divide in the neighbourhood of a vein in a number of thin threads which appeared to reunite at the other side of the vein (PIOTH, 1957).

The substomatal cavities become also filled by mycelium, and from this mycelium emerge one to three, but usually two conidiophores, which grow out through the stomata into the open (Plate IV, A). As stomata occur here on both sides of the leaf, conidiophores too are found on the upper as well as on the lower side. We found no definite swellings of the hyphae in the substomatal cavities, as were described by some previous authors, e.g. MILBRATH (1923); because the substomatal cavities are wider than the other intercellular spaces, the hyphae may reach here a larger diameter.

Apart from the leaves which form the head, those on the flowering shoot too may be attacked. In structure the latter resemble the leaves of the plants that are grown at a low temperature or in strong sunlight. The flowering shoots themselves may also become infected, but here the mycelium does not penetrate beyond the outer layers of the cortical parenchyma. From the point of attack the hyphae spread in apical and in basipetal direction, but this happens very slowly. At a temperature of 20 °C and a high air humidity the mycelium needed ten weeks to spread over a distance of 5 to 10 mm.. The factors that are respon-

sible for this slow rate of spreading, may be of a similar nature as those that play a part in the vicinity of the thicker nerves. In the capitula no infection with *Bremia* could be obtained; 20 to 30 hours after the inoculation a brown discoloration was noted, and soon afterwards *Botrytis cinerea* made its appearence.

The mycelium of *Bremia* does not spread from the infected leaves by way of the leaf base and the stem to other leaves. The infection remains confined to the parenchyma of the infected lamina and to that in the adjoining part of the petiole. In this respect it differs from the infection which *Phytophthora infestans* causes in the potato, for here the mycelium may spread through the shoots (cf. e.g. VAN DER ZAAG, 1956), and from that caused by *Plasmopara viticola* in the vine, where even in the xylem of the stems the presence of mycelium could be demonstrated (BARRETT, 1939; PIOTH, 1957).

7. PHYSIOLOGY OF THE PARASITISM

7.1. INFLUENCE OF TEMPERATURE, AIR HUMIDITY AND THE PRESENCE OF LIQUID WATER

7.1.1. *Literature*

The globose to ovoid, hyaline conidia of *Bremia lactucae* germinate only in water, not in air which is saturated with water vapour (SCHWEIZER, 1919; MILBRATH, 1923; SCHULTZ, 1937; SCHMIDT & BÖHM, 1954). All authors, with the exception of MILBRATH (1923), describe the germination as a direct one, which means that the conidia form at once a germ tube, but MILBRATH reports that occasionally 8 or more swarm spores may be produced, i.e. that the conidia may also behave as sporangia. Especially conidia that are produced in the darker and cooler months of the year would behave in this way.

According to SCHWEIZER (1919) the range of temperature which is most favourable for the germination of the spores, would lie between 20 °C and 25 °C; at lower temperatures the germination process would proceed at a slower rate. Other authors mention a much lower optimum temperature. According to MELHUS (1921) the latter would lie between 6 °C and 10 °C, according to SCHULTZ (1937) between 5 °C and 10 °C, and according to POWLESLAND (1954) between 1 °C and 10 °C. The highest temperature at which the conidia may germinate, lies according to SCHULTZ (1937) at 20 °C and according to POWLESLAND (1954) at 25 °C. The lowest temperature would be found in the vicinity of the freezing point. Especially in the neighbourhood of the optimum temperature the germination proceeds at a fast rate. Between 15 °C and 21 °C the germination percentage and the length of the germ tubes increase at first in direct proportion to the time (POWLESLAND, 1954). With conidia that have been produced at higher temperatures, the germination percentage is lower than with those that were formed at lower temperatures (SCHULTZ, 1937).

The entrance of the germ tubes in the epidermal cells may proceed rapidly. According to SCHULTZ (1937) the optimum temperature for this process would lie between 15 °C and 17 °C. At such a temperature a few germ tubes were found to have entered the leaf 7 hours after the inoculation. After 24 hours already 34–46 μ long hyphae were present in the leaf.

POWLESLAND (1954) estimated for temperatures ranging from 15 °C to 21 °C the time required for the establishment of an infection, i.e. the time required for the germination of the conidia plus that needed by the germ tubes for penetrating into the epidermis. To this end groups of inoculated plants were sprayed after a period of different length with dithane (zineb) in a concentration of 4 gr. per liter. This killed the hyphae on the surface of the plant, but not those that had already penetrated into the leaves. It appeared that 4 hours after the inoculation already some germ tubes had entered the leaf, for when the spraying with zineb took place 4 hours after the inoculation, about 15 per cent of the plants proved to be infected.

The development of the mycelium in the interior of the leaf is favoured by a high degree of humidity in the air (cf. e.g. WINGRAVE, 1952; POWLESLAND,

1954). In view of the comparatively low optimum temperature which they found for the germination of the conidia, SCHULTZ & RÖDER (1938) suppose that the growth of the mycelium in the leaf too will be served best by a comparatively low temperature. Several other authors are of the same opinion, e.g. ERWIN (1921), MELHUS (1921), and COX (1955). According to POWLESLAND (1954) the lowest temperature for the growth of the mycelium would be 2 °C, the highest one 20 °C, whereas according to BRIEN et al. (1957) the optimum temperature would lie between 15.5 °C and 17 °C.

All investigators agree that for the development of the conidiophores a high atmospheric humidity is required (SCHULTZ, 1937; YARWOOD, 1937; OGILVIE, 1943; POWLESLAND, 1954). According to SCHULTZ & RÖDER (1938) it would have to be at least 98 or 99 per cent; according to POWLESLAND (1954) it lies between 80 and 100 per cent. The temperature is of but secondary importance, as the range of temperature in which conidiophores may be produced, is very wide (4°–20 °C). According to GROGAN et al. (1955) the most suitable temperature is found between 10 °C and 15.5 °C, whereas, according to POWLESLAND (1954), it would lie between 6 °C and 11 °C.

7.1.2. *Methods of investigation*

As it had been found that germination of the conidia takes place only in water, we used in our experiments on the germination of the conidia suspensions of the latter. These suspensions were made partly with pure water and partly with a decoction of lettuce leaves. The influence of the temperature on the germination of the conidia and on the growth of the germ tubes was studied in hanging-drops; the latter were placed in incubators that were kept at different temperatures. At the end of 24 hours the germination percentage was determined in a sample containing 500 conidia, whereas of 100 germ tubes the length was measured. In order to estimate the rate at which the germination of the conidia and the growth of the germ tubes proceeds, similar estimations were carried out after periods of differing length.

The range of temperature within which the fungus can enter the epidermis, was studied in detached leaves which had been inoculated with drops of a suspension of conidia; these leaves were kept in Petri dishes that were placed for 24 to 48 hours in incubators at different temperatures. At the end of the sojourn in the incubator, the leaves were cleared in lactophenol-aethanol and stained with cotton blue dissolved in the same liquid; after that it was possible to see whether the fungus had entered the leaf or not.

The influence of the temperature on the rate at which the germ tubes enter the leaf, was studied by the aid of the method of POWLESLAND (1954). To this end groups of 20 plants were sprayed with 0.4 % zineb each at a different moment after the inoculation; then we waited in order to see whether the plants would become diseased. By repeating the experiment at different temperatures the influence of the latter could be determined. As appears from the literature (POWLESLAND, 1954), and as we could confirm in our own experiments, the hyphae on the leaves are the only ones that are killed by the treatment with zineb.

In order to study the influence of the temperature on the production of conidiophores with conidia, plants were taken on which conidia were already present; these plants were carefully rinsed, and placed in pots which contained

water; in this way the air around the plants remained saturated with water. The pots were placed in incubators at various temperatures, and the plants were regularly inspected in order to find out at what moment the production of conidiophores was resumed. The problem was studied also with the method by the aid of which CRUICKSHANK (1958) had studied the production of conidia in *Peronospora tabacina*. In that case discs with a diameter of 1 cm were cut out from infected leaves in which the fungus was unable to produce conidia because they were not covered by the required film of water. These discs were placed on top of cups with a diameter of 0.9 cm, which were filled with water; care was taken that between the leaf disc and the water in the cup no air was present. A number of these cups were placed in a Petri dish containing some water, and the Petri dishes were put in incubators. The discs were inspected at regular intervals in order to see whether conidia had been produced.

In order to dispose always over fresh conidia, every other day on the average a hundred plants were inoculated. This was done at temperatures which varied, according to the season, between 15 °C and 25 °C; however, in each experiment the temperature varied at the most 4 °C. Part of the plants were kept at a constant temperature. As every morning all plants were inspected in order to see whether conidiophores with conidia were present, it was possible to determine how many days the inoculated plants needed to produce their fructifications. Afterwards it appeared that in case the humidity of the air remained continually high, the appearance of conidiophores with conidia must be taken as the first symptom of the disease. Thus, under this environmental condition the end of the incubation time could be estimated.

7.1.3. *Results and discussion*

That for the germination of the conidia water is required, and that air saturated with water vapour is insufficient to this end, appeared clearly from the behaviour of conidia that had been brought on a slide which for one half was covered with water, and which was placed in a Petri dish lined with wet filter paper; the conidia that were immersed in water, were the only ones that germinated.

On the diseased leaves two kinds of conidia appeared to be present, viz. conidia with a hyaline, minutely granular content and conidia with an opaque, coarsely granular content. The last-mentioned conidia did not germinate in water, and are probably dead. Table 3 gives for some suspensions of conidia produced under different circumstances the percentages of conidia with an opaque and with a hyaline content as well as the percentage of the conidia which after 24 hours at 4 °C had germinated.

The germ tube emerges from the conidium exactly opposite the place where the latter originally was attached to the conidiophore. It grows at first in a straight line, but after a short time it begins to follow a meandering course. Very rarely a branched tube was found. The germ tubes often produce a swelling when they come into contact with other ones or with conidia, but after some time the swelling may produce in its turn a tube of the normal aspect. During the development of the germ tube the protoplast leaves the conidium. Production of swarm spores was never observed, neither with conidia that had been produced at higher temperatures, nor with those that were formed at lower ones.

TABLE 3. Percentage of conidia with opaque and with hyaline contents present in a number of suspensions, and the percentage of conidia which had germinated after a sojourn of 24 hours at 8 °C.

Percentage conidiën met granulaire en met hyaline inhoud in een aantal sporensuspensies en het percentage gekiemde conidiën na 24 uur bij 8 °C.

Contents of conidia *Inhoud der conidiën*		Germinated conidia *Gekiemde conidiën*
opaque *granulair*	hyaline *hyalien*	
89.7	10.3	9.4
99.4	0.6	0.1
92.7	7.3	7.5
91.5	8.5	6.4
71.9	28.1	25.4
96.8	3.2	2.1

The influence of the temperature on the germination of the conidia is shown in fig. 2. This figure demonstrates that the lowest temperature at which germination took place, was found at about -3 °C (in a decoction of lettuce leaves), whereas the highest temperature at which germination could be obtained, lay at ± 31 °C. The most favourable temperatures were found between 4 °C and 10 °C. Even at temperatures round the freezing point still a comparatively large number of conidia germinated. It further appeared that of the conidia produced between 20 °C and 22 °C a lower percentage germinated than of the conidia produced at 10 °C to 15 °C. The highest temperature at which germination took place, and the temperature which is most favourable to germination appeared to depend also on the temperature at which the conidia were produced. For those that were produced between 20 °C and 22 °C, they are respectively circ. 31 °C and 4 °–10 °C, and for those produced between 10 °C and 15 °C circ. 29 °C and 2 °–8 °C. At any rate, in the whole range of temperatures in which lettuce is grown, the conidia may germinate, provided that water is available.

The results of the experiments that were carried out in order to determine the rate of germination at different temperatures, are shown in fig. 3, whereas in fig. 4 for two temperatures the observations on which the figures of fig. 3 rest, are given. The graphs prove that especially within the temperature range which is most favourable to the germination of the conidia, the germination is completed in a comparatively short time, viz. in about 4 hours. It is noteworthy that a similar rapid germination has also been found with the sporangia of *Phytophthora infestans*, which are in this respect comparable to the conidia of *Bremia* (CROSIER, 1934).

The length which the germ tubes reach in 24 hours at different temperatures, is shown in fig. 5. Although the values fluctuate rather considerably, the most favourable temperature lies obviously in the vicinity of 15 °C. No difference could be found between the length of the germ tubes produced by conidia which matured at temperatures between 20 °C and 22 °C and that of the germ tubes produced by conidia which ripened at temperatures between 10 °C and 15 °C. Because of the meandering course of the germ tubes, especially of the longer ones, it is difficult to measure their length accurately; for this reason the graph

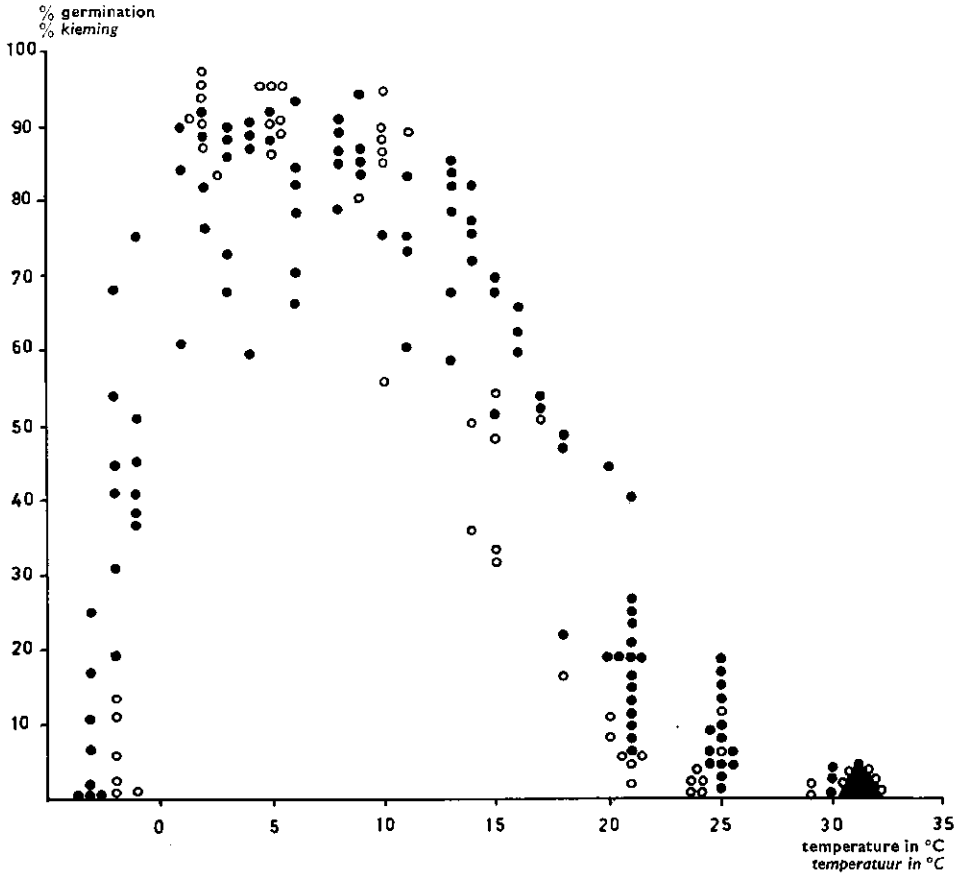


FIG. 2. Germination percentages of the conidia obtained at different temperatures in 24 hours; each figure is the average of 500 observations.

Kiemings-percentages van de conidiën bij verschillende temperaturen na 24 uur; elk punt is het gemiddelde van 500 waarnemingen.

● conidia formed at 20°–22°C
● *conidiën ontwikkeld bij 20°–22°C*
○ conidia formed at 10°–15°C
○ *conidiën ontwikkeld bij 10°–15°C*

has a rather irregular aspect. The longest germ tube was found in a culture in distilled water made at 16 °C; it measured 638 μ after 24 hours. The temperature which is most favourable to the growth of the germ tube is distinctly higher than that which is most favourable to the germination of the conidia. This has also been found for other Peronosporales; for *Phytophthora infestans* these values are respectively 21 °C and 12 °C, for *Peronospora destructor* (BERK.) Casp. 21 °C and 11 °C (CROSIER, 1934; COOK, 1932).

The rate of growth shown by the germ tubes at different temperatures is set out in fig. 6, whereas in fig. 7 the measurements on which the figures of fig. 6 rest, are shown for two of these temperatures. These graphs show that the length

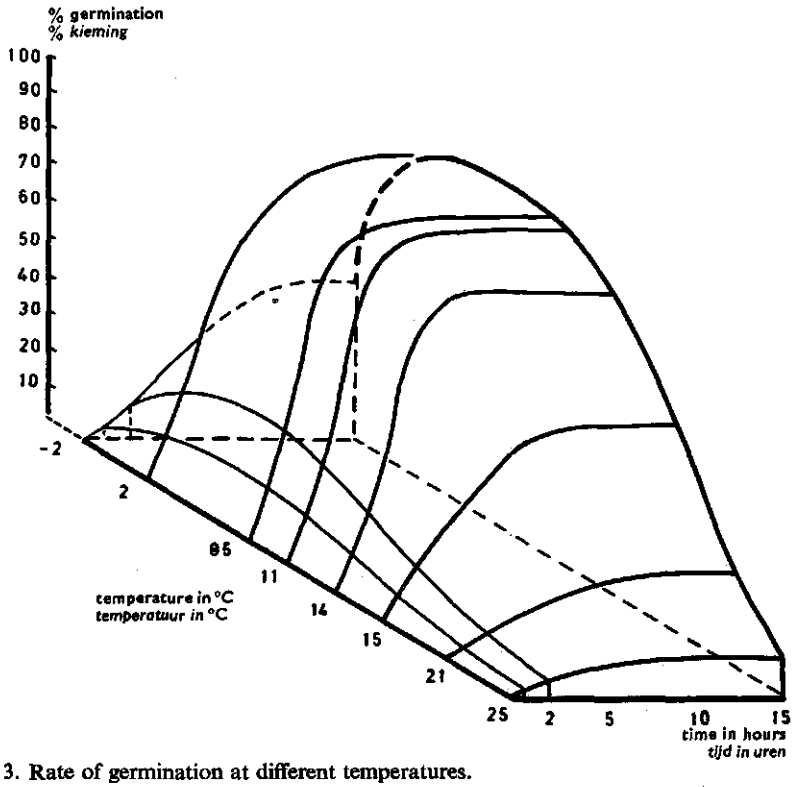


FIG. 3. Rate of germination at different temperatures.
Kiemsnelheid bij verschillende temperaturen.

of the germ tubes increases at first in direct proportion to the time, a relation which has also been found by POWLESLAND (1954).

The entrance into the epidermis may take place between circ. 3 °C and circ. 28 °C. After a sojourn of 48 hours at a temperature of 1 °C a comparatively large number of conidia had germinated, but not a single germ tube had entered the epidermis. Between 28 °C and 30 °C a few conidia still succeeded in germinating, but at these high temperatures too not a single germ tube entered the leaf. Experiments taken at different temperatures, in which the plants at different times after the inoculation were sprayed with zineb, showed that between 10 °C and 22 °C the entrance into the leaf can be prohibited by spraying within 3 hours after the inoculation, but not when more than 3 hours elapse between the inoculation and the spraying; at temperatures between 4 °C and 8 °C the entrance could be prohibited when the spraying took place within 6 to 8 hours after the inoculation. This means that with the various methods of lettuce growing that are practized now, temperature can not act as a limiting factor in the infection with *Bremia*. Between 10 °C and 22 °C the fungus can enter the leaf within three hours after the inoculation. As the germ tubes are, as a rule, still very short when they enter into the leaf, it is comprehensible that this requires but little time. Similar results were obtained by POWLESLAND (1954). At lower temperatures the entrance requires more time.

