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AN ANALYSIS OF THE VARIABILITY OF THE GERMINATIVE POWER OF CONIDIA IN A NUMBER OF FUNGI BELONGING TO THE PERONOSPORALES[1]

Een analyse van de kiemkrachtvariabiliteit der conidiën bij een aantal schimmels, behorende tot de Peronosporales

BY

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Many research workers have at one time or another carried out investigations into the influence of the environment upon the germinative power of conidia in the Peronosporales. In reading their papers the statement is frequently encountered that the sporal material examined is extremely variable in its germinative capacity. Results concerning the influence of external factors are not easily reproduced; they are often contradictory. Single series of observations or repetitions in which the same systematic error has been assimilated easily give rise to wrong conclusions. On several occasions this may have happened. It is not impossible that the denounced *variability* of sporal material is also, at least partly, responsible for the fact that the radiation responses of several Peronosporales, *inter alia* the potato blight fungus, remained undiscovered. They were described only recently by DE WEILLE (1961, *Peronospora arborescens;* 1963, *Phytophthora infestans*).

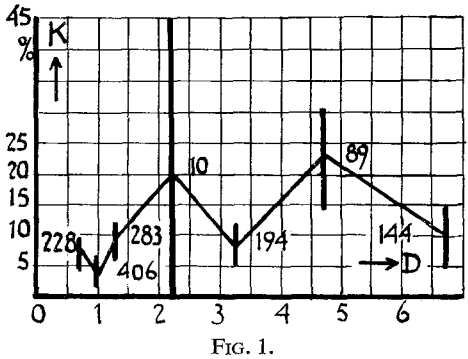

FIG. 1.

Peronospora viciae. U.v. irradiation results. Intensity i = 40 000 ergmm^{-2}h^{-1}; $i_{max.}$ at λ = 3300 Å. Numbers within quadrant reflect sample size n. Vertical bars indicate local width $4|s_K|$ of confidence belt. K = germinative power in per cent. D = duration of irradiation in hours.

Peronospora viciae. *Resultaten van u.v. bestraling. Intensiteit i = 40.000 ergmm^{-2}h^{-1}; $i_{max.}$ bij λ = 3300 Å. Getallen binnen het kwadrant duiden monstergrootte n aan. Staande strepen geven de plaatselijke breedte $4|s_K|$ van de betrouwbaarheidsband weer.* K = *kiemkracht in %.* D = *bestralingsduur in uren (h).*

Fig. 1 may demonstrate how real variability or seeming variability, caused by systematic errors, may conceal any real environmental effect. The graph sets out the results obtained by exposing conidia of *Peronospora viciae* to weak ultra-violet (u.v.) irradiation. They conflict with the inactivation curve published previously (DE WEILLE, 1961, *Peronospora arborescens*), according to which activation by small dosages of u.v. energy is followed by inactivation by greater doses. From a first glance at fig. 1 it might be assumed that, at best, the above inactivation curve apparently does not hold good for *P. viciae*. Yet is does.

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This article may make clear that the pictured "voluntary" result is entirely attributable to systematic errors.

Looking back at the experiment of fig. 1, it can be stated that nearly all possible errors were present.

Within the scope of agrometeorological research into the relationship between plant disease and weather, the author carefully investigates the influence exerted by each individual environmental factor (i.e. radiation, temperature (T) and humidity) upon the germinative power (K) of the conidia in a number of disease organisms, viz. *Phytophthora infestans* and some Peronosporaceae. The knowledge thus obtained is of importance for the interpretation of field results. Though there is a great deal of literature on these subjects, this does not deal with *radiant energy*. Moreover tentative research concerning the influence of relative (atmospheric) humidity (r.h.) has led to results which conflict with those published earlier, *inter alia* by CROSIER (1934, *Phytophthora infestans*).

As to the term *germinative capacity*, the author refers to a previous paper (DE WEILLE, 1961). In principle it is *the percentage of conidia* in a sample of 500 elements, *capable of germination* under optimal conditions. Hence K expresses the *potential* capacity to germinate.

The term *conidium* here embraces all vegetative spores found in the Peronosporales, including the *zoösporangia*, as they are called, of *Phytophthora*.

The term *germination* here denotes both conidial or direct germination and sporangial or indirect germination. It does not include (secondary) germinations of zoöspores.

In laboratory work at the K.N.M.I. the already mentioned variability was encountered. Considerable amplification of statistical material by repeating experiments many times failed to elicit the information about the action of the individual external factors necessary to obtain an insight into the effects of weather.

On closer investigation it became possible to detect the *errors of method* responsible for the greater part of the variability. No doubt other workers have made the errors to be described here just as the author did at the start (fig. 1). As a matter of fact it was possible to avoid those errors in later series of tests. On the basis of the findings it even proved possible to apply an approximative graphical correction to several early series of observation results, thus giving rise to series conformable to analogous uncorrected series obtained with elimination of the errors of method described further on.

In the following pages an analysis of variance will be made, which is biological for the greater part. The variance of the previously published curve of inactivation due to u.v. irradiation (DE WEILLE, 1961) will serve as a starting point since the typical character of that curve had already been verified by sound mathematical evidence. Departures from the inactivation curve will now be examined in order to find their cause.

TEST-SAMPLE SIZE

The influence of the sample size n on the reliability of the observed germination percentages can be approximated in two ways, i.e. statistically and biologically.

