TRANSLATION NO. 33

DATE: July 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

#33

(In Russian)

N. Novotelnova

INFLUENCE OF TEMPERATURE AND HUMIDITY ON GERMINATION OF PHYTOPHTHORA INFESTANS CONIDIA

Plant Protection 11/2; 79-88, 1937

Since the time of the initial study of Phytophthora infestans De Bary (1861) the influence of temperature and air humidity on germination of conidia was recorded (Jones, Giddings and Lutman, 1912; Melhus, 1915; Murphy, 1922 and others).

There are few data in regard to the influence of air humidity; they are based mostly on observations. Experimental data on the influence of temperature are known more or less in general.[1]

In order to develop the prognosis indicators of development of Phytophthora infestans, the purpose of this work was to study the peculiarities of spore germination and to establish cardinal points of temperature and humidity.

Methods. We kept the culture of Phytophthora infestans, isolated from potato leaves, for a year on fresh potato slices. All the experiments were conducted with a 7-day culture. Conidia from the potato slices were transferred with a platinum needle to a hanging drop in a moist chamber (depression slide). Water from the tap was taken, but it was heated up to required temperature before the experiment.

Experiments on spore germination under different temperatures were conducted in Kiuster's multi-incubators; for continuous observations Leitz's apparatus "Nuttle" proved extremely convenient, it is an electric water-bath incubator with a glass front wall.[2] Inside the "Nuttle" is placed a microscope, the upper part of its tube emerging. Such an apparatus enables continuous observation of the spores under a microscope at any set temperature. In experiments on the influence of air humidity the spores were kept in dessicators with various concentrations of sulfuric acid. Caluclations were made according to the Regnault[3] scale. The following variants of relative air humidity were tested: 100, 83, 62, 48, 34 and 21%.

---

[1] After the completion of this work, a voluminous work by Crosier (1934) appeared in the press, in which, with sufficient fullness, is presented the problem of the influence of meteorological factors on germination of spores Phytophthora infestans.

[2] We want to use this opportunity to express our thanks to the director of the National roentgenological-radiological and cancer institute, Prof. Nemenov, for the permission to use this apparatus.

[3] Landolf-Bornstein, Physikalisch-chemische Tabellen.

Similar to the procedure during the experiments on the influence of temperature, conidia were transferred to cover glasses, not in a water drop but on dry surface. Then these cover glasses were placed, on special supports inside the desiccators, drops of water were deposited on the conidia and the glasses which were examined under the microscope were placed on depression slides, after which the moist chambers were placed in the multi-incubator at an 18° temperature.

All the tests continued for 48 hours, because preliminary experiments showed that if, during 48 hours, the spores did not germinate, further changes do not take place; besides, bacteria develop later on which upset observations. The germination percentage was calculated on the basis of a microscopic examination of not less than a hundred spores, but usually their number was considerably higher. Characteristic stages were drawn with the help of Leitz's drawing ocular (camera Lucida?).

1. Influence of temperature on the manner of germination of conidia

We studied, in our experiments, temperatures from 0 to 35°C. in 1-2° intervals. Drawings of peculiarities in spore germination observed under conditions of various temperatures are given in fig. 1.

Fig. 1. Influence of temperature on manner of germination of spores P. infestans.

The following types of germination of conidia were observed: (1) forming of germ tubes (so-called direct germination); (2) originating of zoo-spores (the so-called indirect germination); (3) the so-called combined germination which was observed within higher temperatures. The first type of germination was observed within the 4-30°C. range. The second, within the 6-21°C. range. Crosier indicates a lower temperature minimum -3°.