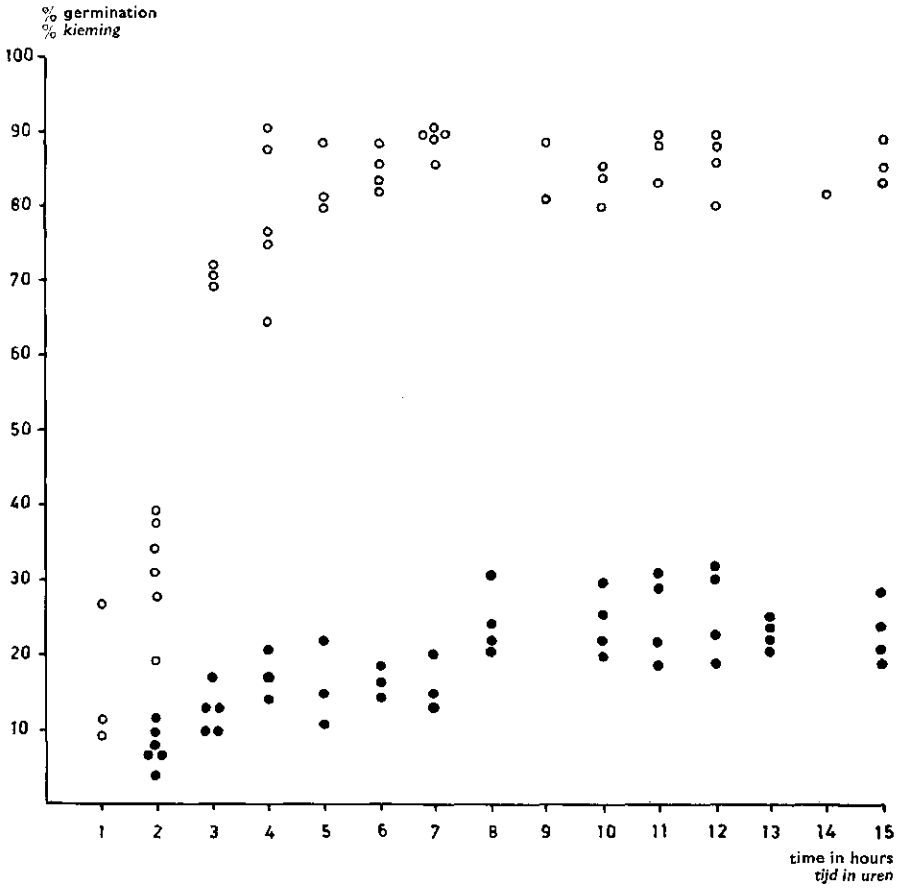


FIG. 4. Rate of germination at 8.5 °C and at 21 °C; each figure is the average of 500 observations.
Kiemsnelheid bij 8.5 °C en bij 21 °C; elk punt is het gemiddelde van 500 waarnemingen.

Although *Bremia lactucae* is kept apart from *Peronospora* on account of a slight morphological difference, it appears that it resembles the species of the latter genus to a large extent also in the way in which the conidia germinate and in that in which the germ tube enters the leaf of the host.

Within the Peronosporales two groups may be distinguished on account of the way in which the conidia germinate; in the first group the germ tube is produced by the conidium itself; in the second the conidium is to be considered a sporangium, which produces a number of swarm spores, and it are the latter which produce the germ tube. To the first group belong *Bremia lactucae* and various species of *Peronospora*; the group in which the conidia behave as sporangia, comprises e.g. *Phytophthora infestans* though here direct germination is possible and *Plasmopara viticola*. These two groups differ to some extent also in the way in which the germ tube enters the leaf. The germ tube of *Plasmopara viticola* enters the leaf through a stoma, those of *Phytophthora infestans* follow,

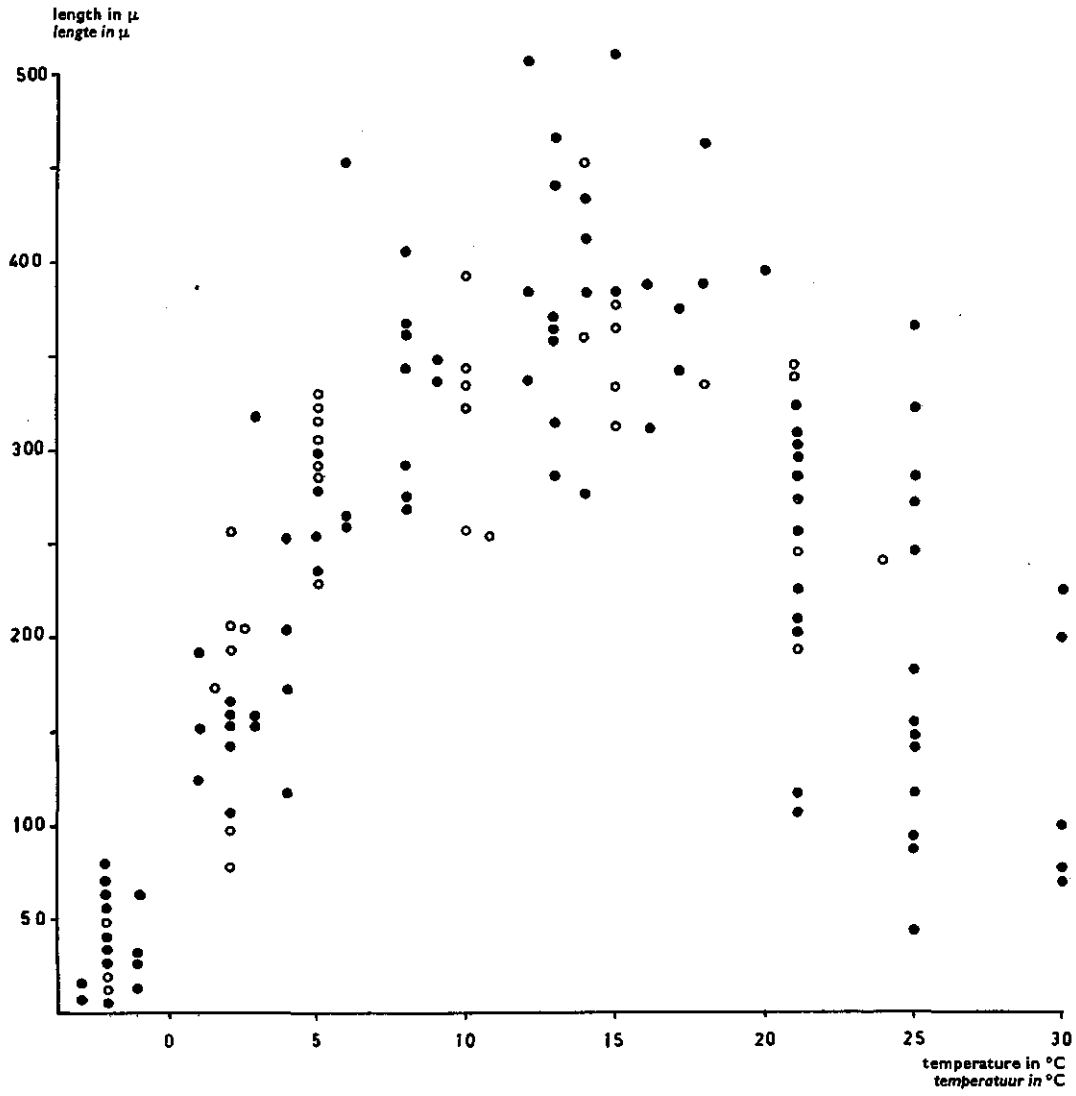


FIG. 5. Length reached by the germtubes in 24 hours at different temperatures; each figure is the average of 100 measurements.
Kiembuislengte na 24 uur bij verschillende temperaturen; elk punt is het gemiddelde van 100 metingen.

as a rule, the same way, although some of them may pass through epidermal cells. In *Bremia lactucae* and in most of the *Peronospora* species the germ tubes enter the leaf in the main or exclusively by way of the epidermal cells.

Before conidiophores can be produced, the diseased leaves must become covered by a film of water. On completely dry leaves the production of conidiophores appears to be impossible. The presence of such a film of moisture on the

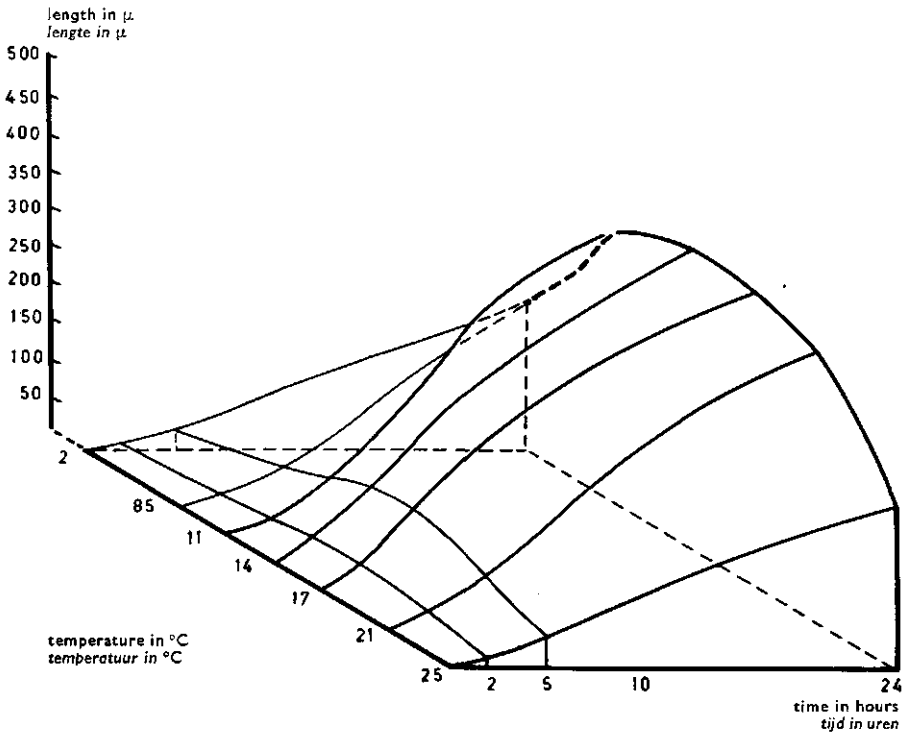


FIG. 6. Growth rate of the germ tubes at different temperatures.
Groeisnelheid van de kiembuizen bij verschillende temperaturen.

surface of the lettuce leaves is recognizable already by a slight change in its colour, the green assuming a somewhat less bright shade. When drops of water are present on a diseased leaf, the conidiophores are produced only in a narrow ring immediately round these drops. In the zone below the central part of the drop no conidiophores are produced. According to some authors, e.g. SCHULTZ & RÖDER (1938), the temperature would be of secondary importance for the production of conidiophores, but as a film of water on the surface of the leaves is formed only when the temperature of the leaves sinks below the dew-point, the importance of the temperature can doubtless not be denied. Conidiophores are produced between 5°–6°C and 22°–24°C. This is more or less in agreement with the observations of SCHULTZ (1937), who reports that this happens between 2°C and 24°C. Between 10°C and 21°C the number of conidiophores is larger than it is at lower or higher temperatures, but a distinct optimum could not be found. According to CROGAN et al. (1955) it would lie between 10°C and 15.5°C, and according to POWLESLAND (1954) between 6°C and 11°C.

That *Bremia lactucae* requires for the production of its conidiophores that the leaves of its host are covered with a film of water, seems to have escaped the attention of earlier investigators; at least, it is not mentioned by them. Several of them, however, point out that a high degree of atmospheric humidity is needed (e.g. SCHULTZ, 1937; YARWOOD, 1937; OGILVIE, 1943; POWLESLAND, 1954).

For some related fungi too a high degree of humidity is considered sufficient, e.g. for *Plasmopara viticola* (YARWOOD, 1937; OSTERWALDER, 1941; MÜLLER-STOLL, 1950). MÜLLER & SLEUMER (1934), however, were of opinion that the humidity of the air ought to be so high that the development of a film of water on the surface of the leaves becomes possible. According to COOK (1932) this would apply also to *Peronospora destructor*. That *Bremia lactucae* does not form conidiophores in places where the leaf is covered with drops of water, finds a counterpart in the behaviour of *Peronospora tabacina* (PINCKARD, 1942) and of *Plasmopara viticola* (GAUDINEAU, 1954), which form no conidiophores either below drops of water.

The growth of the mycelium in the interior of the leaf is, according to various investigators, e.g. WINGRAVE (1952), POWLESLAND (1954) and GROGAN et al. (1955), favoured by a high humidity of the air. This effect may be due to the fact that the latter reduces the transpiration of the plant, with the result that the degree of humidity in the intercellular spaces becomes very high. Moreover in plants that are continually exposed to an atmosphere with a high degree of humidity, the intercellular spaces reach larger dimensions, and it might be that this facilitates the development of the mycelium in the latter.

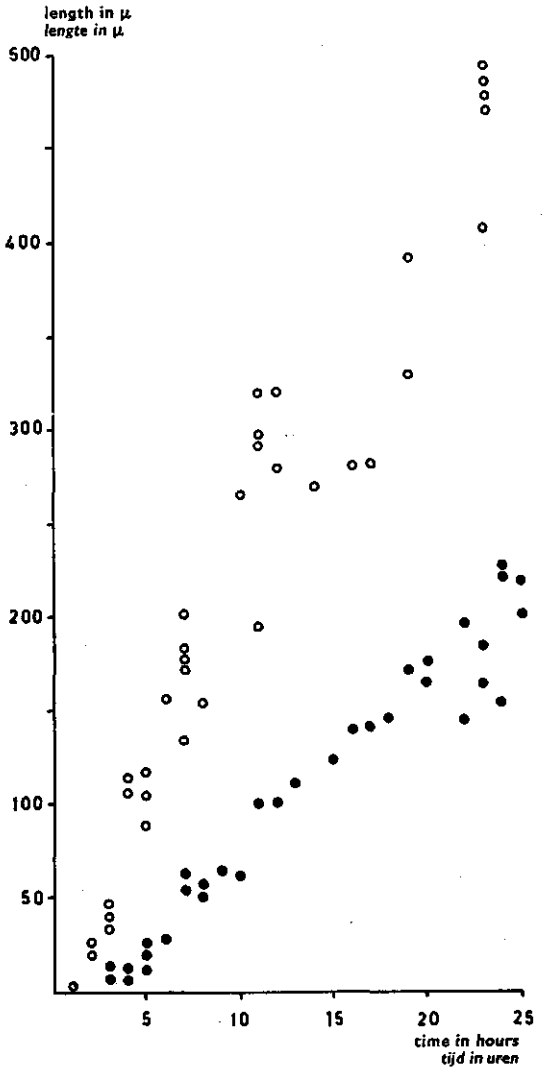


FIG. 7. Growth rate of the germtubes at 2°C and at 14°C; each figure is the average of 100 measurements.
Groeisnelheid van de kiembuizen bij 2°C en bij 14°C; elk punt is het gemiddelde van 100 metingen.

The young mycelium will first of all fill the substomatal cavity which is nearest to the place where the germ tube entered the leaf, and from this centre it will gradually spread to the substomatal cavities in the surrounding zone. Only after a substomatal cavity is completely filled up with mycelium, the latter

will begin to produce its conidiophores. After inoculation with a dense suspension of conidia (circ. 10.000 conidia per ml.) the conidiophores always appear on the spot where the inoculation took place and in the zone around the latter. When a strongly diluted suspension (circ. 10 conidia per ml.) is used for the inoculation, it takes longer before the first conidiophores appear, but under these circumstances too the conidiophores appear on the spot where the inoculation took place and on the adjoining zone. The mycelium apparently must fill a rather large number of substomatal cavities before it can start with the production of conidiophores, and in case the inoculation is carried out with a small number of conidia, this requires more time. Why the mycelium under otherwise suitable circumstances does not start with the production of conidiophores as soon as it fills the first substomatal cavity, is not clear.

The presence of a thin film of water on the surface of the leaf is, as we have seen, decisive for the production of conidiophores, and determines in this way the development of the first symptom of the disease that is outwardly recognizable, and accordingly also the length of what is generally called the incubation time. If the relative humidity of the atmosphere remains so high that from the moment of the inoculation the leaves are permanently covered with a film of water, the first symptom, i.e. the presence of conidiophores on both sides of the leaf, appears already within a few days (Plate II, A). In this case the incubation time coincides therefore with the time required for the production of the conidia (GÄUMANN, 1951). The shortest incubation time, viz. 5 days, was found when the inoculated leaves were kept at a temperature of 20°–22°C, provided that the film of water was present at the right time, e.g. since the moment of inoculation. From this result we may conclude that 20°–22°C is the temperature which is most favourable to the growth of the mycelium. This value is higher than that mentioned by BRIEN et al. (1957), viz. 15.5°–17°C.

At the moment the fungus produces its conidia, the infected leaves are still green, but at least at temperatures between 5°C and 15°C, two or three days later they begin to turn yellow, and after a few days more, they begin to die off. At higher temperatures two or three days after the ripening of the conidia the leaves prove to be completely gelatinized. When the infection remains confined to definite spots, it takes a longer time before the leaves begin to die off, and in this case there is, as a rule, no gelatinization. The area in which the conidiophores develop, gradually increases in size, and starting in the middle a change in colour sets in, somewhat later it dies off.

When the leaves are continuously covered with a film of water, there appears to be a distinct relation between the length of the incubation time and the temperature. This may be seen in fig. 8, which shows that the shortest incubation time is found between 20°C and 22°C. At lower temperatures its length increases at first slowly and then with growing rapidity.