Statistical approach

Considered *statistically*, the reliability of a specimen, a sample taken from a universe, is known to increase according as n, the number of its elements, here conidia, increases. Experience has shown that only samples of at least 500 conidia ensure some accuracy, which means that their results K_k resemble those of their duplicate and triplicate, K_k' and K_k'' (DE WEILLE, 1961).

For readers interested in the elementary statistics underlying the above, some further elucidation may be of value.

In germination trials each element has 2 chances, viz. + 1 (germination) or 0 (no germination). In a universe having a germinative power K = 5 per cent (≡ 5%), in our mathematical consideration to be denoted by y = 0.05, a spot-check of 1 element will fail to produce 0.05 for result; in general we will find 0.00 and theoretically in (on average) 1 in 20 cases 1.00. A sample of 2 elements will result in y = 0.00 or 0.50 or 1.00 with great preference for 0.00. Consequently samples of 1 or 2 conidia are worthless. The correct result y = 0.05 will only become possible in a sample of at least 20 elements. In practice, however, this result y = 0.05 is unlikely to be obtained in such small samples. And even if it *is* obtained by chance, repeat samples will show values of y strongly diverging from 0.05. In general we can state that results obtained by using small samples will *not* resemble those of their replicates. This phenomenon is related to the fact that, in a sample of n elements, the standard deviation of the mean (of the so-called internal frequency of the sample) s decreases as n increases, but increases as n decreases. This is shown in fig. 2, for which 3 fixed values of n were chosen. The results for n = 500 are compared with those for samples 3 times larger or 3 times smaller.

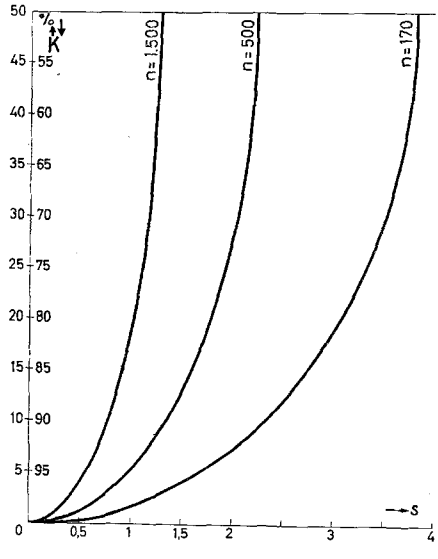

FIG. 2. Relationship between germinative power K, sample size n and standard deviation s.
Verband tussen kiemkracht K, *monstergrootte* n *en standaardafwijking* s.

As was demonstrated in fig. 1, one can, in each point of an inactivation curve y, and determined separately for each observational point, plot 25 upwards or downwards, parallel to the ordinate. Thereafter two additional curves can be drawn, one through the points $y_k + 2\,s_k$ and the other through the points $y_k - 2\,s_k$. The zone between these two new curves (which, of course, contains the original curve y) is called the confidence belt of y (comp. DE WEILLE, 1961, fig. 10). The probability P of transgression of these limiting curves y ± 2 s, the *exceedance probability*, is 5 per cent. At least if a (nearly) binomial dispersion of germinated spores between the ungerminated ones is assumed. If the exceedance probability is so small as to amount to 5% or less ($P \leqslant 0.05$) we call the observation point statistically *reliable*.

As is shown by a good many experiments conducted at the K.N.M.I., a potential germinative level of 5 per cent frequently occurs in *Phytophthora infestans*. In the following discussion we shall assume the *real* K (in the universe) to amount in fact to 5 per cent. If a band width 4 s = 4 per cent is considered sufficient, so that (with a probability of 95 per cent) the *observed* K will lie between 3% and 7% (s = 0.01; y = 0.05 ± 2 s), what size should n have in this case, or in other words: up to what number should counting of conidia be continued?

Filling in the formula $s^2 = y\ (1 - y)/n$, we find $0.0001 = 0.05 \times 0.95/n$, from which it follows that n = 475 (comp. DE WEILLE, 1961, p. 427). The result obtained conforms very well to the experience recorded in the article referred to. It called $n \approx 500$ the significant sample size.

As appears from the formula $s^2 = y(1 - y)/n$, the value of s not only depends on n, but also on the *level* of K. This is also demonstrated in fig. 2, which was composed by reading K and 3 for 3 values of n in an s-nomogram bij TAS (1960). From the curve n = 500 one may learn that, according as K approaches 50%, s as such increases, but that its relative impact upon K — which we can denote by s/K — diminishes.

Any increase of n decreases s. This effect is all the more marked according as K is smaller (fig. 2). At low values of K the ratio s/K attains a high value. We can therefore state that

on statistical grounds there is a good case to reject series of observations in which each individual observation results in a value of $K \leqslant 5\%$, even at n = 500, because *s and K are of the same order of magnitude*. Experience has shown that such series are useless.

Biological approach

Even if the systematical errors still to be discussed are avoided, the *empirical variability* (found in sporal samples of all Peronosporal fungi hitherto examined for the obtainment of knowledge concerning their responses to environmental conditions) still *exceeds what we should expect* mathematically.

Although, for practical reasons, the author retains the adopted sample size of 500 conidia, with which useful results were obtained, mainly owing to manifold repetition of the experiments, the fact remains that the empirical exceedance probability P of the boundary curves y ± 2 s exceeds the theoretical 5%. Counted over 1000 preparations (germinated samples), P appeared to amount to 9%.