The formation of zoo-spores took place as follows: at first the contents of conidia is divided mostly in 6 parts after which awhile emerge, moving with the help of flagella; then they stop, become round, grow in size and germinate. At low temperatures, such as 6-8°, the zoo-spores, as a rule, germinated inside conidia and then their germ tubes emerged from the tip of the conidia as well as from any point of its wall. In one case, when at a low temperature, condia germinated to form zoo-spores, the following phenomenon was observed: on zoo-spores were formed short germ tubes with a swelling at the end similar to secondary zoo-spores. Being transferred to more favorable temperature, the "secondary zoo-spores" germinated and the germ tube reached normal size.

The third manner of germination is the so-called combined germination. The germ tube which is formed at the beginning does not develop later on, it produces a thickening which changes then into a secondary conidium; the latter can germinate like an ordinary conidium, producing a shoot or zoo-spores.

The secondary conidia usually differ morphologically from the normal ones by larger size and a more distinctly papilla; but generally speaking their form is highly variable. As a rule they are formed - and that in a large quantity - at temperatures of 24° to 30°C., but single instances were sometimes observed at lower

temperatures as well. The higher the temperature, the longer the time of their originating, and at low temperatures this period decreases. Thus at 30°C. secondary conidia are disclosed after 72 hours, and sometimes even after 96 hours. At 26° and 24° they appear after 48 and sometimes even 24 hours.

There are hypotheses that secondary conidia represent a resting stage, which appears under the influence of unfavorable conditions. Blackwell and Waterhouse observed that during early development stages the secondary conidia can produce a germ tube and zoo-spores, but in dried-out media with inadequate nutrient substances, they exist for weeks and months forming chains of conidia which gradually diminish in size. In the old artificial culture on potato agar (20 days old and older) we also used to find secondary conidia but, according to drawings which we have, they have little similarity to the secondary conidia obtained in water.

Murphy observed secondary conidia only in water when oxygen was gradually decreasing, and he calls them hydro-conidia. De Bary (1863) indicates that secondary conidia are formed only on the surface of a water drop, but if they are put into water, a normal production of zoo-spores begins.

Szymanek (1927) refers the formation of secondary conidia to anomalies of direct germination. The author finds that secondary conidia discovered, after 4 months, during the study in a drop of glucose in Van-Tegem cell, were absolutely identical with bodies which he found in infected tissues of potatoes and that they are the resting stage of the fungus.

At a 26-28° temperature, the secondary conidia germinate in 48 hours and produce tertiary conidia, which in turn germinate into germ tubes at the end of which thickenings may be formed and they are then transformed into conidia. Due to the fact that the secondary conidia being formed have walls no thicker than those of normal conidia, that no formation of additional nutrient substances is observed in them and, finally, due to just as rapid a formation in them of zoo-spores as in normal conidia—we have no foundation to refer the secondary conidia to the resting stage of the fungus. In order to solve this problem definitely, it is necessary to study the viability of secondary and tertiary conidia during a longer period of time.

2. Critical points of temperature in spore germination

The type of germination into germ tubes has a small range of temperatures, with the lower point at 4°C. and a germination percentage at 9.2.

(p. 82) Fig. 2 (Caption) Influence of temperature on germination percentage of spores of P. infestans.

The higher the temperature, the higher the germination percentage, which reaches its maximum at a 10-15° temperature (100%). This is an optimum for formation of zoo-spores. Still higher temperatures begin to depress formation of zoo-spores and the germination percentage decreases.

The low germination percentage at 10-20° can be explained by the fact that the formation of zoo-spores is already depressed and the germination into tubes only begins to prevail. But at 21° the germination percentage begins to increase again due to formation of germ tubes until it reaches the second optimum at 25° (93%). This temperature is optimal for formation of germ tubes. Between 25-30° a new decrease in germination percentage takes place and at 32° there is no germination.

The percentage of zoo-spore germination was not recorded because it is difficult to distinguish in the mass the non-germinated zoo-spores; usually none of them germinate. 21° is a maximum temperature for formation but not germination of zoo-spores, because their germination takes place at 30°C. Crosier (1934) mentions a slightly lower temperature at which germination was still noticed—28°. It is necessary to emphasize that a 100% germination takes place at temperatures optimal for formation of zoo-spores (10-15°), while the formation of germ tubes never reaches 100%.