No conidiophores are produced when the humidity of the air is such that at the prevailing temperature no film of water is formed on the surface of the leaves. In this case the mycelium in the substomatal cavities apparently undergoes no change. However, in the rest of the leaf it continues its growth, and as the chloroplasts in the cells of the host begin to degenerate under the influence of the parasite, the leaf turns yellow, and succumbs in the end. In case the infection is confined to definite areas, the latter begin to show the yellow discoloration. The spots gradually increase in size, and may run together, in which case here

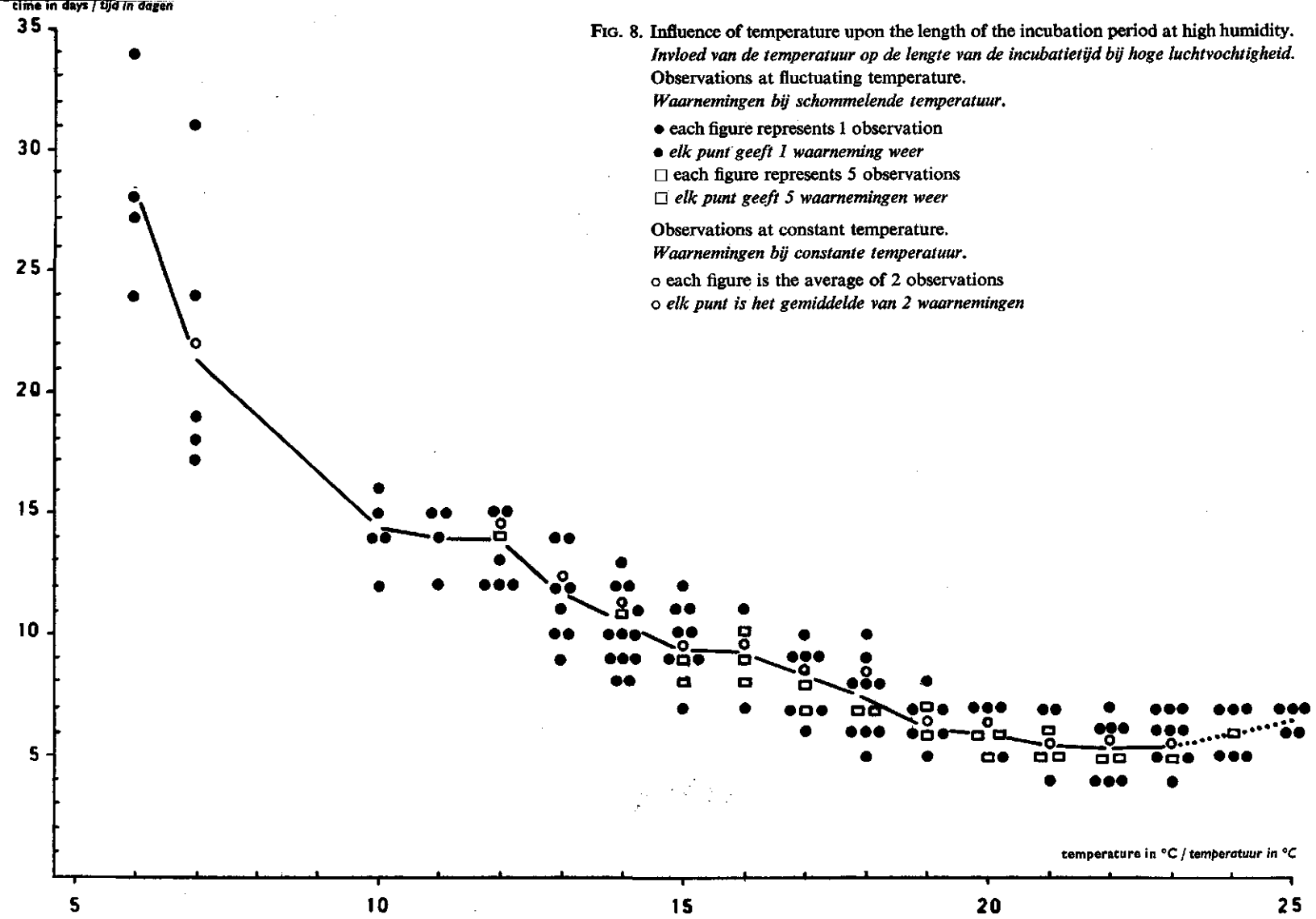


FIG. 8. Influence of temperature upon the length of the incubation period at high humidity.
Invloed van de temperatuur op de lengte van de incubatietijd bij hoge luchtvochtigheid.
Observations at fluctuating temperature.
Waarnemingen bij schommelende temperatuur.
● each figure represents 1 observation
● *elk punt geeft 1 waarneming weer*
□ each figure represents 5 observations
□ *elk punt geeft 5 waarnemingen weer*

Observations at constant temperature.
Waarnemingen bij constante temperatuur.
○ each figure is the average of 2 observations
○ *elk punt is het gemiddelde van 2 waarnemingen*

too the whole leaf in the end turns yellow. However, in older leaves the yellow discoloration of the infected spots never spreads over the whole surface. The parts which remain green, are enclosed between some of the thicker veins.

If in the leaves meant in the preceding paragraph the circumstances become favourable to the production of conidiophores, then the latter appear everywhere where the leaf has turned yellow, and in addition in a wide zone around the discoloured parts. However, no conidiophores are produced on the green parts that are enclosed between the thicker veins.

In case circumstances remain unfavourable to the production of conidiophores, the infected leaves finally wither, and are thrown off in the form of small shrivelled shreds. This drying-up proceeds more rapidly when the temperature is high, but even then it takes some weeks before the process is completed. However, even when the withering has made already considerable progress, the production of conidiophores and conidia is still possible, provided that there is a favourable turn in the weather conditions.

When no film of water is present on the surface of the leaf, and when therefore no conidiophores are produced, the incubation time may be assumed to end when the yellow discoloration becomes noticeable. This incubation time is always longer than that which is measured, in the presence of a film of water, by the first appearance of the conidiophores. Between the incubation time, measured by the first appearance of the yellow discoloration, and the temperature no distinct relation could be ascertained. Within a group of plants that had been inoculated simultaneously, sometimes differences of 8 days were noted in the incubation time when this criterion had to be used. At 5 °C the first discolorations were noted after 5 to 7 weeks, between 10 °C and 15 °C after 3 to 5 weeks, and between 18 °C and 22 °C after 2 to 3 weeks.

When the water on the surface of a leaf on which conidiophores are present, is lost by evaporation, and when the circumstances for the production of new conidiophores therefore become unfavourable, the infected parts dry up; as a result they become necrotic and nearly transparent (Plate 2, B). A necrotic spot does not increase in size, though it is possible that after some time it becomes surrounded by a slowly expanding zone showing a yellow discoloration. When circumstances once more become favourable to the development of conidiophores, then the latter appear along the margin of the necrotic spot and in a zone around this spot. In the centre of the latter but rarely conidiophores appear. When leaves that are covered over their whole surface with conidiophores, are allowed to wither, this leads in one or two days to their death.

In leaves with a firm texture, i.e. with small parenchyma cells and narrow intercellular spaces, local inoculation occasionally resulted in the development of glassy spots. This happened when up to the time at which the development of conidiophores was to be expected, the atmospheric humidity remained very high. When at that moment the humidity underwent a marked decrease, so that the film of water on the surface of the leaves disappeared, the glassy spot became necrotic and nearly transparent. When the humidity did not undergo such a decrease, then conidiophores were produced, at first over the whole surface of the glassy spot, but afterwards only along its margin and in a narrow zone round the latter. In leaves with larger parenchyma cells and larger intercellular spaces such glassy spots were never observed.

When young plants were inoculated in such a way that more than half their

leaves would become infected, the first symptom of the disease appeared to be a growth stagnation which became noticeable in 4 or 5 days. A few days later the usual symptoms of the disease became visible either in the form of conidiophores and conidia or in that of a yellow discoloration of the leaves. The infection by *Bremia lactucae* appears therefore to be the cause of the growth stagnation, and not the result of the latter, as the growers so far thought. They observed the appearance of the usual symptoms about three days after the beginning of the growth stagnation, but as this period of three days lies well within the incubation time found under the most favourable circumstances, i.e. with a permanently high atmospheric humidity, it is clear that the plants must have been infected already before the growth stagnation became noticeable. It is true that plants whose growth is retarded in one way or another, may be attacked by *Bremia*, but such plants are not more susceptible than plants with a normal rate of growth.

Under favourable circumstances conidiophores may, as already remarked, appear on the upper side of the leaf as well as on its lower side. The development of conidiophores on the upper side of the leaf is not to be regarded as a symptom of an infection of more than usual severity, as sometimes has been assumed, but merely as an indication of the presence of a covering with water on that side. This appears already in cultures on a commercial scale. Especially with the growing of "hot-bed lettuce", where the humidity of the air is kept very high, infected leaves are found to be covered with conidiophores on both sides. With other methods of cultivation this is observed only on leaves that have but little space around them. As a rule, the development of conidiophores remains confined to the lower side of the leaves, because the latter become easily covered with a film of water on account of the vicinity of the evaporating soil surface and the absence of movement in the air which fills the space between them and the soil.

Leaves on which *Bremia* is producing its conidiophores, gelatinize and rot easily, especially at temperatures above 15 °C. This may find its cause in the circumstance that the parasite deprives its host of certain nutrients, but it may also be due to a reduced respiration caused by the blocking of the stomatal apertures and the substomatal cavities by the mycelium; the dead cells of the host provide saprophytic organisms with a suitable substrate.

With lettuce that is cultivated on a commercial scale, an infected leaf will but exceptionally turn yellow and die before the parasite has found time to produce its conidiophores. At one time or another the circumstances will, as a rule, be suitable for the development of the latter. The most common symptom of the disease is therefore the presence of white fructifications on lightgreen to yellow leaf spots.

That owing to the disappearance of the film of water on the surface of the leaves necrotic centres appear in the leaf spots that are caused by the infection with *Bremia*, was observed only in lettuce grown under Dutch lights. With this method of cultivation the humidity of the air around the plants may undergo rapid changes, as when the cultures are aired, the wind sweeps along the plants causing a rapid evaporation of the water film by which the leaves are covered. However, glassy spots, such as appeared in our experiments, and which reminded us of the so-called "oil spots" on vine leaves that are attacked by *Plasmopara viticola* (cf. e.g. ISTVANFFI & PALINKAS, 1912; GAUDINEAU, 1954), were never observed in cultures on a commercial scale.

That the size of the conidiophores depends on the degree of humidity in the air round the host plants, may be observed already with the naked eye. In "hot-bed lettuce", which at the end of the period of growth is surrounded by air with a very high degree of humidity, the conidiophores are more than twice as long as they are on "winter lettuce."

That the length of the incubation period measured in plants which are continuously exposed to a high atmospheric humidity, depends so distinctly on the temperature, is in itself not strange. However, it is rather astonishing that there is such a striking difference between the optimum temperature pertaining to the incubation period at high humidity and that pertaining to other stages of the life cycle of the parasite. The shortest incubation period is found between 20°C and 22°C, i.e. at a comparatively high temperature, whereas for the germination of the conidia the most favourable temperature lies between 4°C and 10°C, and for the growth of the germ tube at circ. 15°C (table 6). At a lower humidity of the air, when yellow leaf spots are the first symptoms of the disease, the shortest incubation period was also found at higher temperatures. In *Plasmopara viticola* too the shortest incubation period was met with at comparatively high temperatures, viz. between 20°C and 26°C, but with this fungus the most favourable temperatures for the other life processes are also high; for the germination of the conidia as well as for the growth of the germ tubes they lie between 18°C and 24°C. With *Peronospora tabacina* the most favourable temperature for the production of the conidia lies between 15°C and 20°C and for the germination of the conidia between 10°C and 15°C.

7.2. Influence of leaf extracts on the germination of the conidia and on the growth of the germ tubes

According to Schultz (1937) lettuce leaves and extracts of the latter would exercise a stimulating influence on the germination of the conidia of *Bremia lactucae*; in lettuce water (obtained by boiling 100 gr. fresh lettuce leaf for 30 minutes in 1 l. distilled water; the extract was subsequently brought back to a volume of 1 l.) at 20°C 85% of the conidia germinated, whereas in distilled water practically no germination was observed.

The question whether the host plant exercises a stimulating influence on the germination of the conidia and on the growth of the germ tubes, was studied by means of germination experiments carried out in five different media, viz. 1. in lettuce water prepared in the same way as that of Schultz, 2. in press sap of plants that had been cultivated in the usual way; 3. in press sap of plants that, in order to exclude influences exercised by organisms that might be living on the leaves, had been cultivated under sterile conditions; 4. in distilled water; and 5. in distilled water that had been left for 3 to 4 hours in the form of drops on leaves of plants cultivated in the usual way. The last-mentioned medium was included in the experiments because it is known from the literature (cf. e.g. Brown, 1922) that the exudations of the leaves of some plants may exercise a stimulating or inhibiting influence on the germination process. As at a temperature of 15°C the germ tubes may have entered the leaf 3 to 4 hours after the inoculation, the drops were left on the leaves during a period of about the same length. At the end of that period they were cautiously sucked off, and used

for the experiments. The water used in the experiments was distilled in an apparatus of Pyrex glass.

The plants of which the press sap was used in the third set of experiments, were grown from seeds that had been disinfected by shaking them for 30 minutes in 0.5% mercuric chloride, after which they were washed in sterilized water and sown in sterilized erlenmeyer flasks with a content of 3000 ml., the bottom of which had been covered with a thin layer of sterilized soil. In order to make the opaque press sap more transparent, it was diluted with ten times its own volume of distilled water.

The suspensions of the conidia were prepared with distilled water mixed with an equal volume of the medium whose influence was to be tested, and these suspensions were studied in hanging-drop cultures. The latter were kept for 24 hours at a temperature of 8°–10°C. The germination was studied at first at 2°C, because an accelerating or retarding influence of the medium might perhaps be more marked at a temperature at which the germination did not proceed at the fastest rate; when it proceeds at a slower rate, the difference may become larger, because in that case the medium is able to exercise its influence during a longer period. However, when it appeared that at this temperature no effect could be detected, a temperature of 8°C to 10°C was chosen. In each

TABLE 4. Germination of the conidia in % and length of the germ tubes in microns as found after a sojourn of 24 hours at 10°C in different media.
Kieming van de conidiën in % en lengte der kiembuizen in μ na een verblijf van 24 uur bij 10°C in verschillende media.

Experiment *Proef*	Press sap from plants grown under sterile conditions *Perssap van steriel opgekweekte planten*	Press sap from plants not grown under sterile conditions *Persap van niet-steriel opgekweekte planten*	Extract of lettuce-leaves *Afreksel van slabladeren*	Distilled water left during 3–4 hours on lettuce leaves *Gedestilleerd water, dat 3–4 uur op slabladeren heeft gelegen*	Distilled water *Gedestilleerd water*
	Germination of the conidia in % *Kieming der conidiën in %*				
1	86.4	86.7	84.1		86.4
2	94.6	93.7	93.6		92.2
3	94.4	97.4	96.4	95.1	91.5
4	90.8	92.1	91.4	92.6	90.6
5	92.6	92.8	94.8	90.7	94.5
6	93.0	90.1	92.9	90.4	93.8
7	88.3	86.3	86.1	88.4	88.4
Average/*Gemidd.*	91.4	91.3	91.3	91.4	91.1
	Average length of the germ tubes in microns *Gemiddelde kiembuislengte in μ*				
1	217.7	284.2	266.6		286.8
2	382.5	343.1	371.8		323.5
3	322.0	298.0	374.6	390.7	326.5
4	370.4	282.0	299.4	258.7	298.7
5		285.4	323.0	328.7	290.4
6		320.6	348.2	298.4	329.1
Average/*Gemidd.*	323.2	305.9	330.6	319.1	309.2

set of experiments 500 conidia were inspected in order to estimate the percentage of germination, whereas of 100 germ tubes the length was measured.

Table 4 shows that none of the media exercised either a stimulating or an inhibitory influence on the germination of the conidia or on the growth of the germ tube. This result is in contradiction with that obtained by SCHULTZ (1937), but in agreement with that of POWLESLAND (1954), who did not succeed in obtaining a better germination percentage by adding either fragments of lettuce leaves or a maceration of the latter to the culture medium. With *Peronospora tabacina* too no increase of the germination percentage of the conidia could be obtained by adding sap of tabacco leaves to the culture medium (ANGELL & HILL, 1932).

With this kind of experiments the control experiments may not always be fully reliable. This may be due to the presence of certain ions. It is known that even minute amounts of the latter may exercise a strongly inhibiting influence on the germination of conidia. In our own experiments too it seemed at first that lettuce water in comparison to distilled water exercised a stimulating influence on the germination of the conidia and on the growth of the germ tubes, but it appeared that the distilled water used in these preliminary experiments contained substances which inhibited the germination. The experiments were therefore repeated with water that had been distilled in an apparatus of Pyrex glass, and in these experiments, as stated above, no longer any influence of the leaf extracts was recognizable. It is not impossible that the diverging results obtained by Schultz may be explained in this way.

Whether any substances may have diffused out of the lettuce leaves into the drops of water that were placed on their surface, could not be ascertained. The liquid obtained in this way might perhaps exercise some influence if it could be applied in a strongly concentrated form.

7.3. INFLUENCE OF LIGHT ON THE PRODUCTION OF CONIDIOPHORES AND CONIDIA

According to YARWOOD (1937) light exercises in *Bremia lactucae* an inhibiting effect on the production of conidiophores. When infected plants of which the conidiophores had been carefully removed, were placed in air with a high degree of humidity, it appeared that within 11 hours already a large number of new conidiophores with conidia were produced. However, this applied only to plants that were kept in the dark, for no conidiophores were produced when the plants under otherwise identical circumstances were exposed to the light, at least so long as they were left in the latter; when they were after some time placed in the dark, the production of conidiophores was resumed. Yarwood is of opinion that the results of his experiments do not prove that the production of conidiophores is influenced directly by the presence or absence of light. He favours the view that light is only indirectly of importance, and that the production of conidiophores by the parasite might be influenced by changes in the metabolism of the host.

For the study of the influence of light on the production of conidiophores plants were used of which the substomatal cavities in the leaves were filled with mycelium, and where the fungus was therefore in a position to start at once with the production of conidiophores. As soon as a film of water would be

deposited on the leaf, the development of the latter could be expected (cf. p. 157). Provided that the atmospheric humidity does not act as a limiting factor, that the mycelium of *Bremia lactucae* fills the substomatal cavities and that as yet no conidiophores are found in the evening, the latter may be expected on the following morning, i.e. after a sojourn of some length in the dark during the night.

With inoculated plants placed in glass boxes in which the temperature was kept at 15 °C to 17 °C, and in which permanently a high degree of air humidity was maintained, the length of the incubation time was approximately known. During the night in which the end of the latter was to be expected, three groups, each consisting of 100 plants, were inspected every hour in order to determine exactly the moment at which the conidiophores would appear.

On all plants the conidiophores showed themselves between 2 and 3 in the morning, i.e. after the plants had been kept for 6 to 7 hours in the dark. A repitition of the experiment on another night produced the same result; now too at 3 in the morning, all plants were covered with a dense layer of conidiophores, whereas at 2 no such covering was present.

In order to continue these observations at more convenient hours, the plants were placed in a dark space, in which they could be exposed to the influence of light at any desired moment. To this end 12 TL tubes, each of 40 W, were mounted above the glass box which contained the experimental plants; in this way at the level of the plants a light intensity of circ. 10.000 lux was obtained. The temperature in the box fluctuated between 15 °C and 20 °C, and the air in it was kept at a high degree of humidity be means of a vaporizer. The whole box was screened by means of black cloth. The lighting was regulated in such a way that the plants remained in the dark during the day, and were exposed to the light of the TL tubes during the night, each period lasting 12 hours. Other glass boxes, in which a corresponding temperature and air humidity were maintained, were neither screened nor artificially lighted; in these boxes the plants were therefore each day during $14\frac{1}{2}$ hours exposed to the ordinary daylight and during $9\frac{1}{2}$ hours to darkness. Each box contained 30 plants which had been inoculated at the same time. After some days and during one of the periods of darkness conidiophores and conidia made their appearance, viz. 7 to 8 hours after the preceding period of lighting had been ended. On the plants which at the same time were exposed to daylight, no conidiophores were as yet noticeable; here they appeared in the following night. Daylight, therefore, seemed to inhibit the production of conidiophores.

In a subsequent experiment inoculated plants of which the incubation time was on the point of expiring, were from now on exposed to continuous light. The production of conidiophores, which in the dark would have taken place in 7 to 8 hours, now took 9 to 11 hours. The exposition to continuous light, therefore, retarded the production of conidiophores, but did not prevent it.