This phenomenon is due to untypical observations, so-called "outliers", which could not possibly be sufficiently taken into account when drawing the y-curves and their confidence belts. This might be called a selection effect.

Moreover a sample size of n = 170, which ought to be sufficient at high values of K (see fig. 2) is always dissatisfactory.

In the first instance it was endeavoured to approach this problem *biologically*. In this connexion the author thought of stimulating and inhibiting substances, to which fungal spores are highly sensitive (BROWN, 1946; DE WEILLE, 1961). See also DUGGAR (1901), UPPAL (1924), MCCALLAN et al. (1941) and HORSFALL & RICH (1953).

The untypical observations, disregarded when plotting the y-curves, may possibly be due to the ability of a germinating conidium to stimulate some surrounding conidia also to germinate. Due to this phenomenon, germinated conidia are not equally dispersed in the preparation but are most often found within more or less distinct spheres of germination, outside which only few germinated spores are found. This situation makes n seem smaller than is observed. In statistical terms this phenomenon is called *persistence*. In this case we could rather speak of "contagion". In very recent statistical research this contagion could be reliably demonstrated. This work indicated that in the germination in question an apparent internal inhibition has to be overcome. The persistence found indicates that the assumption that an antagonistic hormone complex (stimulus *vs.* inhibition) is probably involved, is very probably correct.

The original biological approach of the visually observed persistence found was stimulated by a publication by OUINTANILHA (1933), who had been able to show mutual stimulation in germinating spores of *Coprinus fimetarius*.

In the laboratory the germinations to which this article relates, take place in artificial dew (DE WEILLE, 1961). This dew consists of a number of droplets, one part of which contains a number of germinated conidia, while the other part does not. During preparation, at first a partial evaporation prevents the contents of the individual drops from being mixed. Thereafter the conidia from the various droplets are included in one single film of fluid. Thus the unevenness of the germination pattern is more or less maintained.

CONDITIONS OF GERMINATION

Substantial differences between the observed germination percentages may have been caused by unequal conditions under which sporal samples to be compared after exposure to the storage medium, were germinated.

The object slides to which the conidia are applied at the beginning of any storage experiment should be painstakingly cleaned so that inhibiting (heavy metal ions) or stimulating (vaseline) actions are barred. After rinsing, autoclaving is to be preferred to drying with a cloth.

In the experiments germination takes place in cool Petri dishes in which a saturated atmosphere is maintained by the presence of two wet circular sheets of filter paper, one lying on the bottom and one located in the lid, to which it adheres as long as it is sufficiently wet. The slides, with conidia in artificial dew, are placed on the bottom of the dish. The "dew" can be obtained in two manners, either by breathing on the slides in a cold room, so that they fog up, whereafter the condensation droplets remain liquid in the cool Petri dishes, or by means of a plastic atomizer. In the latter case *aqua bidestillata* (distilled in an all-glass apparatus) is used. Care should be taken to ensure that the droplets are small (otherwise lack of oxygen may inhibit germination) and are equal in size and number on all slides.

Drying up of some slides by evaporation in some of the dishes will usually make the test worthless.

Duration

Until a maximum is reached, the number of germinated spores in a sample will go on increasing owing to progressive activation, so that, as long as the medium of germination is maintained, the duration (d) of germination influences the percentage observed.

In *Colletotrichum falcatum* CHOWDHURY (1937) found the maximum ($K_x = 24.1\,\%$) to have been reached after d = 124 hours, in *Cladosporium herbarum* ($K_x = 6.7$ per cent) already after d = 70 hours. The germination of *Phytophthora*- and *Peronospora*-conidia also shows a dependence on the duration. If conidia belonging to one experiment are germinated at different times, d should be equal in all cases. This d can either be standardized at preferably 16 hours (DE WEILLE, 1961) or be made very long, e.g. several days, so that the maxima are reached or at least the mutual differences become relatively insignificant. In the latter case possible influences retarding germination are likely to be completely overcome too, whereas lump preparation of the complete collection of samples becomes possible.

When in the author's experiments two parallel series of preparations were made, only differing in the germinative duration d of their constituent samples, the longer germinated preparations always showed higher percentages provided the generally observed K exceeded 5 per cent. If, in general, the percentages observed were lower than 5 per cent no relationship between d and K could be detected. The explanation of this phenomenon is readily given by fig. 2 and the corresponding formula.

Temperature

MELHUS (1915) and CROSIER (1934) showed that the highest percentage of germination in *Phytophthora infestans* is achieved at a temperature (ϑ) of 11 to 13 °C. As there is no cold-storage room at de Bilt, in which during summer time a constant temperature of 13 °C could be maintained, this item was continually difficult. In case ϑ rises during non-coincident germination periods of a series of samples, the resultant K-values are modified in such a way that they do not directly reflect the storage period (D) before germination.

Mr. YPERLAAN of the K.N.M.I. has devised the following method. At a dark and cool site in a brick shed with windows only having an aspect to the north, water-logged empty flat earthen seed pots are placed upside-down. Upon these are placed the Petri dishes which are cooled by evaporation from the seed pots, thus enabling ϑ to be kept at ca. 14 °C for a fairly long time. Until fairly late summer ϑ can still be kept as low as 16 °C, evaporation compensating modest temperature rises of the environment.