The results which we obtained do not coincide with data by Jones, Giddings and Lutman, who indicate 10-20° as an optimal temperature for formation of zoo-spores and do coincide with the optimum which they established for germ tubes (about 25°). Crosier also sets the optimum at 24°. Agreement is noted with data by Melhus and Murphy whose optimum temperature for formation of zoo-spores is 10-13° and 10-15°, according to Crosier—13°. Melhus considers the optimal temperature for formation of germ tubes to be 22-23°, i.e., lower than that obtained in air tests. The minimum temperature for zoo-spores is indicated as 5-6°, for germ tubes—above 6°; we observed an opposite phenomenon.

Germination of conidia into zoo-spores took place in a much shorter time period than germination into germ tubes; thus only one hour is needed for formation of zoo-spores at an optimum 15° temperature, while 5 hours are needed for formation of a germ tube at an optimal temperature. This is what Melhus writes about it: "The time necessary for germination varies in relation to temperature, namely because to produce a germ tube more energy is needed than to produce zoo-spores. In the experiments of the author, at a 12-13° temperature, the observed germination time was 1-8 hours. The originating of zoo-spores is connected with the maturity of conidia. In our tests zoo-spores were formed only in young cultures, not older than 7 days. Melhus maintains, also, that young spores produce zoo-spores more frequently and germinate into germ tubes less frequently.

Caption of fig. 3. Dependence of the length of the germ tube of P. infestans on temperature. (p. 83)

The length of the germ tube also depends on temperature. They are longest at 22°C. (813 microns) which coincides with the highest termination percentage as indicated above at corresponding temperatures. The connection between the germination percentage and the length of the germ tube can be established in older (9-days old) cultures. In these cases the germination percentage and the greatest length of the tubes are approximately at the same temperature (23°). And in cases where there is germination into zoo-spores, the highest percentage of germination cannot possibly coincide with the greatest length of the tube. The obtained data are confirmed by Crosier: rapid lengthening of the germ tubes is observed at 21-24° and the highest germination percentage—at 21-26°.

In the light of the obtained results we come to the conclusion that in the methods of recording germination of conidia Phytophthora infestans, the germination should be judged according to the germination percentage and not the speed of elongation of the germ tube. The criterion of germination percentage is conditioned by biological peculiarities of this fungus.

The results of the experiment in regard to the manner of spore germination and to cardinal points of temperature are given in table 1.

Table 1 (p. 83)

Manners of germination	Temperatures - Celsius			
	Limits	Optimum	Maximum	Minimum
Formation of zoo-spores	6-21	10-15	20	6
Formation of shoots (tubes)	4-30	25	30	4
Formation of secondary conidia	24-30	24-28	30	24

3. Change of temperatures.

The following test was conducted in order to clarify the effect of temperatures--which are beyond the critical ones--on spores; whether they destroy the latter's viability or only inhibit the process of their germination. For this purpose the spores were kept a certain time at high temperatures and then were transferred to optimal temperatures. Below is given a table with results of the change of temperature from 35 to 18° (table 2).

Table 2 (p. 84)

Effect of super-maximum temperatures on conidia

Temperature-Centigrade	Time of exposure	% of germination of conidia	Length of germ tubes in microns
35	24	0	0
35	4	93	99,8
35	1	100	168,0
35	30 (min?)	77,7	197,1

It was brought out that a 24-hour exposure to a 35° temperature caused destruction of conidia, while a 4-hour exposure to the same temperature was even stimulating for germination the sense of increase of germination percentage. Blackwell and Waterhouse (1931) state a hypothesis that heating accelerates the maturing of conidia. One hour of heating strongly stimulates germination, while 30 minutes are insufficient for stimulation. Effect of high temperature of short duration is often not lethal, while a longer exposure at considerably lower temperature destroys conidia.