In the preceding experiments the relative humidity of the air was kept so high that this factor could exercise no inhibiting influence on the production of conidiophores. However, what would happen when an inoculated leaf in which the mycelium is ready to proceed to the production of conidiophores, was kept dry for some time? And when then at some moment the leaves became covered with the required film of water, at what time of the day or of the night would the fungus then proceed to the production of its conidiophores?

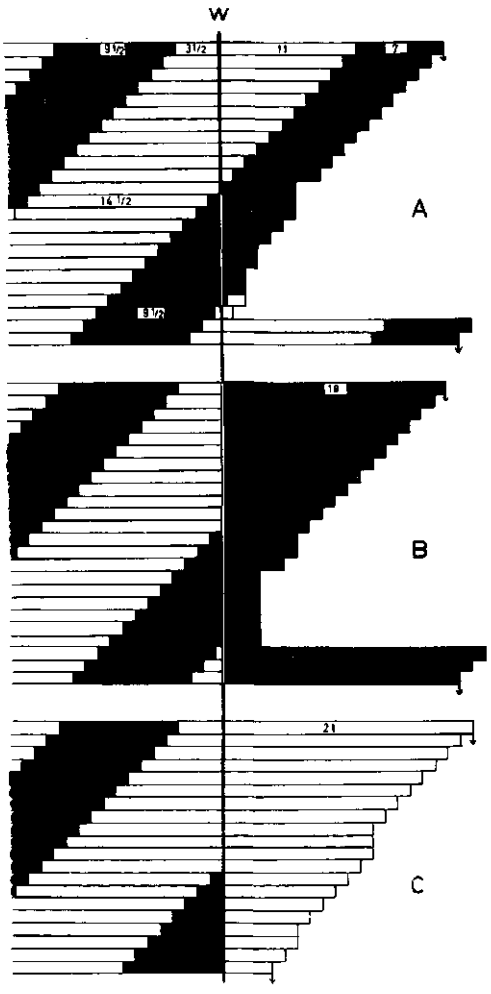


FIG. 9. Scheme of the experiments on the influence which light exercises upon the production of conidiophores.

Schema van de proeven over de invloed van licht op de vorming van sporendragers met sporen.

A normal succession of day and night
normale wisseling van dag en nacht

B continuous darkness after treatment
continu donker na de behandeling

C continuous light after treatment
continu licht na de behandeling

W time upon which the waterfilm had been formed
tijdstip, waarop de waterfim werd gevormd

$3\frac{1}{2}$; $9\frac{1}{2}$ . . . . . time in hours/*tijd in uren*

↓ sporulation/*sporulatie*

in light/*in licht*

in darkness/*in duisternis*

In order to find an answer to these questions, the following experiments were carried out in two parallel series. However, as the results of the corresponding experiments of the two series agreed completely, in fig. 9 only one of each pair of experiments is reproduced.

The first experiment was carried out with the alternation of day and night as it happens to be at the end of April, i.e. 14½ hours of daylight and 9½ hours of darkness. Inoculated plants which were ready to start with the production of conidiophores, were kept dry for some time, then wetted and placed in one of the glass boxes in which the air was kept at a high degree of humidity. From 9 in the morning to the next day at 9 every hour 15 plants were sprayed with water. The first group of 15 plants had been exposed to the light for 3½ hours before they were wetted, and were exposed to it for 11 hours after that. The other groups had been exposed for a longer time to the light before they were wetted, and after they had been wetted they had to be exposed once more to the light, but now for a shorter time, before conidiophores developed. However, in almost all instances the conidiophores were produced at the same time, viz. after 6 hours.

When the plants before they were wetted, had already spent some time in the dark, the time which the parasite required to produce its fructifications, appeared to decrease with the length of this period. When it had lasted 9 hours, the conidiophores appeared already 2 hours after the leaves were wetted, and if it had lasted 9½ hours, and when the plants thereafter had stood for ½ hour in the crepuscular morning light, the time the parasite required for the production of its conidiophores, was reduced to 1 hour. However, if the plants, after spending 9½ hours in the dark, stood for 1½ hours in the morning light before they were wetted, then the parasite was found to be unable to produce its conidiophores that same day; instead they were produced in the following night in the usual way, i.e. after the plants had stood for 7 hours in the dark.

It appeared, therefore, that when the parasite has reached the stage in which it can start the production of its conidiophores, the rate at which the latter are produced, is accelerated when the host plants are kept in the dark in the period before they are wetted; an exposition of half an hour to weak light did not change this rate. However, when the host plants were exposed for 1½ hours to light, they were no longer able to produce their conidiophores within the following period of darkness; in this case it required a day of 14½ hours followed by the usual period of 7 hours of darkness to produce them.

In the next series of experiments the plants in which the parasite had reached the stage in which it could start the production of its conidiophores, were exposed during a period of varying length to the influence of the light before they were wetted and placed in the dark (fig. 9, B). When the period during which they were exposed to the light, lasted 3½ hours, the conidiophores were produced 18 hours after the plants had been wetted and placed in the dark. With a longer exposition to the light, the conidiophores were produced after a shorter sojourn in the dark; after an exposition to the light of 14½ hours, the period in the dark required for the production of the conidiophores, was reduced to 7 hours. The length of the period of exposition to the light plus the length of the period in the dark needed for the production of the conidiophores was in all these experiments 21½ hours. An exposition to light, therefore, proved to enable the parasite to reduce the length of the period of darkness follow-

ing the wetting of the leaves which it requires to produce its conidiophores.

When the plants before they were wetted, were kept already for some time in the dark, the parasite produced its conidiophores in the subsequent period at an earlier moment. After such a previous period in the dark of 9 hours, only 3 hours were needed to this end. A sojourn in the dark, therefore, seems to increase the rate at which the conidiophores are produced, but here too a short exposition of the host plants to daylight before they had been wetted, proved to impede the process; after ½ hour in daylight, the production of conidiophores proved to require a sojourn of 20 hours in the dark. In case the host plants after they had been wetted, remained exposed to the light, then a long previous sojourn in the dark was found to be most favourable for the production of conidiophores. However, in that case after the wetting 3 to 4 hours more were required than when the plants were permanently kept in the dark (fig. 9, C).

The preceding experiments show that a continuous exposition to light retards the production of conidiophores, but that it does not suppress it, as had been reported by YARWOOD (1937). In this connection it is worth mentioning that in *Peronospora tabacina* too, according to WOLF & MCLEAN (1940), the production of conidiophores was not suppressed by exposing the infected plants to continuous light.

It seems likely that the production of the conidiophores is the result of a chain of reactions taking place in the mycelium. Part of these reactions might take place in the light, and another part in the dark, but in order to complete the chain a light period followed by a period of darkness would be required. After such a succession the production of conidiophores would take place within a short time, no matter whether the leaves of the host plant are covered with water for a short time or during a longer period. However, when the period in the dark is followed by an exposition to light lasting one hour, this seems to cause a return to a previous stage in the chain, for in that case the production of conidiophores requires a new period of light and a new period of darkness (fig. 9, A). With leaves kept continuously in the dark the time the reactions leading to conidiophore development required from the moment the leaves are wetted, is the shorter, the longer the plants were previously exposed to light. With plants, that had previously been exposed to light, but that before they were wetted, were kept in the dark during some hours, the development of conidiophores was the more rapid the longer the length of the period during which they were exposed to light plus the length of the period passed in the dark (fig. 9,B). However the whole chain may be completed also in continuous light, although in that case it takes 2 or 3 hours more. That an exposition to the light of one hour causes the above mentioned shifting to a previous stage in the chain, is unexpected. More experiments will have to be carried out, before we may hope to explain this.

The conclusions at which we arrived on account of our experiments, may be summarized in the following points.

1. The production of the conidiophores is a process which consists of at least two stages. During the first stage the parasite fills a large number of substomatal cavities in the leaf of its host; when it is so far, it is in a position to start with the production of conidiophores.
2. The second stage does not start unless the leaf of the host is covered with a thin layer of water.

3. The longer the host plants are kept in the dark, the shorter the time which the parasite requires for the production of its conidiophores.
4. When in the second stage the plants are continuously exposed to light, the production of conidiophores takes a few hours more than it would have done in the dark. However, a short period of light causes a relapse to the first stage.

8. EPIDEMIOLOGY

8.1. VIABILITY OF THE CONIDIA; DISPERSAL AND SURVIVAL OF THE PARASITE

Experiments by ANGELL & HILL (1931a) have shown that the viability of the conidia of *Bremia lactucae* is longest at lower temperatures and at a relatively low humidity of the air. Of conidia that had been kept for 17 days at a temperature of 2 °C and at a relative atmospheric humidity of 60 to 70 %, 1 % still was able to germinate. Some preliminary experiments carried out by SCHULTZ (1937) indicate that the conidia can stand some degree of desiccation. At higher temperature the conidia lose their viability in a short time (ANGELL & HILL, 1931a; POWLESLAND, 1954).

The influence which the atmospheric humidity exercises at different temperatures on the viability of the conidia, was studied by placing leaves on which mature conidiophores were present, in open dishes that subsequently were enclosed in larger vessels which contained different concentrations of sulphuric acid and which were sealed with vaseline. The degree of atmospheric humidity which in this way was maintained in these vessels, is given in table 5. The dishes remained in the latter either for a period of 24 hours or for one of 48 hours, and during this time the temperature in these vessels was maintained either at 2 °C, at 15 °C or at 21 °C. After that the conidia were washed from the leaves by means of distilled water, and with the suspensions that were obtained in this way, hanging-drop cultures were made; the latter were stored for 24 hours at 4 °C. At the end of this period the number of conidia that had germinated, were counted. Some other leaves were washed directly instead of being placed in the open dishes, and the suspensions of conidia that were obtained from these leaves were treated in the same way, and here too the number of conidia that after 24 hours in the hanging-drop cultures had germinated, were counted. The proportion between the germinated conidia and the total number of conidia in these cultures was taken as the norm, and the proportions found

TABLE 5. Relative humidity in % obtained in closed vessels by means of different ratios of 98% sulphuric acid and distilled water.
Relatieve luchtvochtigheid in % in gesloten potten verkregen met behulp van verschillende mengels zwavelzuur 98% en gedestilleerd water.

Volume parts sulphuric acid *Volume delen zwavelzuur*	Volume parts distilled water *Volume delen gedestilleerd water*	Relative humidity *Relatieve luchtvochtigheid*
0.0	100.0	100
14.2	85.8	85
22.4	77.6	70
24.5	71.5	55
30.7	69.3	50
34.5	65.5	40
41.4	58.6	25
51.6	48.4	10

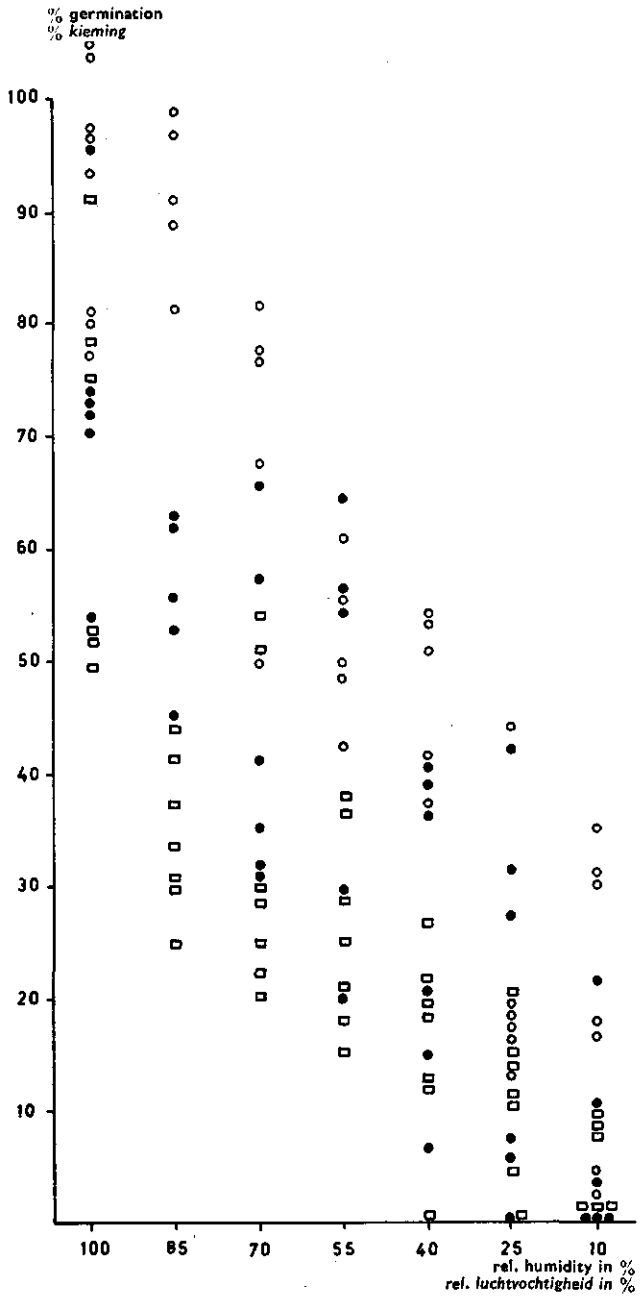


FIG. 10. Influence of humidity on the germinating power of the conidia at 2 °C (o), at 14 °C (•) and at 21 °C (□) estimated at the end of 24 hours; each figure is the average of 500 observations.

Invloed van de luchtvochtigheid op de kiemkracht der conidiën bij 2 °C (o), *14 °C* (•) *en 21 °C* (□) *na 24 uur; elk punt is het gemiddelde van 500 waarnemingen.*

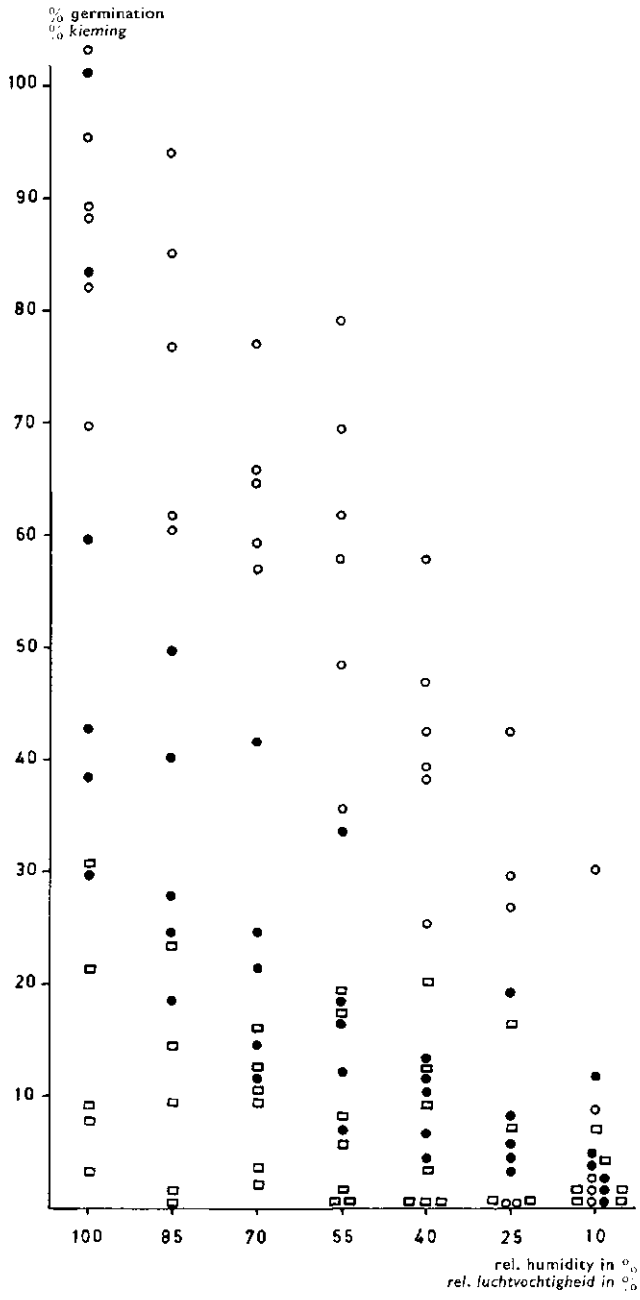


FIG. 11. Influence of humidity on the germinating power of the conidia at 2 °C (○), at 14 °C (●) and at 21 °C (□); estimated at the end of 48 hours; each figure is the average of 500 observations.

Invloed van de luchtvochtigheid op de kiemkracht der conidiën bij 2 °C (○), bij 14 °C (●) en 21 °C (□) na 48 uur; elk punt is het gemiddelde van 500 waarnemingen.

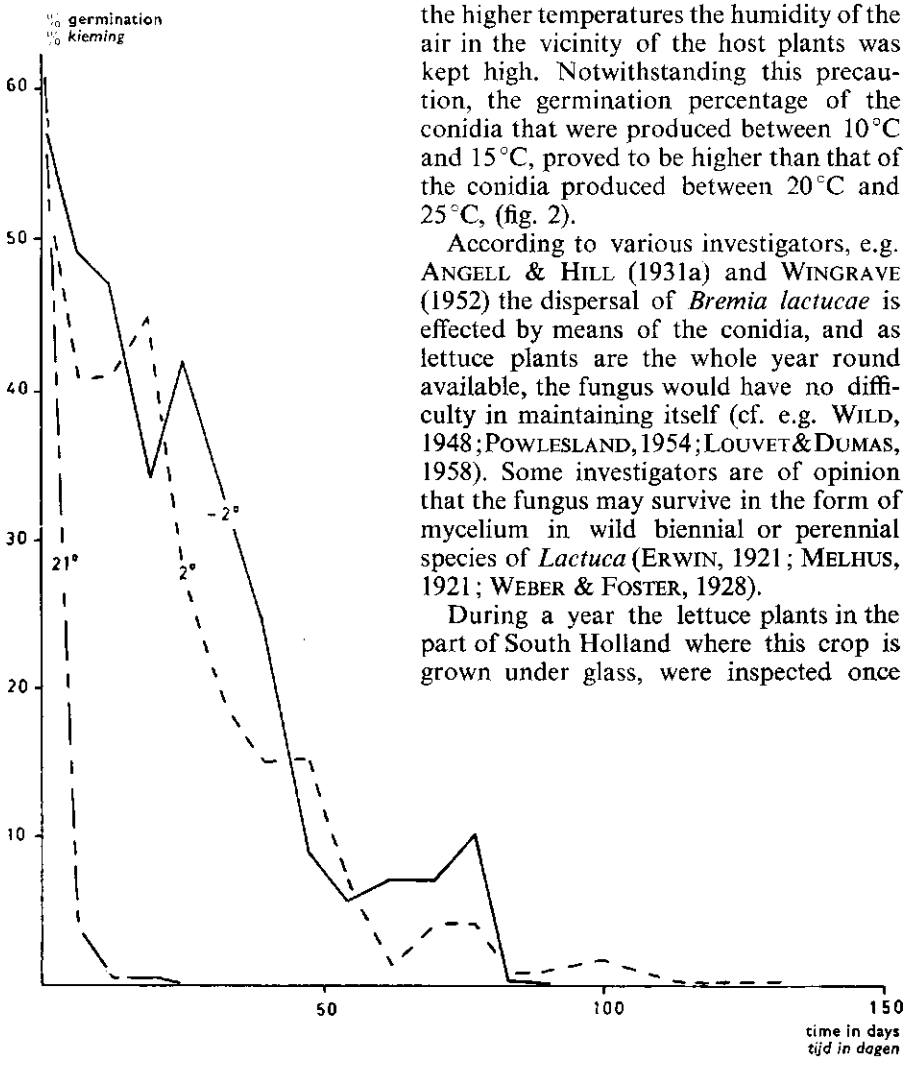
in the other cultures were expressed as a percentage of this norm. The results are shown in fig. 10 and in fig. 11. It appears that the conidia are very resistant against desiccation, especially at a temperature of 2 °C.