SAMPLING

In connexion with two phenomena still to be elucidated, viz. the process of *maturation* and the subsequent *decline* in germinative capacity, it is of the utmost importance that sporal material on which the influence of environmental factors is studied constitute as much as possible a *homogeneous population* or homogeneous universe (DE WEILLE, 1961, p. 423). Heterogeneity of the sporal populations examined by several authors has undoubtedly often been the cause of the great variability. For being statistically homogeneous the material's elements must have had one and the same "previous history"; out of a homogeneous substratum they must be harvested at the same moment and when being harvested all must be at the same age. The latter characteristic can be ensured by taking care that the substratum, with the mycelium, continuously remains in a medium inhibiting spore formation until the research worker removes it to a medium inducing sporulation (or simply modifies the medium to obtain this effect). Then, without interruption, the substratum remains in the sporulation medium during a period, dependent on the age of the conidia required at the start of the experiment.

At 100% r.h. and under continuous illumination both *Peronospora arborescens*, the downy mildew of the maw-seed poppy (DE WEILLE, 1961), and *Phytophthora infestans* can form conidiophores, but they fail to produce conidia. Under a Philips TL 33 discharge tube the former will already remain sterile at 250 lux, whilst the latter can only produce sporadic conidia at an illumination intensity of 400 lux. After one dark night in an otherwise unchanged medium both the maw-seed poppy leaves and the halved potatoes serving as a substratum for the respective fungi are covered by more or less homogeneous conidial material that can produce marked test results regarding the influence of the environment upon their germinative capacity provided it is harvested at one and the same moment and also further treated without systematic errors.

Light counteracts sporulation in the Peronosporales, at the same time advancing spore formation in the subsequent dark period. This was noticed by YARWOOD (1937, *Pseudoperonospora humuli, Bremia lactucae, Plasmopara viticola, Peronospora destructor;* 1943, *P. destructor*) and corroborated by VAN DOORN (1959, *P. destructor*) and DE WEILLE (1961, *P. arborescens*).

The deniers of the influence of light upon sporulation have always been numerous; we only mention HECKE (1898, *Phytophthora infestans*), CROSIER (1934, id.) and CLAYTON & GAINES (1933, *Peronospora hyoscyami*). From the fact that sporulation can take place in the alternation of night and day as well as in continuous darkness the conclusion was sometimes drawn that sporulation was not associated with light.

THE SUBSTRATUM

The substratum on which conidia develop does not fail to influence their germinative capacity; this influence can hardly be considered irrespective of stimulative, inhibitive or toxic substances present in the substratum. Progressive formation of decay products may cause the germinative capacity of conidia, growing on certain parts of the substratum, to decline rapidly since the action of a poisonous compound on the germinative power of fungal spores is exponentially related to its dosage (MCCALLAN et al., 1941). It is therefore definitely not unimportant whether spores are harvested from a fresh or a rotten leaf, or from a white or a brown potato slice. If such mutually diverging samples are used in conjunction, the results obtained with them will be found to be useless.

Within a strain of *Phytophthora infestans*, cultured on potato tubers, the germinative capacity may increase considerably after its conveyance to leaves. Mixed samples originating from tubers and from leaves cannot be used for one and the same trial.

The author also failed to obtain usable results with sporal *Phytophthora* material obtained from tubers on which, beside the potato blight fungus, a *Penicillium* species had also developed. Then the germinative capacity of *Phytophthora* is so little above nought per cent that series of such samples do not permit conclusions with regard to the environmental factors during a period of storage. This phenomenon takes us back to what was said about toxic substances. In the relevant experiments it was not proved, however, that an antibiotic *is* involved.

At all events samples of detached conidia, used in research into the influence of environmental factors upon the germinative power, must originate from a uniform undecayed substratum in which no organisms occur that may antagonize the fungus under observation.

AGE AND ENVIRONMENT

It has already been stated that sporal samples, to be usable for comparison for the purpose in view, must be of the same age. The situation could also be imagined in which different age classes are present in the same proportion in all samples to be compared. Such material is obtained under a uniform light regime. It is probable that some workers have used such populations, the second kind mentioned, without realizing the implications. If so, it is easy but, again, usually incorrect to take refuge in the concept of variability.

It seems worth while to inspect more closely the germinative power K as a function of age; the medium in which the conidia exist should also be taken into account.

For the present we shall only consider the behaviour of K of conidia *attached to conidiophores* in a *saturated atmosphere*. Loose (detached) conidia will not be discussed in this context.

Maturation

In most experiments conducted in 1960 the procedure was as follows: At (m + 1) successive moments (of time t), viz. $t = t_0$, $t_0 + \Delta t$, $t_0 + 2\,\Delta t$, ..., $t_0 + m\,\Delta t$, samples were taken from one and the same substratum. Every time a sample was harvested it was brought immediately to the storage medium. After an exposure of D_k hours to this medium all slides with conidia were simultaneously taken out of it and conveyed to the medium of germination (described on p. 119). Supposing that the last samples had been put into the storage medium at $\Delta t = \frac{1}{2}$ h before the termination of the storage, the effect studied was that of a period of storage $D = \Delta t, 2\,\Delta t, ..., m\,\Delta t, (m + 1)\,\Delta t$ hours. The advantage of the method adopted was that the circumstances of germination could easily be made uniform, the drawback being the fact that the condition of harvesting all samples at one and the same moment was not satisfied.

The curves for the germinative results K per series turned out to differ greatly in characteristics. This leads to the assumption of either a kind of super-variability or at least one error of method. Since all actions were performed very hygienically some fundamental fault was assumed. It might be hidden in the ages of the compared samples, since the average age of the conidia in the last samples taken (and shortly thereafter put into the storage chambers) exceeded by m half hours that of those harvested first, age here following both t and D, but the latter quantity in the reverse direction.