When transferred, after 48 hours, from 32° to 13°, a formation of secondary conidia (after 48 hours) was observed. At high temperatures, in the majority of cases a side germination of conidia took place. Secondary conidia obtained at 26° formed zoo-spores after a transfer to 15°, similar to the normal conidia. When the temperature was alternating from 20 to 30°, secondary conidia were also obtained. Zoo-spores obtained at 9°, when transferred to 35° (24 hours), stopped moving and did not germinated. Neither did the zoo-spores germinate after the return transfer to a 9° temperature. At a low temperature of 1-2° during 48 hours, germination is lacking, but with a change of temperature to 10°, conidia germinate in a direct and indirect manner. Thus it appears that sub-minimum temperature is more readily endured by conidia than the supra-maximum temperature.

4. Influence of air humidity.

The spores of Peronospora are particularly sensitive to air humidity. Doran's experiments with Peronospora viticola, Ravaz and Verge's (1912)—with Plasmopora viticola and Aren's with the same organism, confirm the high sensitivity of these organisms to air humidity. According to McAlpine (1910), drying out of conidia, even for a very short period, completely destroys their power to germinate.

Melhus does not indicate the amount of air humidity but he says that conidia did not germinate after a 6-hour stay in the laboratory.

Lounis (?) points out that conidia do not survive when the drying out process is fast.

In our experiments conidia do not germinate without water even at a 100% air humidity. The next problem was that of the effect produced by various percentages of air humidity on conidia which are in a dry state. We tested their viability, or its loss, by placing conidia in water after a certain exposure to various air humidities. At the beginning of the experiment a 24-hour exposure was chosen. But since the first tests already showed that at this procedure the spores lost completely their viability, it was necessary to change to shorter exposures of 6, 4 and 2 hours. Later on it became clear that even these exposure periods are destructive to conidia. Only tests with a half-hour exposure at a 100% humidity gave positive results, i.e., subsequent addition of water resulted in a small percent of germination of conidia. Therefore conidia of Phytophthora infestans are extremely non-resistant and lose very rapidly their viability when they fall from the conidia-bearers. These results coincide with results obtained by many researchers who observed the influence of dry air on spores of perenosporaceae. Discrepancy is found with experiments by Crosier. The author indicates that at 20-40% air humidity the conidia lost their viability after 1-3 hours and in humid air (50-80%)—after 5-15 hours.

5. Age of culture.

Numerous studies of spore germination among Perenosporaceae indicate that the younger the conidia the greater their power of germination. Thus Rosenbaum (1917)

observed the formation of zoo-spores of Phytophthora cactorum only in very young cultures. Uppal (1926) obtained zoo-spores of Phytophthora colocasiae in cultures not older than 7 days. According to Ashby (1920) in Phytophthora palmivora the percentage of zoo-spore formation decreases with the increase of age of culture. Zattler (1931) writes on Pseudoperenospora humuli that the higher the age of spores the more they deviate from the optimum in their germination. Blackwell and Waterhouse (1931), on the basis of their observations of Phytophthora colocasiae and Phytophthora cactorum, when zoo-spores were formed in eight- and ten-day culture (and not in twelve-day ones), attribute the cause of such a phenomenon to deficiency in oxygen and accumulation of products of metabolism as factors accompanying a greater age.

Doran (1922) also explains the inhibition in the germination process by oxygen deficiency. However, there is more than one opinion on the influence of oxygen on germination. Murphy (1922) and Wals (1868) maintain that oxygen stimulates formation of zoo-spores and according to Uppal oxygen is necessary to direct germination and not for formation of zoo-spores. According to Uppal's conclusions, the process of zoo-spores formation is only a reconstruction of protoplasm, while the formation of the germ tube requires energy and represents growth.