In order to study the viability of the conidia under less extreme circumstances and during longer periods, leaves on which conidiophores were present, were collected from lettuce that had grown for the sale. From these leaves the conidia were wiped off and collected dry in watch glasses. These watch glasses were stored at temperatures of –2 °C, 2 °C, 1 °–10 °C and 21 °C, and at a relative air humidity either of 100 % or of 50 %. A part was also stored air-dry at a relative air humidity of circ. 70 %. A certain number of conidia were at once brought in distilled water at a temperature of 4 °C, and of this sample at the end of 24 hours the germination percentage was determined; this served as norm. The germination power was determined of the samples after they had been stored for some time under different environmental conditions. By comparing the percentages found in the samples with the norm, the changes which the germination power of the former had undergone, could be expressed in figures. The percentage of germination of the samples was determined every 2 or 3 days, afterwards once in every 6 or 7 days. To this end part of the sample was brought in distilled water, and kept during 24 hours at 4 °C. When the germination percentage fell below 10 %, then some lettuce plants were inoculated with the conidia in order to find out whether they still were able to cause infection. The results of the experiments taken at –2 °C, 2 °C and 21 °C and with a relative air humidity of circ. 70 %, are given in fig. 12. Conidia that were stored at lower temperatures appeared to retain their viability during a very long time, viz. at 2 °C up to 140 days, and at –2 °C up to 95 days. However, with conidia that had been stored during 25 days at 21 °C no germination was obtained in vitro, although they still appeared to be able to infect lettuce plants, be it to a slight degree. After they had been stored for 28 days at 21 °C, infection with these conidia proved to be impossible. With the conidia that had been stored for 100 days at –2 °C, and with conidia that were kept for 140 days at 2 °C, infections could still be obtained, but with conidia that had been stored for a longer period, this was possible no more. In fig. 13 A, B and C the results are shown of germination tests made with conidia that had been stored at –2 °C, 2 °C, 21 °C and at a temperature fluctuating between 1 °C and 10 °C; in the last-mentioned case the conidia were stored, from the middle of December to the middle of February, in an unheated glasshouse. The relative atmospheric humidities here too were 100 %, circ. 70 % and 50 %. Although the germination percentage of the conidia which were allowed to germinate when the experiment was started, was comparatively low, these graphs too show that at lower temperatures the conidia retain their viability for a long time.

Although in the literature indications are to be found of a long viability of the conidia of *Bremia lactucae* (ANGELL & HILL, 1931a; SCHULTZ, 1937), it was so far not known that they may retain their capability to germinate for a period lasting no less than 140 days. The conidia of *Peronospora tabacina* too proved to retain their viability for a very long time at lower temperatures (ANGELL & HILL, 1931b).

The percentage of conidia with granular contents increases when the samples grow older. In dead conidia the contents are coarsely granular. For this reason care was taken that the conidia which were used in the experiments, did not

show granular contents, that they were of the same age, and that they had been produced as much as possible under identical environmental conditions. Among the latter the relative humidity of the air around the host plants occupies an important position. The lower the relative humidity stayed, the sooner the contents of the conidia became granular, and the less viable they were. The temperature is in this respect also of importance, because at higher temperatures the relative humidity of the air is usually lower than at less high temperatures. For this reason we always took care that at the higher temperatures the humidity of the air in the vicinity of the host plants was kept high. Notwithstanding this precaution, the germination percentage of the conidia that were produced between 10 °C and 15 °C, proved to be higher than that of the conidia produced between 20 °C and 25 °C, (fig. 2).



According to various investigators, e.g. ANGELL & HILL (1931a) and WINGRAVE (1952) the dispersal of *Bremia lactucae* is effected by means of the conidia, and as lettuce plants are the whole year round available, the fungus would have no difficulty in maintaining itself (cf. e.g. WILD, 1948; POWLESLAND, 1954; LOUVET & DUMAS, 1958). Some investigators are of opinion that the fungus may survive in the form of mycelium in wild biennial or perennial species of *Lactuca* (ERWIN, 1921; MELHUS, 1921; WEBER & FOSTER, 1928).

During a year the lettuce plants in the part of South Holland where this crop is grown under glass, were inspected once

FIG. 12. Viability of the conidia at a relative humidity of circ. 70% and at temperatures of -2°C, 2°C and 21 °C.

Levensduur der conidiën bij een relatieve luchtvochtigheid van ongeveer 70% en temperaturen van -2°C, 2°C en 21°C.

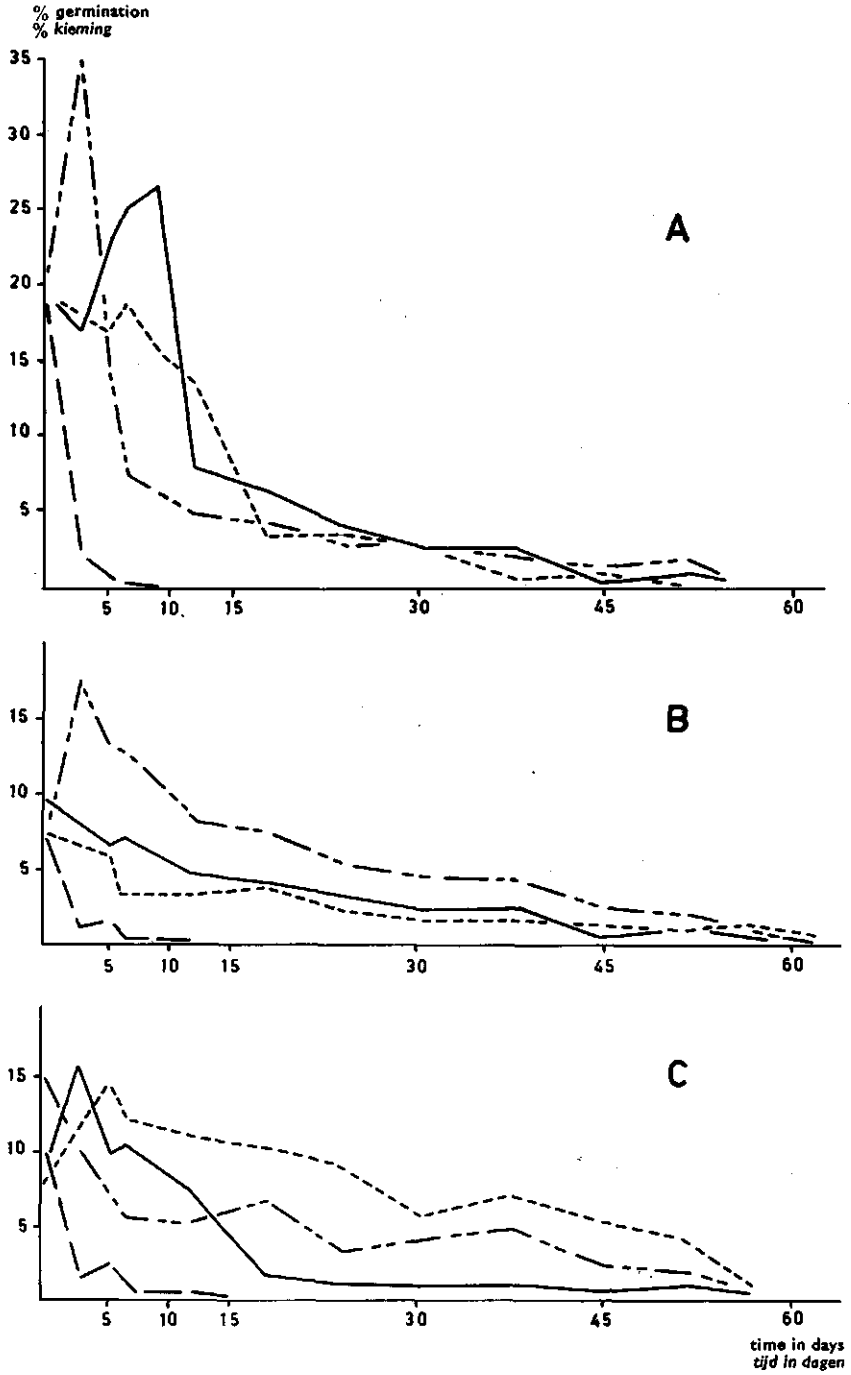


FIG. 13. Viability of the conidia at relative humidities of 100% (A), circ. 70% (B) and 50% (C), at temperatures of –2°C (— - - —), 4°C (- - -), 1°–10°C (——) and 21°C (— —).
Levensduur der conidiën bij een relatieve luchtvochtigheid van resp. 100% (A); ongeveer 70% (B) en 50% (C), bij temperaturen van –2°C (— - - —), 4°C (- - -), 1°–10°C (——) en 21°C (— —).

a week in order to see whether diseased ones were present or not. Between July 1958 and July 1959 leaves with the fructifications of the fungus could regularly be noted, except in the first half of July 1958 and in February 1959; in June 1958 they were also absent. There are apparently but a few periods in which circumstances are unfavourable to the development of the parasite, and these periods are usually but short. They may occur in January and February and in June and July; in these months the relative humidity of the air is rather low.

At the lower temperatures at which "winter lettuce" is grown, viz. from 3 °C to 10 °C, and at the temperatures at which the early "spring lettuce" is cultivated, viz. from −2 °C to 15 °C, the incubation time is, provided that the humidity of the air remains constantly high, at least 4 weeks, but when the humidity of the air does not remain constantly high, it becomes much longer. It will, therefore, be clear that the temporary absence of the necessary conditions for the production of conidiophores and conidia in this part of the year does not really endanger the survival of the fungus. Moreover, the conidia prove to have a long period of viability under these circumstances.

The occurrence of a hot and dry period does not offer insurmountable difficulties either. At 18 °C to 20 °C the time between the penetration of the parasite in the leaf and the first appearance of the yellow spots in the latter varies between two and three weeks. In case the leaves do not become covered with a film of water before the end of this period, the yellow spots are seen to increase slowly in size, but it takes at least three weeks more before the infected leaves are totally withered, and up to that time the production of conidiophores is still possible. Therefore, also during such a hot and dry period *Bremia* is not in very great danger; it may easily survive in the leaves in the form of mycelium.

Related fungi pass periods that are unfavourable to their development either in the form of oospores, so e.g. *Peronospora tabacina*; like *Phytophthora infestans* because they are able to spread through a large part of the host; or, as *Plasmopara viticola*, in both ways. Infection of the seeds was found in beans that were attacked by *Peronospora manshurica* (NAUM.) Syd.. In spinach attacked by *P. spinaciae*, however, no dispersal by means of the seed could be demonstrated, although the mycelium of this fungus was found in the outer parts of the ovules, viz. in the funicle, the integument and in the nucellus (LEACH & BORTHWICK, 1934). This applies also to beets attacked by *P. schachtii* Fuckel (LEACH, 1931), and to tobacco attacked by *P. tabacina* (ANGELL & HILL, 1932).

In our own experiments the capitula of lettuce could not be infected, and we found no indications for a dispersal of the fungus by means of infected seeds. WILD (1948) noted in lettuce seedlings that were grown in Petri dishes, the presence of infected cotyledons, and thought that this could only be explained by assuming that the seeds had been infected. OGILVIE (1943) regards infection of the seeds not excluded, because he found that the flowering stems could be infected. We too could obtain an infection of the stem, be it that on account of the presence of a layer of wax a special method of inoculation had to be applied. However, we also observed that the stems were never infected by the spreading of mycelium that was present in the leaves, and that the infections which could be obtained by direct inoculation, remained confined to a small area around the place where they had been inoculated; the growth of the mycelium in apical as well as in basal direction appeared to be very slight. In the thousands of seedlings that were used in our experiments, we did not find a single infection with

Bremia which could not be placed on the account of conidia that had arrived from outside.

That *Bremia* would persist in the soil on rests of infected leaves, as was assumed by BRIEN et al. (1957), is hardly believable, as such leaves rot very rapidly. In large-scale cultures too no indications for this assumption were ever found.

In the literature oospores of *Bremia lactucae* have been mentioned but rarely, and never from lettuce, but only from some other Compositae. They were never found in the diseased lettuce plants that were studied by us, and we regard it therefore as unlikely that the fungus would persist in the soil in this way.

The only mode of dispersal used by *Bremia lactucae* is, in our opinion, that by means of conidia. During unfavourable periods it may persist either in the form of mycelium in the leaves of the host or else by means of conidia.

8.2. COMPARISON OF THE RESULTS OBTAINED IN OUR EXPERIMENTS WITH THE EXPERIENCES OF THE GROWERS

The question why especially in cultures of "autumn lettuce" the disease spreads so easily, can now be answered without difficulty by the aid of what we have found out with regard to the demands which *Bremia lactucae* puts on its environment. The circumstances under which "autumn lettuce" is grown, appear to induce a rapid growth of the young plants, and this leads to the development of parenchyma cells of a comparatively large size, separated from each other by rather wide intercellular spaces. In case of an interruption of the normal growth, e.g. after a transplantation, such plants are apt rapidly to lose their turgescence and to stay for some time in a flaccid condition. As the air by which they are surrounded, may reach, either on account of evaporation from the soaked soil or else on account of the weather conditions, a high degree of humidity, the young leaves are apt to be wet. In that case one of the most important demands which the fungus puts on its environment, is fulfilled, as under these circumstances conidia that may be present on the leaves, can germinate. As especially at this time of the year a large number of diseased lettuce plants are found in the open, there is no lack of conidia, which may be transported by the wind to the young crop. The temperature, which in this period sinks in the night to 10 °C, and which rises in daytime to circ. 15 °C or to even higher values, is favourable to the germination of the conidia as well as to the entrance of the germ tube in the leaf (table 6). The infections of the young plants that are established in this way, may expand very rapidly, as the humidity of the air reaches periodically a very high degree. For this reason the fungus may produce its fructifications, and this may lead to new infections. In the cultures of "autumn lettuce" the infection of large numbers of plants therefore is to be ascribed to the circumstance that at this time of the year the external conditions are particularly favourable to the development of the fungus. In the autumn of 1959 the number of sunshine hours was larger, and the temperature higher than usual, and in this autumn the number of plants infected by *Bremia* accordingly remained low.

The preceding considerations indicate that the infection by *Bremia* may be prevented to a large extent when some precautions are taken. In order to keep

TABLE 6. Schematic summary of the effects exercised by environmental conditions on the various stages in the development of the disease caused by *Bremia lactucae* in lettuce plants.

Overzicht van de invloed van uitwendige omstandigheden op de verschillende stadia van de levenscyclus van Bremia lactucae *in sla.*

	Germination *Kiming*	Growth of the germ tubes *Groei van de kiembuizen*	Penetration via epidermal cells *Binnendringing via epidermis cellen*	Spread of the mycelium, formation of haustoria, filling of the substomatal cavities *Uitbreiding van het mycelium, vorming van haustorien, opvullen van de ademholten*	Production of conidiophores *Ontstaan van conidioforen*
Water	drops *druppels*	drops *druppels*	drops *druppels*		waterfilm
Relative humidity *Relatieve luchtvochtigheid*				high *hoog*	
Favourable temperature in °C *Gunstige temperatuur in °C*	2–10	15	10–22	20–22	6–23

the plants in a healthy condition, it is essential that the soil-blocks in which the seedlings before the development of their first leaf are transplanted, are not too wet, as otherwise the cotyledons may remain covered with water for a too long time. However, they should not be too dry either, for in that case it takes too long before the seedlings become once more turgescent and resume their growth, and in this interval they may sink down and come into contact with the wet soil. When the seedlings are transplanted, care should be taken that there is enough space between the cotyledons and the soil-block, because in this way a rapid evaporation of water that eventually may be present on their surface, is secured. For the same reason the transplanted seedlings should be aired when the weather is dry. The grower should not wait too long with the transplantation in order to prevent that drops of water which remain suspended between the seedlings, do not evaporate or evaporate too slowly. An early transplantation, moreover, prevents that the roots become too long, and that too many of them are damaged when the seedlings are taken out of the soil. When too many of them are damaged, it takes the seedlings more time before they are able to restore their turgescence, and they remain too long in a drooping position. It should be realized that these growth stagnations are indirectly favourable to an infection by *Bremia*, because drops of water on the lower side of the drooping leaves do not easily evaporate; in this way eventually present conidia obtain the chance to germinate, and to effect an infection. Growth stagnations of this kind are due, therefore, to unsuitable methods of cultivation, and should not be confused with the growth stagnations that are caused by a serious infection.

9. PHYSIOLOGICAL SPECIALIZATION; SPECTRUM OF HOST PLANTS

9.1. LITERATURE

SCHWEIZER (1919) has carried out a large number of cross inoculations with conidia of *Bremia lactucae* of different origin. As all host plants that are known so far, belong to the Compositae, he confined himself in the choice of his test objects to representatives of this family. It appeared that within the species *Bremia lactucae* REGEL several formae speciales occur. Each of these formae speciales appeared to be bound to a definite genus; one was found on *Sonchus*, another one on *Crepis*, etc.. On the species of the genus *Lactuca* too one forma specialis was met with. Schweizer succeeded in transferring this form from *Lactuca sativa* to *L. sativa* var. *capitata*, to *L. serriola*, to *L. virosa* and to *L. viminea*, and vice versa.

The experiments of Schweizer have been repeated in part by other investigators. ERWIN (1921) and MELHUS (1921) succeeded in transferring *Bremia lactucae* from cultivated lettuce to other species of *Lactuca*, and also the other way round. JAGGER (1926) tried in vain to transfer *Bremia lactucae* from *Sonchus*, *Senecio* or *Cirsium* to *Lactuca sativa* var. *capitata*, and LING & TAI (1945) came to a similar conclusion. WILD (1948) could transfer *Bremia lactucae* from cultivated lettuce only to *Lactuca virosa* and *L. saligna*, whereas POWLESLAND (1954) could infect with inoculation material obtained from cultivated lettuce only *L. sativa*, *L. serriola* and *L. muralis*. According to some authors the forma specialis found on species of the genus *Lactuca* would comprise various physiological races. In this connection it is interesting that JAGGER (1926) reports that 4 varieties of lettuce obtained from North America, proved in England to be resistant against *Bremia lactucae*. Two French lettuce varieties too proved to be resistant. JAGGER & CHANDLER (1933) accepted 5 physiological races within the forma specialis occurring on representatives of the genus *Lactuca*; 2 of the latter were found in England, 3 in North America (Florida and California). When a lettuce variety which originally was considered resistant, in the long run became infected, this was ascribed to an attack by a new physiological race. WINGRAVE (1952) arrives on account of experiences of this kind to the conclusion that there are at least two physiological races in Tasmania. In Germany SCHULTZ & RÖDER (1938) could prove the presence of two physiological races. They compared a large number of lettuce varieties as to their susceptibility under natural circumstances, and discovered in this way that some of them were under such circumstances resistant. However, some varieties which under these circumstances proved to be susceptible, were found to be resistant in their experiments, and they explain this discrepancy by assuming that in these experiments another physiological race of the parasite had been used (SCHULTZ & RÖDER, 1937). In England OGILVIE (1945) found 2 physiological races; the first could infect all lettuce varieties and also *Lactuca virosa*, whereas the second was unable to infect the latter, and infected but part of the lettuce varieties.