About *Plasmopara viticola* ISTVÁNFFI & PÁLINKÁS (1913) had stated that only $1\frac{1}{2}$ days after their formation the majority of the conidia are mature enough to infect the crop.

In the agricultural laboratory of the K.N.M.I. the author subjected conidial material of *Phytophthora infestans* to maturation experiments. In this context *maturation or ripening is the phenomenon that the germinative power* (K) *increases with time.*

The rate of change of K with time is called "maturation speed" and is expressed in differential form by $\frac{dK}{dt}$.

At various moments (t_k) sporal samples were brushed off their substratum. They constituted a young homogeneous population, formed in the night before the start of the tests (t_0 = 8 a.m.). Without previous storage they were germinated at a fairly constant temperature (ϑ = 14–16 °C) and for a long duration (d = ca. 48 h.) in order to smooth out possible mutual differences in germinative circumstances. The results are given in fig. 3. Curve a represents the means of 5 closely related lines. In later tests the high percentages of germination obtained with this particular plant material were very rarely observed again. Graph 3 therefore also shows curve b, which is partly based on one series of very reliable observations. The course of b from t = 8 to t = 18 is the outcome of a great number of series. Some other maturation curves lay between a and b, usually nearer to b than to a.

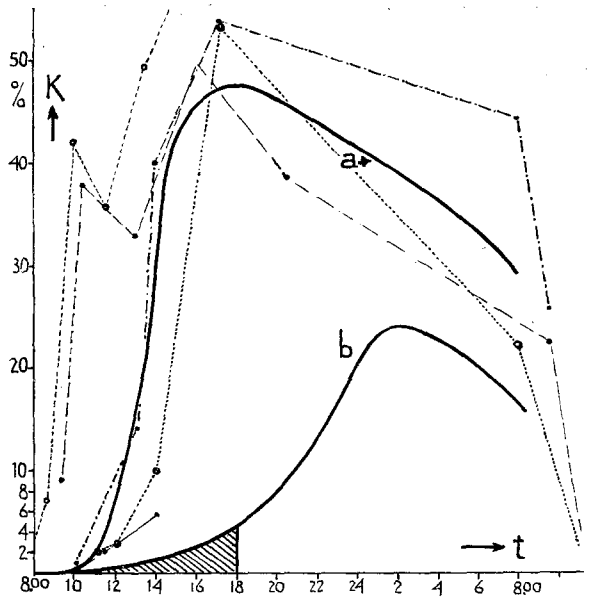

FIG. 3. *Phytophthora infestans.* K as a function of time t (C.E.T.) during a normal summer day, while the r.h. is constant at 100 per cent; conidia *attached* to sporophores. Ancillary lines indicate series of data on which a is based. Highest value of s among these data amounts to 2.3. Two lines at extreme left are based on slightly heterogeneous sporal populations; others on homogeneous universa.

Phytophthora infestans. K *als functie van tijd* t *(M.E.T.) gedurende gewone zomerdag, terwijl de r.v. steeds 100% bedraagt; conidiën* vastzittend *aan dragers. Hulplijnen geven reeksen gegevens weer die aan* a *ten grondslag liggen. Hoogste waarde van s bij deze gegevens bedraagt 2,3. Twee lijnen geheel links kwamen voort uit enigszins inhomogene sporenpopulaties, andere uit homogene universa.*

From a and b we must conclude that if (while investigating the influence of the medium upon loose conidia) the easy way is adopted of constantly putting freshly harvested sporal samples into the climatic cells and finally taking one control sample (D = 0), it incorrectly appears that a wise course has been followed. In the type of climatic chambers used it is easier to add new slides than to take slides out during the experiment; uniform conditions of germination cause too much of a headache; no preparation work is needed outside normal working hours: all material is stained and fixed *en bloc.* In actual fact, however, the complication will inevitably arise that the maturation process is uncontrollably involved in the results. A control sample should therefore in fact be taken every time a sample is harvested; the control sample is then germinated immediately. However, all advantages of the gradual harvesting method are then lost in any case.

Let us assume that in a test series maturation occurs according to b and that the longest exposure to the storage medium (which is, of course, equal to the whole duration of the experimental storage) lasts from 8 to 18 h. C.E.T. (= Central European Time). We now make a series of systematical errors, increas-

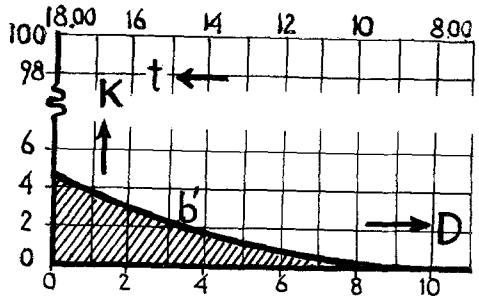

FIG. 4. Singular systematic error; for explanation see text.
Enkelvoudige stelselmatige fout; voor verklaring zie tekst.