In observing spore germination we frequently came across ~~the phenomenon~~ that under similar conditions conidia germinated either in larger numbers or singly and the difference concerned the length of the shoots (?) as well. We contributed such variations to difference in the degree of maturity of spores. For this purpose experiments were carried out on germination of spores of 7- and 9-day cultures. The germination percentage in a 9-day culture lagged considerably behind the germination of conidia of a 7-day culture and the germ tubes were considerably shorter. It is interesting to note, that in a 9-day culture there was usually no formation of zoo-spores. In this regard there is a complete analogy with the results obtained by Rosenbaum with Phytophthora cactorum, by Uppal with Phytophthora colocasiae and Blackwell and Waterhouse with the same fungi. The more dubious seems the fact that DeBary obtained zoo-spores from conidia Phytophthora infestans, which persisted on slowly drying out leaves of the plant-host during three weeks after maturing. It is more probable that on these leaves continued formation of new conidia which produced zoo-spores.

SUMMARY (published in English on p. 38)

. . . . . .

In conclusion I consider it my duty to express my appreciation to N. A. Naumova for constant guidance and advice during the work process and to Prof. N. A. Naumova for valuable direction and criticism.

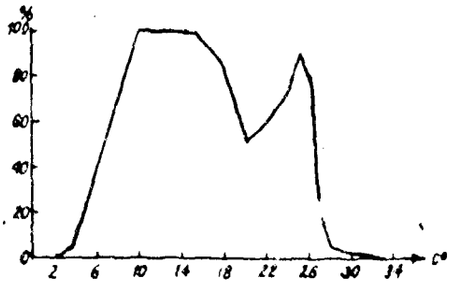

Fig. 2

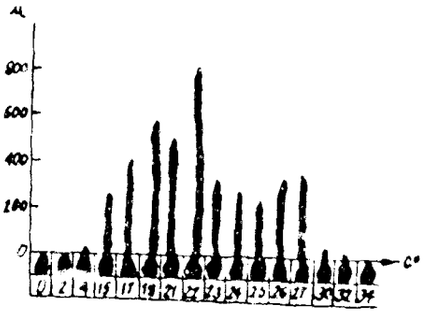

Fig. 3

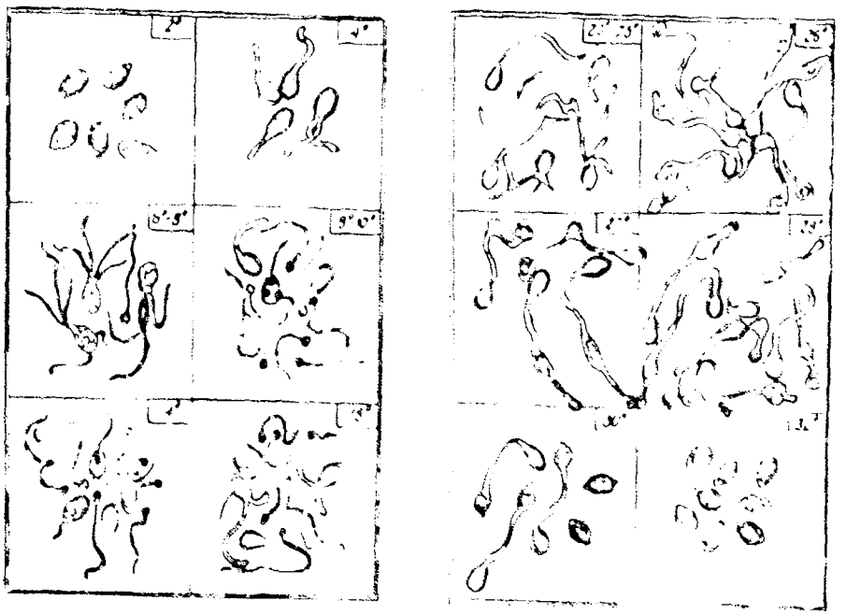

Fig. 1. Influence of temperature on manner of germination of spores P. infestans.