9.2. METHODS OF INVESTIGATION

The presence of physiological races within the forma specialis of *Bremia lactucae* which occurs on *Lactuca sativa* var. *capitata*, the head lettuce, could be studied by means of a collection of lettuce varieties brought together at the "Institute of Horticultural Plantbreeding" at Wageningen. This collection comprised 133 varieties of head lettuce *(Lactuca sativa* var. *capitata)*, 18 varieties of curled lettuce *(L. sativa* var. *crispa* L.*)*, 9 varieties of cos lettuce *(L. sativa* var. *longifolia* LAM.), and 12 varieties of roman lettuce *(L. sativa* var. *romana* GARS.) and the following wild *Lactuca* species, viz. *L. quercina* L., *L. sagittata* L., *L. saligna* L., *L. sativa* L., *L. serriola* L. and *L. virosa* L..

The various lettuce varieties as well as the various wild *Lactuca* species were cultivated in the usual way, the seedlings being transferred to soil-blocks. After the development of the first leaf they were inoculated by immersing them in a suspension of conidia, after which they were placed in glass boxes in which a high relative atmospheric humidity was maintained, and in which the temperature was kept between 15 °C and 20 °C. The external conditions, therefore, were favourable to the development of the fungus. The conidia were obtained from various places where lettuce is grown on a commercial scale, and were derived from various varieties of this vegetable (table 7). Care was taken to collect conidia only at such places where no other plants that might be infected by *Bremia lactucae*, were present. It is unfortunately not excluded that the conidia that were obtained in this way, may have belonged to more than one physiological race. Originally it had been our intention to study the susceptibility of the various hosts by means of monospore cultures. However, in preliminary experiments in which suspensions of conidia were tested in various dilutions, it appeared that when the suspension was diluted so far that it contained about 10 conidia per ml., the incubation time at a constantly high air humidity, i.e. in the most favourable circumstances, was already 3 to 5 days longer than when dense suspensions were used (cf. p. 160), and as such an addi-

TABLE 7. Place of origin and name of lettuce variety from which *Bremia lactucae* was collected. *Herkomst en naam van de sla-variëteit, waarvan* Bremia lactucae *werd verzameld.*

Place of origin *Herkomst*	Lettuce variety *Sla-variëteit*
Bergsenhoek	Proeftuin's Blackpool
Duiven	,, ,,
Huissen	,, ,,
Naaldwijk	,, ,,
Nootdorp	,, ,,
Sloten	,, ,,
Venlo	,, ,,
Vleuten	,, ,,
Zwijndrecht	,, ,,
Huissen	Meikoningin
de Lier	,,
Rotterdam	,,
Huissen	Attractie
den Hoorn	May Princess
Leidsendam	Regina

tional period increases the chance on contamination from the outside with other *Bremia lactucae* races to a considerable degree, this plan was given up. The conidia collected at one site were tested on at least 18 plants of every lettuce variety of every wild *Lactuca* species and of the other Compositae that were used in these experiments.

The plants of which the susceptibility was to be tested, were inspected to see whether they were macroscopically free of infection, and when they proved to be quite healthy, some of their leaves were put in Petri dishes, and then some drops of the suspension of conidia were placed upon them. After 24 hours or sometimes after 48 hours the leaves were cleared and stained in order to see whether the fungus had succeeded in entering them.

9.3. Results and discussion

The 15 samples of conidia of which the origin is recorded in table 7, were not only tested on the above-mentioned lettuce varieties and on various wild *Lactuca* species, but also on some lettuce varieties obtained from North America, where they had proved to be resistant against the attacks of *Bremia*. Further the samples were tested on some other Compositae, viz. on *Cichorium endivia* L., on *Cirsium arvense* (Savi) Ten., on *Senecio vulgaris* L. and on *Sonchus oleraceus* L., as it has sometimes been thought that the forma specialis of *Bremia lactucae* which is found on lettuce, might infect these Compositae too. In this case it might be expected to pass also from these plants to our lettuce cultures.

At the end of the normal incubation time on most of the *Lactuca* species and of the lettuce varieties that are grown in the Netherlands, the development of conidiophores and conidia was noted. Between the various lettuce varieties there appeared to be but little difference in susceptibility, and with regard to the conidia of different origin there was no difference in pathogenicity at all. No conidiophores were produced on the leaves of the four Compositae not belonging to *Lactuca*, on those of *Lactuca serriola* and of *L. saligna*, on those of the cos lettuce "Grand Rapids", on the curled lettuce "Jade" and "Imperial" and on the North-American varieties. An incubation time which was 2 to 3 times as long as the normal one, was found in the cos lettuce "Salad Trim" and in the curled lettuce "Great Lakes" and "Lake Superior". However, the number of conidiophores that was produced on these varieties, was but small. Microscopical investigation revealed that in those species of *Lactuca* and in those lettuce varieties in which no fructifications were found, germ tubes nevertheless had entered the leaves. However, the vesicular swelling of the infection tube in the epidermal cell remained much smaller than it did in the susceptible varieties. Out of these swellings sometimes short hyphae grew out, but as a rule, they did not penetrate beyond the near vicinity of the cell from which they emerged, and if they did grow out to a somewhat greater length, they did not penetrate into the intercellular spaces for more than a few cell lengths, and their diameter remained small (Plate IV, B). Haustoria were not produced. These varieties can therefore not be regarded as fully resistant.

It was thought that the diminished susceptibility of these varieties might be due to substances present in their cells, and that the presence of such substances might perhaps be demonstrated by cultivating the conidia in the press sap of

these plants. However, it appeared that there was no difference between the germination of the conidia in this sap and in distilled water, and that the germ tubes developed in this medium in the normal way.

In *Cichorium endivia, Cirsium arvense, Senecio vulgaris* and *Sonchus oleraceus* no entrance of the germ tubes was ever observed. These plants possess therefore a high degree of resistance against *Bremia lactucae* obtained from cultivated lettuce.

Our experiments gave no indication of the presence of a physiological specialization within the forma specialis of *Bremia lactucae* collected at various places and on different varieties of our lettuce, *L. sativa* var. *capitata*.

10. EXTENT OF THE DAMAGE CAUSED BY THE PARASITE

Our experiments have shown that the growth of young lettuce plants inoculated with *Bremia lactucae* is retarded, provided that at least half the leaves become infected. In cultures on a commercial scale too differences between healthy lettuce plants and plants that are infected by *Bremia*, are recognizable.

In order to obtain reliable figures on the effect of the growth retardation, plots planted with healthy, slightly infected, moderately infected and severely infected plants were compared. For this experiment the lettuce was sown on the 22nd August 1959, and on the 28th August the seedlings were transferred to soil-blocks. On the 8th September the plants had developed three or four leaves, and at that stage they were inoculated by immersing either one leaf (group 1), two leaves (group 2) or three leaves (group 3) in a suspension of conidia. Control plants (group 0) were immersed in water. Until the next morning the plants were kept under a plastic cover in order to prevent the suspension on the leaves from drying up, and to secure an infection by the conidia. On the 9th September the plants were transplanted in the plots. Each plot consisted of 156 plants, and each group was represented four times. On the 21st October the plants of the control group were harvestable, and on this day therefore the plants of all the plots were harvested. The heads harvested in the various plots were weighed, and divided on account of their weight in three qualities, "A-lettuce", "C-lettuce" and "stewing lettuce". "B-lettuce" was represented so sparingly that it was counted as "C-lettuce". It appeared that there was a considerable difference between the percentage of "A-lettuce", the best quality, in group 0 and in the groups 1, 2 and 3 (table 8), and this applies

TABLE 8. Percentage of heads belonging to the qualities "A", "C", and "stewing lettuce", and the average head weight of 624 healthy or variously infected plants.
Percentages „A-sla", „C-sla" en „stoofsla" en gemiddeld kropgewicht van 624 gezonde, of in verschillende mate geïnoculeerde planten.

Groups of 624 plants *Groepen van 624 planten*	Quality/*Kwaliteit*			Average headweight in grammes *Gemiddeld kropgewicht in grammen*
	"A"	"C"	"stewing lettuce" *„stoofsla"*	
Healthy plants . . . . *Gezonde planten*	82.3 (100.0[1])	15.1	3.6	220.1
1 leaf inoculated . . . *1 blad geïnoculeerd*	64.4 (78.2[1])	28.8	6.8	185.7
2 leaves inoculated . . *2 bladeren geïnoculeerd*	65.8 (80.0[1])	25.9	7.3	181.8
3 leaves inoculated . . *3 bladeren geïnoculeerd*	25.8 (31.3[1])	54.5	19.7	160.1

[1] Yield of "A-lettuce" when the percentage of "A-lettuce" in the group of healthy plants is put at 100%.
[1] *Opbrengst van „A-sla" als het percentage „A-sla" van de groep gezonde planten op 100% wordt gesteld.*

also to the average weight found in the four groups. If we put the percentage of "A-lettuce" in group 0 at 100, it becomes 78.2 in group 1, 80.0 in group 2, and 31.3 in group 3, which means that the harvest of the best quality has decreased in the groups 1, 2 and 3 with resp. 21.8%, 20.0% and 68.7%. The differences between the percentages of "A-lettuce" in the four groups are significant. The differences between that in group 0 and group 1 and between group 0 and group 3 have a reliability of 95%. The differences between the average weights of the heads in the four groups are even strongly significant. The differences between group 0 and group 1 and between group 0 and group 2 have a reliability of 95%, those between the average weight of the groups 0, 1 and 2 on the one hand and group 3 on the other are reliable for 99%.

It appears therefore that the harvest of "A-lettuce" obtained from the plots with the plants of which in the 3- or 4-leaved stage three leaves were inoculated, remained circ. 68%behind that of the plots with the healthy plants, but that the harvest of the plots with the plants that had been infected less severely, differed but slightly from that of the plot with the healthy plants. That in the less strongly infected plots the difference with the not infected plots was so much smaller, is due to the circumstance that in the autumn of 1959 the weather was sunny and warm, with the result that the plants grew rapidly, and that the loss of the one or two infected leaves was rapidly compensated by the development of new leaves.

The difference between group 3 and group 0 may be ascribed to the fact that group 3, although of the same age as group 0, was harvested too soon. In order to reach the normal harvestable stage these plants ought to have been given 2 or 3 weeks more time. If they had received this respite, the money value of the harvest would have been higher than that of the harvest of group 0, because in the meantime the prices had risen. However, the grower has to harvest all the plants of the same culture at the same time, viz. at the moment the healthy plants are harvestable. The diseased plants which remained behind in their development, must be harvested at the same time with the healthy ones, and in this way, of course, the percentage of lettuce of the "A" quality decreases.

11. EFFECT OF FUNGICIDES

For combating *Bremia lactucae* formerly various combinations of the Bordeaux mixture have been recommended (ERWIN, 1921; DORAN, 1933; WILD, 1948; WINGRAVE, 1952). SPRAU & VON MINCKWITZ (1949) advice steaming of the soil as a preventive measure. The use of pulverized sulphur has also been recommended, but the opinions with regard to the effectivity of such a treatment are divided; ERWIN (1921) expects some success if dusting is performed at a low atmospheric humidity. DORAN (1933) reports that he could check the production of conidiophores with this agent, but KENDRICK (1929) and CROGAN et al. (1955) attach no value whatever to a treatment with sulphur. BRAVENBOER (1956) concludes from his experiments that sulphur and agents containing copper exercise a retarding influence on the growth of the host. A rather good effect was obtained with zineb (HAASIS & ELLIS, 1950; POWLESLAND & BROWN, 1954; COX, 1955, 1956, 1957; VERHOEFF, 1957); the residual effect of this substance too appeared to be fairly good. BRIAN (1949) tested the influence of griseofulvin on a large number of fungi, and found that various representatives of the Peronosporales were resistant to this antibiotic; however, *Bremia lactucae* itself was not tested.

We started our investigations on the effect exercised by fungicides on *Bremia*, with experiments carried out in glass boxes. The fungicide that was to be tested, was sprayed or dusted over the test plants either 1 hour or 2½ hours after the latter had been inoculated, and at the end of the incubation time, of which the length under the circumstances of the experiment was known, the 20 or 25 plants with which the experiment was carried out, were inspected in order to see whether they had become infected or not. The number of infected plants was noted. The fungicides with which the experiments were carried out, belonged to various groups (table 9).

The way in which the fungicide was distributed over the surface of the plant, was so efficient that the possibility that the fungus might escape its influence, may be regarded as excluded. Therefore, in case still more than 15 percent of the test plants proved to be infected, the action of the fungicide was regarded as of little effect. The only fungicides with which the percentage of infected plants remained below this limit, were zineb, maneb and B 56. A spray of copper oxychloride also had a fairly good result, but it appeared to cause a slight retardation of the growth. When zineb, maneb and B 56 were added to a suspension of conidia, the latter were killed, and germ tubes that had already been produced before these fungicides were added, were stopped in their growth. TCTNB and brestan too killed the conidia when they were added to a suspension of the latter, but they failed to do this when they were applied to inoculated plants. The efficaciousness of the fungicides can therefore not be determined in suspensions of conidia. When infected plants were dusted with zineb or with B 56, then the conidia which were produced after the dusting were killed when they passed through the film formed by the fungicide, but neither the production of the conidiophores nor the growth of the mycelium in the interior of the leaves were influenced by the latter. The effect of these fungicides is therefore purely preventive.

In the next experiments the influence of a number of fungicides of which in the previous set the effect had proved to be either good, moderate or slight, was studied in a lettuce culture. The experiments were taken in the autumn, the fungicides being 1. zineb and B 56; 2. phaltan; and 3. TCTNB and allisan. Their effect was compared in plots, every treatment being applied to 4 plots, each containing 224 plants. The fungicides were applied every 5 or 6 days in a dose of 1.5 gr. per square meter. In addition one plot was dusted with zineb every 10 or

TABLE 9. Percentage of diseased plants and percentage of germinated conidia found after treatment with various fungicides.
Percentage zieke planten en percentage gekiemde conidiën na behandeling met verschillende fungiciden.

Name of fungicide *Naam van het fungicide*	Percentage of diseased plants *Percentage zieke planten*				Percentage of germinated conidia: dilutions of the fungicides added to the suspensions of conidia *Percentage gekiemde conidiën: verdunningen van de fungiciden toegevoegd aan de sporensuspensies*					
	Time of application *Tijdstip van toediening*									
	1 hour after inoculation *1 uur na de inoculatie*		2½ hours after inoculation *2½ uur na de inoculatie*							
	Application by *Toediening door*		Application by *Toediening door*							
	dusting *stuiven*	spraying *spuiten*	dusting *stuiven*	spraying *spuiten*	0.1	0.05	0.025	0.0125	0.006	0.003
1. zineb	0	0	0	0	0	0	0	0	0	18.9
2. maneb	5	0	10	0	0	0	0	0	0	0
3. TEC 17	–	15	–	15	0	0	0	0	0	0
4. B 56	0	0	0	10	0	0	0	0	0	0
5. Aacuram	–	15	–	5	0	0	0	0	0	0
6. ziram	–	45	–	30	0	17.1	34.3	31.0	73.9	78.4
7. ferbam + 1 + 2	–	5	–	0	0	0	0	0	0	0
8. TMTD	10	20	10	30	0	0	0	0	0	0
9. thioneb	–	45	–	10	0	0	0	0	0	0
10. TCTNB	15	85	20	30	0	0	0	0	0	0
11. PCNB	75[1]	10[1]	45[1]	50[1]	56.7	73.5	98.6	101.6	95.6	100.1
12. phaltan	30	25	30	55	0	0	11.5	25.3	40.6	60.3
13. captan	65	25	25	40	0	0	0	0	0	32.1
14. brestan	60[2]	[2]	65[2]	[2]	0	0	0	0	0	0
15. cyprex	–	35	–	40	0	0	0	0	12.3	55.0
16. karathane	[2]	[2]	[2]	[2]	0	0	0	0	70.3	68.6
17. B 622	65	25	40	30	0	0	18.4	13.5	53.6	38.2
18. allisan	20	70	15	60	0	0	0	0	0	25.3
19. B 500	60	–	65	–	–	–	–	–	–	–
20. pimeracine	80	–	75	–	–	–	–	–	–	–
21. Aaventa	–	10	–	25	0	0	0	0	0	0
22. copperoxy-chloride	20[1]	10[1]	20[1]	5[1]	0	0	0	0	0	15.8
23. G 33	[2]	–	[2]	–	0	3.1	34.1	37.9	64.4	80.9
24. sulphur	100	35	60	45	48.6	100.8	97.1	99.2	101.3	105.3
25. talcum powder	75	–	90	–	–	–	–	–	–	–
26. water	–	85	–	90	–	–	–	–	–	–

[1] Growth inhibition/*Groeiremming.*
[2] Leaves burned/*Bladverbranding.*

1 –zincethylene bisdithiocarbamate
2 –manganousethylene bisdithiocarbamate
3 –bis (dimethyldithiocarbamoyl) ethylene bisdithiocarbamate
4 –bis (dimethylthiocarbamyl) ethylene bisdithiocarbamate
5 –cetylpiridinium dimethyl dithiocarbamate
6 –zincdimethyl dithiocarbamate
7 –ferridimethyl dithiocarbamate +1 +2
8 –tetramethylthiuramdisulfide
9 –polyethylenethiuramdisulfide
10 –trichlorotrinitrobenzene
11 –pentachloronitrobenzene
12 –N-trichloromethylthiophtalimide
13 –N-trichloromethyl thiotetra hydrophtalimide
14 –triphenyltinacetate
15 –dodecylguanidineacetate
16 –dinitrocaprylphenylcrotonate
17 –dichloroanilinetriazine
18 –dichloronitroaniline
19 –halogenated oxychinoline
21 –organic mercury compound
23 –S-carboxymethyl NN dimethyl dithiocarbaminate

12 days. This was done in order to find out wether with a good fungicide a less frequent dusting would suffice.

The lettuce was sown on the 22nd August 1959. On the 27th and on the 28th August the seedlings were transferred to soil-blocks, and on the 8th and the 9th September the young plants were set out in the beds. On the 16th September all plants were inoculated by spraying them with a suspension of conidia. On the 31th August, i.e. 16 days before the inoculation, they were dusted for the first time with the fungicides, the plants being still in the soil-blocks. On the 3rd October the plants were dusted for the last time. At that moment the plants were already so large that the lowermost infected leaves were hardly touched by the fungicide. On the 1st October in each of the plots 20 of the 224 plants were harvested. These 20 plants were chosen in such a way that they occupied corresponding positions in the plots. In each of these samples the number of diseased plants and the degree of infection were estimated (table 10). At this moment the differences between the plots that had been treated with the various fungicides, were still distinctly visible, but it would not have been wise to wait with these estimations until the end of the experiment, because it was to be feared that all parts that had been infected with *Bremia*, would become infected with the much more damaging *Botrytis cinerea*, so that the effect caused by *Bremia* would no longer be clearly recognizable. Actually, at the end of the experiment many leaves that originally had been infected by *Bremia*, had rotted off on account of the secondary infection with *Botrytis*. On the 23rd October the rest of the crop was harvested, and the heads in each plot were weighed, and divided in groups according to their quality.