ing during the interval mentioned according to the curve segment above the shaded area in fig. 3. These errors will be concealed in the results of the storage trial. Let us consider how this comes about. The control sample taken at t = 18 h. has been exposed to storage during D = 0 hours. In the same way t = 16.00 corresponds to D = 2 and so on, until D = 10 for samples entered at t = 8.00. Thus the positive direction of D along the abscissa is the reverse of that of t, so that the systematic error in any curve representing storage influences relative to K, will give rise to a subtraction of the shaded part of fig. 4, the progressive effect of the error being b'. This b' in fig. 4 is nothing else than the reflected image of b in fig. 3. In a way b' takes the place of the abscissa, along which D is plotted. An example from experimental practice may illustrate this. Curve c in fig. 5 represents the effect of a fairly weak u.v. irradiation on conidia of *Peronospora destructor*. Four series of very reliable observations could be presented by curve c, all series showing the characteristic feature of curve c in the left part of fig. 5. In order not to complicate the figure with two many ancillary curves only one series of data is represented separately by a dotted line. Curve c does not accord with the inactivation curve previously published by DE WEILLE (1961, *Peronospora arborescens*). If d (fig. 5) be the course of maturation (from right to left), e reflects the increase of K during the experiment, so that the shaded areas in the figures 4 and 5 are consistent. Whereas c is based upon observations, d is no more than a theoretical ancillary line, based on data obtained afterwards in other sporal populations. Thus, d represents the *presumable* systematic error. By subtracting the values of the error-curve e from those of c we obtain the inactivation curve c' in fig. 6. Curve c' is given without claiming that it represents the *quantitative* effect of the u.v. irradiation, or, in other words, the exact features we would have observed had no methodic error been involved. We can only assume c' to represent the *qualitative* influence on K exerted by ultraviolet irradiation, i.e. the *nature* of an error-free inactivation curve. This assumption is strongly substantiated by previous experiments (DE WEILLE, 1961).

In the progression of c the fact may have been assimilated that e.g. the "non-irradiated" $K_{2\frac{1}{2}}$ (D = $2\frac{1}{2}$; t = 13.30) has twice the value of the "non-irradiated" K_4 (D = 4; t = 12.00), so that a curve c'', expressing c in percentages of e, would deviate from c', though its typical character would remain unimpaired. See also fig. 12.

At all events, it has been demonstrated that the typical deviation from the genuine inactivation curve, which is due to maturation, can be recognized (and *qualitatively* eliminated). Working in the same manner as in fig. 6 we can also cope with the reverse deviation caused by decline of viability by senescence.

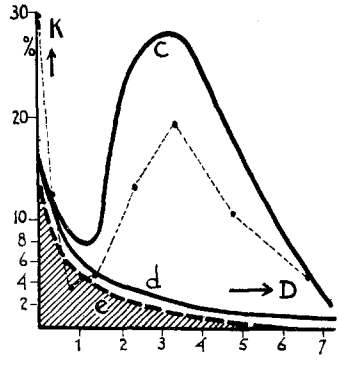

Fig. 5

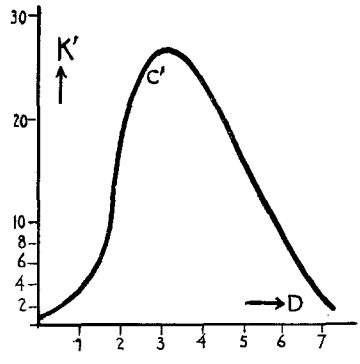

Fig. 6

Peronospora destructor. Influence of u.v. irradiation on germinative power K. R.h. = 80 per cent. i = 47400 erg $mm^{-2}h^{-1}$; $i_{max.}$ at λ = 3400 Å; $\lambda_{min.}$ = 3000 Å. *Detached* conidia.

Peronospora destructor. *Invloed van u.v. bestraling op kiemkracht* K. *R.v.* = *80%. i = 47400 erg* $mm^{-2}h^{-1}$; $i_{max.}$ *bij* λ = *3400 Å;* $\lambda_{min.}$ = *3000 Å.* Losse *conidiën.*

FIG. 5. Systematical error included. Dotted line represents one of 4 series of data on which c is based.
Stelselmatige fout besloten in de kromme. Stippellijn stelt een van de 4 reeksen gegevens voor die aan c *ten grondslag liggen.*

FIG. 6. Maturity differences subtracted.
Rijpheidsverschillen afgetrokken.

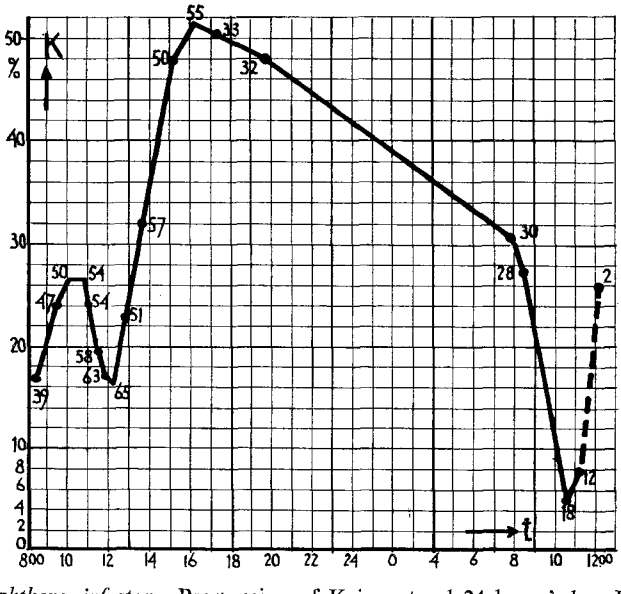

FIG. 7. *Phytophthora infestans.* Progression of K in natural 24 hours' day. R.h. = 100%. Conidia *attached* to sporophores. Figures in graph indicate weight totals [g].
Phytophthora infestans. *Verloop van* K *in natuurlijk etmaal. R.v.* = *100%. Conidiën* aan *dragers. Getallen in grafiek duiden gewichtssommen* [*g*] *aan.*

Decline

The right-hand side of fig. 3 shows a fall in germinative power. K declines as the conidia become senescent. In this context *decline* (of K) *is the phenomenon that the germinative power* (K) *decreases with time.* We may say that if $\frac{dK}{dt} > 0$, a sporal population matures, whereas it declines if $\frac{dK}{dt} < 0$.

The phenomenon is clearly demonstrated by fig. 7. The broken line represents the progression at 100% r.h., of K of conidia of *Phytophthora infestans*, attached to the substratum with their conidiophores. It is a weighted mean of 6 very reliable curves, 5 of which were already represented by fig. 3. After t = 17.00 only 3 curves are represented in the averages.

In order to diminish the influence of less significant samples rigorous discrimation was applied when evaluating the weight (g), which was done according to a scale in accordance with fig. 8 (see also DE WEILLE, 1961).

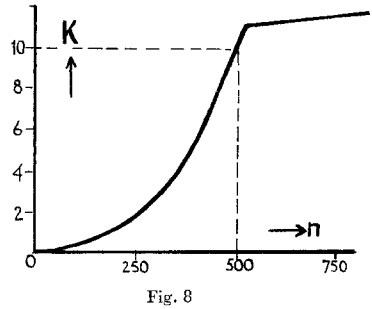

Fig. 8

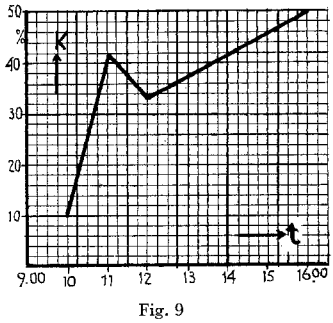

Fig. 9

FIG. 8. Evaluation of g according to n.
Toekenning van g in verband met n.

FIG. 9. *Peronospora destructor.* Progression of K in natural day. R.h. = 100%; conidia *attached* to conidiophores. Based on data by VAN DOORN (1959).
Peronospora destructor. *Verloop van* K *in natuurlijke dag. R.v. = 100%; conidiën* aan *dragers. Naar gegevens van VAN DOORN (1959).*

A sample of 500 elements is allotted g = 10. In fig. 7 the weight totals [g] $\equiv \Sigma g$ are indicated next to the corresponding points. These numbers stress the reliability of the line (comp. fig. 2). The remarkable part near the K-axis is also reliable. In populations exposed to the natural alternation of daylight and darkness that part shows the presence of senescent in addition to newly formed sporal material. The right part of fig. 7 shows the decline of K in an ageing population. In the second morning of the life of a conidium the decline is very rapid. But if the atmosphere remains saturated, young conidia ripen in the morning while the old ones lose viability. Hence fig. 7 shows a picture of *periodicity.*

Both in *Phytophthora infestans* and in *Peronospora tabacina* the decrease of K could be observed during the second morning of existence of (senescent) sporal populations. That the presence of older material next to fresh spores has the same consequences in *Peronospora destructor* may follow from fig. 9, based on a single series of observations by VAN DOORN (1959, table 7, E2). This line in fig. 9 conforms with the separate single lines used for fig. 7.

It is obvious that periodicity in the germinative capacity follows from a preceding corresponding periodicity in the formation of the sporal population in question.

YARWOOD (1943, *Peronospora destructor*) already noticed that
1. exposure of the host plants to light stimulates subsequent sporulation in the dark,
2. light during sporulation adversely influences this process.

In view of these two facts his opinion was that the diurnal variation of sporulation in the field is partly an adaptation to the alternation of light and darkness during a 24 hours' day.

Following YARWOOD (1937), VAN DOORN (1959, *P. destructor*) assumed the effect of light to operate through the metabolism of the host plant; he writes that no sporulation occurs when assimilation predominates (i.e. at day time), but that it does occur, as a function of the amount of previously formed assimilates, when dissimilation prevails (i.e. at night). But it would be wrong to associate sporulation with photosynthesis only; the author mainly worked with conidial material of *Phytophthora infestans*, grown on chlorophyll-free halved potatoes: curve a in fig. 3 and the line in fig. 7 had been obtained with cultures grown on tubers.

Continuous sporulation, less abundant per time unit, occurs in cultures kept *in constant darkness*. K will then show *no diurnal periodicity*.

In fig. 5 it was shown how a concealed maturation effect influences the results of storage experiments. In a similar way the decline of K in one-day old conidia (at 100% r.h.; lower humidities have not yet been dealt with) will affect such results as soon as the classical error of gradual harvesting is made. Exactly as does the maturation line, the senescence line will be expressed in the graphically plotted observational data as its own reflected image (comp. fig. 4). When both phenomena, maturation and senescence, occur simultaneously, we can expect the situation illustrated in fig. 10, in which d and f reflect the maturation and decline processes belonging to two generations of conidia. In fig. 10 e and g reflect the rate of change of K in consequence of the processes represented by d and f. In fig. 11 h(= d + f) shows the progression of K in the mixed sporal population if this remains attached to the host; i (= e + g) shows, again, the rate of change of K due to both maturation and senescence. It must be stated here that d, e, f, g, h and i are all ancillary lines, drawn to explain j in fig. 11. They were derived from data obtained later, when the phenomena of maturation and senescence became better known.