The differences between the percentages of diseased plants counted in the plots that had been treated with different fungicides (column II) are very significant. The differences between the plants that were used as controls, or those treated either with phaltan, TCTNB or allisan on the one hand, and those treated with zineb or with B 56 on the other, are reliable for 99 per cent. It appears that the most active fungicide is B 56, and that zineb comes next. The control plots and the plots treated with TCTNB and with allisan show an about equal number of diseased plants, whereas the plots treated with phaltan occupy an intermediate position between the latter and those that had been treated either with B 56 or with zineb. In column III the average degree of infection found in the leaves of the infected plants is shown. Here too the differences are markedly significant. The difference between the plants used as controls or treated either with TCTNB or with allisan on the one hand, and those treated with B 56 on the other are reliable for 95 per cent. These figures show that the

TABLE 10. Percentage of diseased plants and disease rate of the leaves determined 3 weeks before the final harvest of the lettuce; percentage of lettuce belonging to the quality "A"; and average head weight at the end of the experiment; the samples in which the values of column II to IV were determined consisted of 80 plants.

Percentage zieke planten en de mate van blad aantasting 3 weken voor de oogst; percentage „A-sla" en gemiddeld kropgewicht aan het eind van de proef; de beoordeling weergegeven in de kolommen II, III en IV geschiedde telkens bij groepen van 80 planten.

I	II	III	IV	V	VI
Treatment *Behandeling*	Percentage of diseased plants *Percentage zieke planten*	Average disease rate[1] *Gemiddelde aantasting*[1]		Percentage of heads of "A" quality *Percentage „A-sla"*	Average head weight in grammes *Gemiddeld kropgewicht in grammen*
		of the diseased plants *van de zieke planten*	of all plants examined *van alle gecontroleerde planten*		
zineb[2]	25.0	1.46	0.41	63.3	209.4
zineb[3]	32.5	1.59	0.54	66.2	208.8
phaltan[2]	52.5	1.79	0.97	66.3	209.5
TCTNB[2]	76.3	2.16	1.63	67.1	182.8
allisan[2]	70.0	2.19	1.73	46.8	191.5
B 56[2]	16.3	1.37	0.21	71.9	213.2
untreated *onbehandeld*	71.3	2.15	1.82	45.7	197.2

[1] 0 = no attack; 5 = the whole leaf covered with conidiophores.
0 = geen aantasting; 5 = het gehele blad met conidiophoren bedekt.
[2] Dusting every 5–6 days./ *Stuiven om de 5–6 dagen.*
[3] Dusting every 10–12 days./*Stuiven om de 10–12 dagen.*

plants treated with B 56 and with zineb are much less severely diseased than those treated with the other fungicides. The differences between the figures given in column IV are very significant, and the differences between the control plants and the plants that had been treated either with TCTNB or with allisan on the one hand and those that had been treated with B 56 and with zineb on the other, appear now to be reliable for 99 per cent. In column V the percentages of the heads belonging to the "A" quality are given. The differences between these percentages are significant too. The difference between the plots treated with allisan and B 56, that between the plots treated with phaltan and allisan, and that between the control plots and the plots treated with B 56 are reliable for 95 per cent. It appears that the lowest percentage of "A" quality is obtained from the control plots and from the plots treated with allisan, the highest percentage from those treated with B 56. Column VI gives the average weight of the heads harvested in the various plots, but it appears that there is in this respect no significant difference between the controls and the plants that are treated with one of the fungicides.

The results of this second set of experiments show that the treatment with B 56 gave the best results, as from the plots treated with this fungicide the largest percentage of "A-lettuce" was harvested, and that the next best results were obtained with zineb. No distinct difference was found in these experiments between a dusting once in every 5 or 6 days and once in every 10 to 12 days.

If the results of the preliminary experiments which were summarized in table 9, are compared with those of the experiments with "autumn lettuce" described above and summarized in table 10, it appears that the way in which

the action of the fungicides was tested in the preliminary experiments gave a good forecast of what is to be expected when these substances are applied on a commercial scale. In the preliminary experiments in the laboratory as well as in the experimental plots B 56 and zineb appeared to be good, phaltan a moderate, and TCTNB as well as allisan rather unsatisfactory fungicides. However, the results of the study on the inhibiting influence which is exercised by these substances in vitro on the germination of the conidia, proved to be of no value.

The good results obtained in our experiments with zineb are in complete agreement with the experience of other investigators (HAASIS & ELLIS, 1950; POWLESLAND & BROWN, 1954; COX, 1955, 1956, 1957).

In the case of *Bremia* sulphur appeared to be completely worthless as a fungicide. This is in agreement with the opinion of KENDRICK (1929) and of CROGAN et al. (1955). Growers sometimes claim to have obtained results with this substance at higher temperatures, but this could not be confirmed by our experiments. At a low relative humidity of the air dusting with this substance gave, in contradiction to the opinion expressed by ERWIN (1921), no results either.

12. SUMMARY

1. The aim of this study was to obtain more information on the demands which *Bremia lactucae* REGEL, a parasite found on *Lactuca sativa* L. var. *capitata* L. (head lettuce), puts on its environment in the various stages of its life-cycle, to find measures by means of which this parasite may be kept in check, and to see whether there are varieties of the host plant which are able to resist the attacks of this fungus.
2. In large-scale cultures, the whole year round, and in older as well as in younger lettuce plants symptoms of the *Bremia* infection may be observed.
3. With each of the ways in which lettuce is grown, periods may be found in which circumstances are favourable to an infection by *Bremia*. This are, as a rule, the periods in which the lettuce plants remain wet for some time.
4. The most common and at the same time the most easily observable symptom of the disease is the presence of yellowish green leaf spots. On the lower side of the leaf they are usually covered with white tufts formed by the conidiophores of the fungus. These white patches may be present also on the upper side, but here they are not always accompanied by the yellow discolorations.
5. The white tufts consist of conidiophores with conidia. The conidiophores are a few times dichotomously branched, and at the end of the ultimate ramifications a clavate swelling is seen, which is provided with 4 or 5 sterigmata. Each sterigma bears a single conidium. The length of the conidiophores depends on the relative humidity of the air, and varies, as a rule, between 200 μ and 1200 μ.
6. The conidia are hyaline, ovoid-ellipsoidal to globose, 20.5 μ $\times$ 18.7 μ, and provided with a rather thick, smooth wall. They germinate directly, i.e. without giving rise to swarm spores.
7. The germ tube attaches itself to the surface of the leaf by means of a slight swelling, and from this appressorium a thin infection hypha emerges which perforates the wall of the epidermal cell. Within the latter a vesicular swelling is produced, from which new hyphae arise. Occasionally the germ tube penetrates between two epidermal cells, and once the entrance by way of a stoma was observed.
8. At temperatures varying approximately between 10 °C and 22 °C an infection may be brought about within three hours.
9. The hyphae of the intercellular mycelium produce numerous saccate haustoria which penetrate, in the cotyledons as well as in leaves of various age, into the parenchyma cells. The diameter of the hyphae depends on that of the intercellular spaces. The most favourable temperature for the development of the intercellular mycelium is found between 20 °C and 22 °C.
10. The mycelium is unable to pass the thicker veins; it is also unable to pass from one leaf to another by way of the stem, and it does not even penetrate into the latter. The flowering stem could be infected by direct inoculation, but with the capitula this proved impossible.
11. Before the mycelium can start with the production of conidiophores, it has

first to fill up a considerable number of substomatal cavities. However, before the conidiophores can begin to develop, another condition must be fulfilled, viz. that the surface of the leaf becomes covered with a film of water. A temperature varying approximately between 6 °C and 23 °C is required.

12. Normally the conidiophores are produced in the early hours of the morning when it is still dark. Exposition of the plants to a continuous illumination does not prevent their development, but under such circumstances the conidiophores appear 2 or 3 hours later.
13. The time at which the symptoms of the disease become visible, depends upon the relative humidity of the air. When, from the moment of the infection, the latter remains so high that the leaves are permanently covered with a thin layer of water, then the conidiophores appear at a moment at which the discoloration of the leaf has not yet set in, and they are found in that case at both sides of the leaf.
14. When the degree of atmospheric humidity remains permanently high, a distinct relation is recognizable between the length of the incubation period and the temperature. The shortest incubation period, viz. about 5 days, was found at circ. 22 °C. If the relative humidity is not high enough to keep the leaves covered with a film of water the first symptoms of the disease consist of a yellowing of the leaves. This discoloration marks the end of the incubation period which in this case may last several weeks, dependent on temperature.
15. For the germination of the conidia liquid water is required; a high relative humidity of the air is insufficient. Germination takes place between –3 °C and 31 °C, the most favourable range of temperature lying between 4 °C and 10 °C. It requires but little time, especially when the temperature is favourable.
16. The length of the germ tubes is in the first stages of their growth directly proportional to the temperature. The temperature which is most favourable to the growth of the germ tube is found at circ. 15 °C.
17. No indications in favour of the assumption that substances contained in the lettuce leaves exercise a stimulating influence either on the germination of the conidia or on the growth of the germ tubes, were found in our experiments with extracts and with press sap of these leaves.
18. The various cross inoculations that were carried out by us, did not prove the presence of a physiological specialization within the forma specialis of *Bremia lactucae* which occurs on *Lactuca sativa* var. *capitata*. Some of the lettuce varieties and some of the *Lactuca* species appeared to possess a high degree of resistance to the attacks of this forma specialis, and may perhaps be used as starting points in attempts to produce varieties that deserve to be grown on a commercial scale.
19. At comparatively low temperatures and a low degree of atmospheric humidity the conidia retain their viability for a very long time.
20. The parasite may survive unfavourable periods in the form of the intercellular mycelium that is present in the leaves of the host. Its dispersal is effected by the conidia.
21. The damage caused by the *Bremia* infection finds its expression in a decrease in quantity as well as in quality of the crop. The damage may be

increased by the development of *Botrytis cinerea* Pers. in the infected leaves.

22. So long as no resistant varieties have been produced which can be grown on a commercial scale, the disease may be kept in check by preventive dusting with zineb or with B 56 in doses of 1–1.5 gr. per square meter. The young plants should be dusted once in every 5 or 6 days. Three or four weeks after the young plants have been transplanted, the dusting may be stopped as later infections cause but little damage.

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13. SAMENVATTING

De teelt van kropsla, *Lactuca sativa* L. var. *capitata* L. heeft zich door verschillende oorzaken, zoals de grotere vraag naar dit produkt en de grote uitbreiding van het glasareaal, de laatste jaren sterk uitgebreid. Naast de grote toename van de veilingaanvoer is er ook een betere spreiding van de aanvoer over het gehele jaar ontstaan (tabel 1). Bij het telen van sla in de herfst traden echter moeilijkheden op, niet alleen wat de te volgen teeltwijze en de te gebruiken sla-variëteiten betreft, maar ook door het optreden van een ziekte, die door de kwekers „het wit" wordt genoemd, veroorzaakt door *Bremia lactucae* REGEL. Bij deze teeltwijze nemen de temperatuur, de daglengte en de lichtintensiteit gedurende de ontwikkeling van de planten af, terwijl de relatieve luchtvochtigheid toeneemt (tabel 2). Onder dergelijke omstandigheden trad deze ziekte in veel sterkere mate op, dan bij andere teeltwijzen het geval was. Tengevolge van de aantasting door deze schimmel ontstond oogstderving door slechte ontwikkeling en slechte kropvorm der planten. De bestrijding van deze parasiet liet te wensen over.

Het doel van dit onderzoek was na te gaan, welke eisen *Bremia lactucae* aan het milieu stelt om haar levenscyclus te volbrengen; of door het nemen van bepaalde kultuurmaatregelen aantasting en verspreiding voorkomen kan worden en bestrijding met fungiciden mogelijk is. Ook was het de bedoeling om na te gaan of er resistente sla-soorten (*Lactuca* spp.) bestaan, die als geniteur voor handelsvariëteiten zouden kunnen dienen.

Bremia lactucae is een Phycomyceet en behoort tot de Peronosporales. De schimmel werd het eerst door REGEL (1843) gevonden en beschreven. DE BARY (1863) gebruikte de naam *Peronospora gangliformis* (BERK.) De Bary. De geslachten *Peronospora* en *Bremia* zijn inderdaad zeer nauw met elkaar verwant en zijn alleen morfologisch van elkaar te onderscheiden door de bouw van de sporendragers. Bij *Bremia lactucae* hebben de dichotoom vertakte sporendragers aan het einde van elke vertakking nl. een knots- of handvormige opzwelling terwijl dergelijke opzwellingen bij *Peronospora* spp. niet voorkomen.

Het intercellulair groeiende mycelium bestaat uit coenocytische hyfen. De dikte er van is binnen zekere grenzen afhankelijk van de beschikbare ruimte in de intercellulairen van de waardplant en variëert van ongeveer 5 μ tot ongeveer 12 μ. De schimmel voedt zich door middel van zakvormige haustoria, die door de hyfen in de cellen van de waardplant worden gevormd. Vanuit het mycelium in de ademholten ontstaan één tot drie sporendragers, die via het huidmondje naar buiten treden. De lengte der dragers variëert tussen 200 μ en 1200 μ. De dragers vertakken zich meestal drie tot vijf keer dichotoom. De knotsvormige opzwellingen aan het einde van elke vertakking dragen drie tot vijf vingervormige uitsteeksels. Elk uitsteeksel draagt apicaal één spore. De sporen zijn hyalien, eivormig- elliptisch tot rond, 20.5μ × 18.7 μ.

In de praktijk is het meest voorkomende en het meest opvallende symptoom het optreden van lichtgroen tot gele verkleuringen in het blad, die vooral bij

oudere bladeren vaak scherp begrensd zijn door bladnerven (Plaat 1,A). Aan de onderzijde der bladeren is op dergelijke plekken meestal wit mycelium aanwezig. Dit zijn de sporendragers met sporen. Soms kunnen ook op de bovenzijde van de bladeren sporendragers met sporen worden waargenomen. Bij oudere bladeren kunnen in de geelgroene verkleuringen necrotische centra ontstaan, die in het midden vrijwel geheel doorzichtig zijn. Bij jongere planten worden soms geheel vergeelde bladeren aangetroffen, waarvan alleen de rand nog groen is. Bij jonge slaplanten kan een groeistagnatie optreden, enkele dagen voordat symptomen van een *Bremia lactucae* aantasting zichtbaar worden (Plaat II, B). Volgens vele kwekers wordt na een groeistagnatie de plant zeer vatbaar voor *Bremia lactucae*; het onderzoek toonde echter de onjuistheid hiervan aan. De *Bremia lactucae* aantasting is primair. Wel kan besmetting optreden na een groeistagnatie door andere oorzaak.

Bij de verschillende teeltwijzen kunnen een aantal perioden worden aangewezen, waarop zeer vaak een eerste aantasting door *Bremia lactucae* kan optreden, of waarop een verspreiding van de parasiet tot stand kan komen. In het algemeen zijn het die perioden, waarop de bladeren enige tijd nat blijven. Onder deze omstandigheden kunnen eventueel aanwezige sporen ontkiemen, kunnen de kiembuizen binnendringen en kan infectie tot stand komen. Water is noodzakelijk gebleken voor het ontkiemen der sporen. Een hoge luchtvochtigheid is onvoldoende. De planten kunnen nat worden bijv. als de cotylen van de kiemplanten na het verspenen in perspotten enige tijd tegen de natte perskluit blijven plakken; of als na het uitplanten de blaadjes enige tijd op de, voor het poten nat gemaakte grond blijven liggen. Het kan echter ook het geval zijn, als de plantjes, na het verspenen, niet tijdig worden uitgeplant. De plantjes komen tegen elkaar te staan en aanwezig water kan moeilijk verdampen, waardoor druppels water aan de bladeren komen te hangen. Een eerste aantasting, maar ook een uitbreiding van de ziekte kan optreden als de planten in de kas of in het warenhuis zover zijn uitgegroeid dat zij elkaar raken. Aan de onderzijde der onderste bladeren ontstaat dan condenswater, dat daar gedurende de verdere teelt niet meer verdwijnt. Ook tussen de bladeren kan nu gedurende langere tijd water aanwezig zijn. Is het gewas tot op dit tijdstip echter vrij gebleven van een *Bremia lactucae* aantasting, dan ontstaat geen grote schade, indien de schimmel nog optreedt.

In zeer veel gevallen komt op die plantendelen, die door *Bremia lactucae* zijn aangetast, de schimmel *Botrytis cinerea* PERS. tot ontwikkeling, waardoor de planten te gronde kunnen gaan.

Komt een spore op een nat slablad terecht, dan kan kieming optreden en kan de kiembuis het blad binnendringen. Binnendringing vindt plaats direkt via de epidermis, zowel aan de boven- als aan de onderzijde der bladeren. De kiembuis vormt een appressorium en van daar uit doorboort een dunne infectiehyfe de celwand van de epidermis. In de epidermiscel wordt een zakvormige uitstulping gevormd, van waaruit de hyfen ontstaan (Plaat III, A; fig. 1). Ongeveer 30 uur na het binnendringen kleurt de inhoud van deze epidermiscellen niet meer met katoenblauw, terwijl die van de omliggende cellen lichtblauw gekleurd wordt. Binnendringing kan ook plaats vinden tussen 2 epidermiscellen door. Slechts één keer is waargenomen, dat een kiembuis het blad via een huidmondje binnenging. Hierbij werd geen appressorium gevormd. Het intercellulair ver-

lopende mycelium vult deze ruimten vrijwel geheel op en vormt op vele plaatsen zakvormige haustoria, zowel in cotylen, als in jonge en oude bladeren (Plaat III, B en C). De gehele bladschijf kan doorgroeid worden alsmede de randen van de bladsteel. In het parenchym om grotere nerven en in dat van het centrale deel van de bladsteel groeit geen mycelium. Van een aangetast blad kan de schimmel de stengel niet aantasten en dus ook niet naar andere bladeren groeien. Vanuit het mycelium in de ademholten ontspringen meestal 2 sporendragers. Deze zijn ter plaatse van de huidmondjes iets ingesnoerd (Plaat IV, A).

In bladeren van planten, die opgegroeid zijn bij niet te hoge temperatuur en bij veel licht, dus van een zogenaamd „hard" gewas, vormt de schimmel een minder dicht netwerk van hyfen, dan in bladeren van een zogenaamd „week" gewas, d.i. een gewas dat opgegroeid is bij weinig licht en hogere temperatuur. De vatbaarheid voor *Bremia lactucae* is van beide soorten bladeren even groot. Behalve de bladeren aan de „krop" kunnen ook de bladeren aan de bloemstengels en de bloemstengels zelf worden aangetast. In deze laatste blijft het mycelium beperkt tot de buitenste lagen van het schorsparenchym, terwijl uitbreiding van het mycelium slechts zeer langzaam plaats vindt. Voor de bladeren aan de bloemstengels geldt hetzelfde als gezegd is voor de bladeren van een „hard" gewas. Het is niet gelukt om een aantasting van bloemhoofdjes te verkrijgen.

Op zieke bladeren, waarop *Bremia lactucae* sporuleert blijken 2 soorten sporen aanwezig te zijn, nl. hyaliene met fijn granulaire, en niet hyaliene met grof granulaire inhoud. Deze laatsten blijken niet meer te kiemen, en zijn waarschijnlijk dood (tabel 3).

De sporen kiemen met een kiembuis; indirecte kieming is niet waargenomen. De invloed van de temperatuur op het ontkiemen der conidiën bij verschillende temperaturen is weergegeven in fig. 2. Deze grafiek laat zien, dat de minimum-, optimum- en maximumtemperatuur voor de sporekieming bij respectievelijk ± −3 °C, 4°–10 °C, en bij ± 31 °C liggen. De conidiën bij hogere temperatuur gevormd, (20°–22 °C) blijken minder hoge kiemingspercentages te bereiken dan die, bij lagere temperatuur (10°–15 °C) ontstaan. Voor deze laatsten zijn de optimum- en de maximumtemperatuur naar lagere waarden verschoven. Het ontkiemen der conidiën geschiedt, vooral bij het temperatuuroptimum zeer snel (fig. 3). Voor 2 temperaturen zijn de afzonderlijke waarnemingen weergegeven in fig. 4. De kiembuislengte bij verschillende temperaturen na 24 uur is weergegeven in fig. 5. Het blijkt, dat het temperatuuroptimum voor dit proces bij ± 15 °C ligt. Tussen de lengte der kiembuizen van conidiën bij lagere of hogere temperatuur gevormd, blijken geen duidelijke verschillen. De lengte der kiembuizen neemt aanvankelijk rechtlijnig toe met de tijd, zoals fig. 6 laat zien. Door de onnauwkeurigheid in het meten van de meestal gekronkelde kiembuizen is de spreiding der verschillende punten hier groter dan bij het ontkiemen der conidiën. Voor 2 temperaturen zijn de afzonderlijke waarnemingen in fig. 7 weergegeven.

Stoffen uit slabladeren hebben geen stimulerende of remmende invloed op het ontkiemen der conidiën of op de groei van de kiembuizen (tabel 4).

Het binnendringen der kiembuizen kan plaats vinden tussen ± 3 °C en ± 28 °C Tussen ± 10 °C en ± 22 °C kan dit binnen 3 uren plaats vinden, bij 6°–8 °C na 6 tot 8 uren.

De schimmel kan alleen tot sporuleren overgaan als een groot aantal adem-

holten met mycelium zijn gevuld en als de aangetaste bladdelen met een waterfilm zijn bedekt. Sporuleren vindt altijd plaats op de inoculatieplek en in een zone er omheen. De temperatuurgrenzen voor dit proces zijn ± 6 °C tot ± 23 °C. Normaliter vindt het sporuleren plaats tussen 2 en 3 uur 's morgens; vanaf 9 uur de voorgaande avond moeten de bladeren dan met een waterfilm zijn bedekt. Ontstaat de waterfilm op een later tijdstip, dan heeft sporulatie ook later plaats. Worden de planten na de nacht ongeveer één uur weer aan het daglicht bloot gesteld, voordat een waterfilm is gevormd, dan worden op diezelfde dag geen sporendragers met sporen meer gevormd. Sporuleren vindt dan de volgende morgen tussen 2 en 3 uur weer plaats (fig. 9). Bij continue belichting na het ontstaan van de waterfilm treedt echter wel sporulering op, alleen 2 tot 3 uren later, dan in het donker het geval zou zijn geweest (fig. 9,C).

Het aan- of afwezig zijn van een waterfilm op aangetaste bladeren is ook van betekenis voor het optreden van de eerste, voor het oog zichtbare symptomen van de ziekte, en dus op wat algemeen met de term incubatietijd wordt aangeduid. Blijft de luchtvochtigheid vanaf de inoculatie zo hoog, dat een waterfilm op de bladeren aanwezig blijft, dan is het eerste symptoom het optreden van sporendragers met sporen op de boven- en onderzijde van de aangetaste bladeren (Plaat II, A). Onder deze omstandigheden is er een nauw verband tussen de lengte van de incubatietijd en de temperatuur (fig. 8). Pas enige dagen na het begin van de sporulatie treedt een gele verkleuring in de bladeren op. Is de luchtvochtigheid zodanig, dat bij de heersende temperatuur geen waterfilm op de aangetaste bladeren kan worden gevormd, dan verkleuren de bladeren pas na verloop van enige weken en zij verdrogen langzamerhand. In een vergevorderd stadium van verdroging kunnen nog sporendragers met sporen worden gevormd, als de omstandigheden daartoe gunstig worden. Onder deze omstandigheden is de incubatietijd dus de tijd die verloopt tussen de inoculatie en het optreden van de eerste gele verkleuringen. Bij ± 5 °C is de incubatietijd nu ongeveer 5 tot 7 weken; bij ± 10 °C tot ± 15 °C ongeveer 3 tot 5 weken; en bij 18 °–22 °C ongeveer 2 tot 3 weken. Verdampt al het water van een blad, waarop de schimmel plaatselijk sporuleert, dan drogen die plekken geheel in, worden necrotisch en bijna doorzichtig (Plaat 1, B). Om de necrotische plek heen kan na verloop van enige tijd een verkleuring zichtbaar worden.

Worden bij jonge planten meer dan de helft van het aantal bladeren met een sporensuspensie van *Bremia lactucae* geïnoculeerd, dan is 4 tot 5 dagen na de inoculatie een groeistagnatie merkbaar bij die planten. Enkele dagen daarna kunnen sporendragers met sporen op die bladeren ontstaan; of kan een verkleuring optreden. Als de tijd, door de kwekers waargenomen tussen het merkbaar worden van een groeistagnatie en het optreden van sporendragers met sporen of van verkleuringen binnen de incubatietijd bij voortdurend hoge luchtvochtigheid ligt, is het duidelijk dat de groeistagnatie het gevolg is van een *Bremia lactucae* aantasting.

De conidiën van *Bremia lactucae* zijn goed tegen uitdrogen bestand, vooral bij lagere temperaturen. Resultaten van proeven over de invloed van verschillende relatieve luchtvochtigheden bij 2 °C, 14 °C en 21 °C zijn weergegeven in fig. 10 en 11. Het behoud van de kiemkracht is ook groot onder meer op de praktijk gelijkende omstandigheden. Bij lagere temperatuur en „luchtdroog" bewaard

kunnen de conidiën 140 dagen kiemkrachtig blijven. Bij hogere temperaturen gaat de kiemkracht spoedig verloren (fig. 12 en 13).

Van juli 1958 tot juli 1959 werden symptomen van *Bremia lactucae* aantastingen in de praktijk geregeld waargenomen. Alleen in de maand juni en de eerste helft van juli 1958 was dit niet het geval. Onder de omstandigheden, zoals die in deze periode heersen (tabel 2) is de incubatietijd ongeveer 2 tot 3 weken. Daarna duurt het nog minimaal 3 tot 4 weken voordat de aangetaste bladeren geheel zijn verdroogd. Tot op dat moment is sporuleren nog mogelijk. Een dergelijke droge, warme periode kan de schimmel dus overbruggen in de vorm van mycelium in het blad. Ook in de maand februari 1959 werden geen symptomen van de ziekte in de praktijk waargenomen. Onder deze omstandigheden (tabel 2), is de lengte van de incubatietijd bij voortdurend hoge relatieve luchtvochtigheid 4 tot 5 weken, bij niet voortdurend hoge relatieve luchtvochtigheid langer. Ook een dergelijke periode kan de schimmel dus gemakkelijk overbruggen. Zaadovergang moet uitgesloten worden geacht en overblijven door middel van oösporen lijkt onwaarschijnlijk, omdat deze niet gevonden zijn.
De verspreiding van *Bremia lactucae* vindt plaats door middel van de conidiën.

Uit de verkregen resultaten blijkt duidelijk, waarom nu juist bij de teelt van „herfstsla" *Bremia lactucae* zich zo sterk kan uitbreiden. Vooral in de beginstadia van ontwikkeling der planten zijn de uitwendige omstandigheden voor ontkiemen, binnendringen, myceliumuitbreiding en sporuleren meestal gunstig (tabel 6). Zijn aangetaste planten in een gewas aanwezig, dan kan zeer gemakkelijk een uitbreiding van de ziekte ontstaan, omdat er altijd water tussen de bladeren aanwezig blijft als de planten zover zijn uitgegroeid, dat zij elkaar raken. Aan een belangrijke voorwaarde voor uitbreiding van de parasiet is dus voldaan. De relatieve luchtvochtigheid speelt ook bij de teelt van „broeisla" een rol. Hier moeten infecties bij de jonge plantjes zijn opgetreden, als tegen het oogstbaar zijn van het gewas aantastingen van *Bremia lactucae* worden gevonden. Tijdens de eerste ontwikkeling der planten, in de maand december, zijn de uitwendige omstandigheden voor het optreden van infecties, in ieder geval periodiek gunstig. Wel is niet alleen de groeisnelheid van de planten gering, ook de schimmel ontwikkelt zich slechts langzaam. Bij de lage temperatuur en niet hoge relatieve luchtvochtigheid kan het 6 tot 8 weken duren, voordat het uitwendig zichtbaar wordt, dat planten zijn aangetast. En juist na deze periode vindt het zogenaamde „broeien" plaats, waardoor de temperatuur en de relatieve luchtvochtigheid hoger worden, de laatste zelfs zeer hoog. De ontwikkeling van de parasiet wordt dan zeer begunstigd.

Uit de verrichte proeven om het waardplantenspectrum en de fysiologische specialisatie van *Bremia lactucae* te bepalen, kan niet geconcludeerd worden dat er binnen de forma specialis van *Bremia lactucae* voorkomend op *Lactuca sativa* var. *capitata* fysiologische rassen voorkomen. Een aantal *Lactuca* spp. en variëteiten van *L. sativa* L. blijken in hoge mate onvatbaar voor deze schimmel. Bij deze species en variëteiten kan de schimmel wel binnendringen, maar er ontwikkelen zich meestal geen hyfen. Als dit wel gebeurt blijven zij beperkt in doorsnee en beperkt in groei, terwijl aan deze hyfen geen haustoria gevormd worden (Plaat IV, B). Deze speciës en variëteiten kunnen als geniteur voor handelsvariëteiten gebruikt worden.

De groeiremmingen die optreden bij jonge slaplanten als deze ernstig door *Bremia lactucae* worden aangetast hebben een ongelijke stand van het gewas tot gevolg en daardoor een ongelijk oogstbaar zijn van de planten. Om de hierdoor ontstane oogstderving in cijfers te kunnen uitdrukken werden bij een gewas „herfstsla" dergelijke groeistagnaties kunstmatig opgewekt, door de planten in verschillende mate ziek te maken. Dit gebeurde door 1, 2 of 3 van de 3 tot 4 ontwikkelde bladeren van de planten te inoculeren. De resultaten van deze proef zijn weergegeven in tabel 8.

Om een goed bestrijdingsmiddel voor *Bremia lactucae* te vinden werden diverse fungiciden eerst op kleine schaal beproefd onder optimale omstandigheden voor de ontwikkeling van de schimmel. Ook de eventueel kiemremmende werking van de middelen in vitro werd nagegaan. De resultaten hiervan zijn weergegeven in tabel 9. Met enkele middelen werd daarna een grotere bestrijdingsproef uitgevoerd bij een gewas „herfstsla" (tabel 10). Uit beide tabellen blijkt, dat de beoordelingen, zoals deze in de oriënterende proeven zijn verricht, een betrouwbare indruk kunnen geven van de werkzaamheid van een fungicide voor *Bremia lactucae*. De remmende invloed van de middelen op de sporekieming in vitro is hiervoor niet geschikt gebleken. Hoewel het middel B 56 een iets betere werking vertoonde dan zineb in deze proef, moet dit laatste middel voor de praktijk worden geadviseerd, daar van B 56 nog niets bekend is omtrent nevenwerkingen. Omdat zineb alleen preventief werkzaam is, is het duidelijk, dat met het stuiven van dit middel over de slaplanten in een vroeg stadium van ontwikkeling van de planten moet worden begonnen. Zodra de planten zover zijn uitgegroeid in de kas of in het warenhuis dat zij elkaar raken, heeft stuiven weinig zin meer. Het is dus van groot belang om voor *Bremia lactucae* resistente, of in hoge mate onvatbare variëteiten te kweken. Inmiddels is met het inkruisen van de resistentie afkomstig van *Lactuca serriola L.* en enkele andere in hoge mate onvatbare *L. sativa* variëteiten in bestaande handelsvariëteiten een begin gemaakt. Het zal echter een aantal jaren duren, eer een voor de praktijk bruikbare variëteit zal zijn verkregen. Misschien biedt het uit Amerika ontvangen materiaal mogelijkheden. Men is daar reeds sinds 1932 met het kruisingswerk bezig. Ook in dit materiaal is de resistentie, waarschijnlijk berustend op één enkel dominant gen, afkomstig van *L. serriola.*

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A

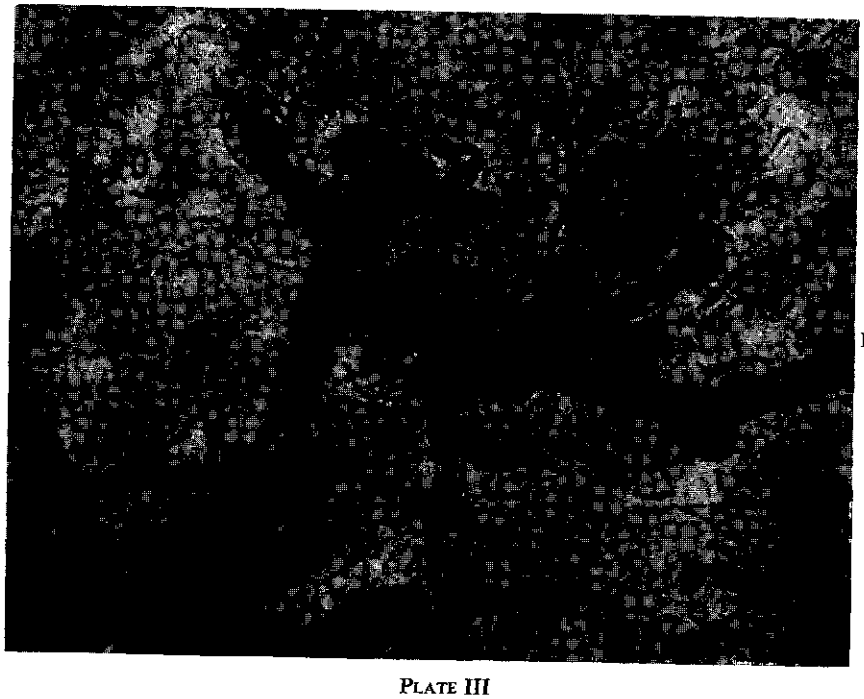
B

PLATE II

A. Production of conidiophores by *Bremia lactucae* on both sides of inoculated leaves.
Vorming van sporendragers door Bremia lactucae *op beide zijden van geïnoculeerde bladeren.*

B. Growth inhibition caused by a *Bremia* attack; on the right a plant of which 2 cotyledons and 1 leaf are infected; on the left a healthy one.
Groeiremming ten gevolge van een Bremia *aantasting; rechts een plant waarvan de 2 cotylen en 1 blad zijn aangetast, links een gezonde plant.*

A



B

PLATE III

A. Appressorium, vesicle formed by the infection hypha within the epidermal cell, and development of the first hyphae.
Appressorium, uitstulping van de infectiehyfe in de epidermiscel en ontwikkeling van de eerste hyfen.

B. Mycelium in the intercellular spaces and in the substomatal cavities, seen from above.
Mycelium in de intercellulairen en in de ademholten, van boven gezien.

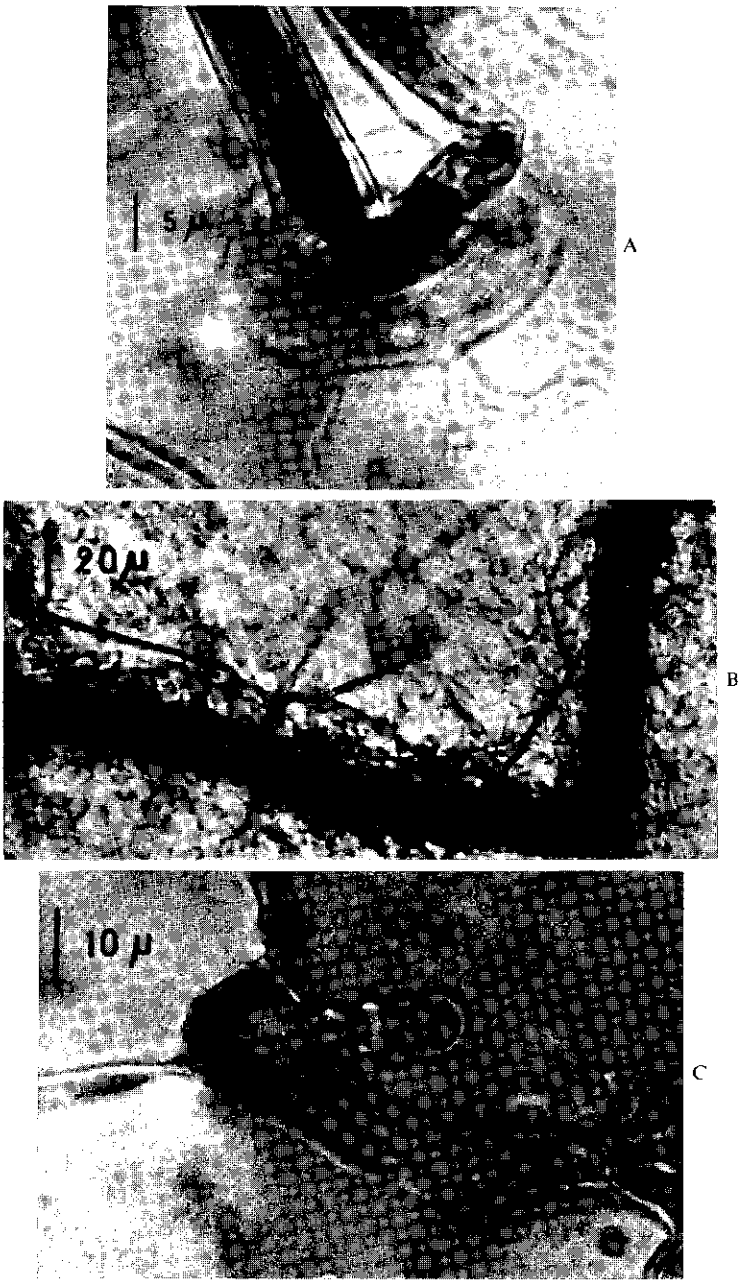


PLATE IV

A. The lower part of two conidiophores which have passed through a stoma.
De bases van twee conidiophoren, die via een huidmondje naar buiten komen.

B. Sparingly developed hyphae in the leaf of the resistant lettuce variety "Grand Rapids" belonging to *L. sativa* var. *longifolia* Lam.
Spaarzaam ontwikkelde hyfen in het blad van de resistente sla-variëteit „Grand Rapids", behorend tot L. sativa *var.* longifolia *Lam.*

C. Cross section through an inoculated leaf with a hypha filling the intercellular space; haustorium penetrated into a parenchyma cell.
Dwarsdoorsnede door een geïnoculeerd blad met een hyfe, die de gehele intercellulaire ruimte opvult en een haustorium in een parenchymcel.