Before the diurnal variation of K was known, two irradiation experiments, each in two replications, were conducted with conidia gradually harvested out of a very heterogeneous population of *Peronospora viciae* (i = 12500 erg $mm^{-2}h^{-1}$; $i_{max.}$ at 3100 Å; $\lambda_{min.}$ = 2700 Å). At first the results gave rise to serious doubt about the correctness of the idea of direct biological influence by radiation already published (DE WEILLE, 1961), the more so because they are statistically reliable. More recent investigations have, however, fully substantiated the earlier work. In retrospect it is no wonder that the results, illustrated by j in fig. 11, turned out as they did.

If we plot the differences between the K-values of the curves j and i for various values of D (i.e. the shaded area in fig. 11) along the D-axis, we do obtain an inactivation curve, viz. k in fig. 12. Curve k represents changes of K, not due to periodicity. Meanwhile we are not sure to have plotted the true shape of k in this picture. We can also suggest other curves, e.g. curves starting from (j–h) instead of (j–i). In fig. 12 inactivation curve m gives the changes of K

with regard to h, viz. (j–h), expressed in percentages of j, whereas 1 gives the changes (j–i), expressed in percentages of j. When expressing the value (j–i) or (j–h) in percentages of i or h we produce still steeper curves, all of the same type; these curves may be called "optimum curves". Hence fig. 12 affirms the reality of the inactivation phenomenon, but owing to the methodical errors, this phenomenon still remains *quantitatively indeterminable.*

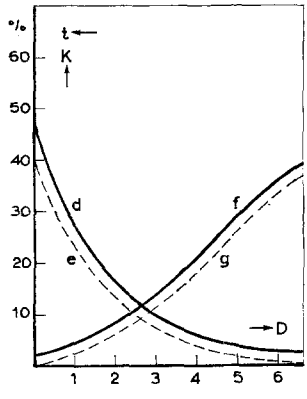

Fig. 10

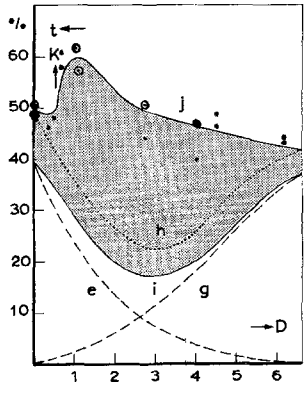

Fig. 11

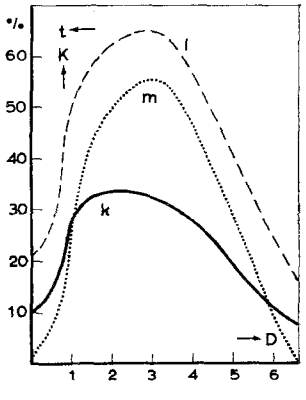

Fig. 12

Peronospora viciae. Effect of u.v. irradiation on a heterogeneous conidial population (j in fig. 11). For further explanation see text.

⊙ : very reliable data; • : less reliable data.

Peronospora viciae. *Effect van u.v. bestraling op een inhomogene conidiënpopulatie (j in fig. 11). Voor nadere uitleg zie tekst.*

⊙ : *zeer betrouwbare gegevens;* • : *minder betrouwbare gegevens.*

Age and atmospheric humidity

So far we have exclusively investigated the connexion between the germinative capacity (K) and time or duration of time in saturated air. From an epidemiological point of view this is understandable, since in the field it is in saturated air that the dangerous situation arises which may lead to sudden outbreaks of diseases like potato blight and downy mildews as soon as a sufficiently long leaf wetness period renders infection possible. In very unsaturated air this situation will not arise.

In such an atmosphere maturation and decline of K will not take place as described in the preceding pages. We must in any case know the course of events lest it be inadvertently incorporated into the observations relating to loose, detached conidia. In an unsaturated atmosphere conidia, attached to their host by means of the sporophores, will *not* ripen as they will in saturated air, whilst germinative power already obtained will rapidly diminish. Fig. 13 gives an example based on the work of VAN DOORN (1959, table 7, D1 and E1). The author can confirm this course of events for *Peronospora tabacina* and *Phytophthora infestans.* The lines p and q relate to young conidia. According to DORAN (1922) mature spores endure a wider range of environmental conditions than immature spores.

It is noteworthy that conidia, after having been taken from the conidiophores when mature, react to exposure to low air humidity in quite another way than do those still attached to the host plant. Laboratory investigations indicate that to loose conidia a temporary dryness of the ambient air might even constitute a certain stimulus for subsequent germination (comp. JAHN, 1905, Myxomycetes). Already free conidia are in any case much less dependent on air humidity conditions than are the still attached conidia. This gives rise to the presumption that withdrawal of water from attached conidia, due to the host plant's evapotranspiration, takes place via the conidiophores, thus causing loss of viability in the conidia.

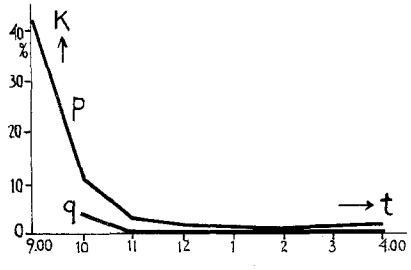

FIG. 13. *Peronospora destructor.* Curves for K in unsaturated air. Curves p and q each represent a young conidial generation. R.h. $< 95\%$; conidia *attached* to conidiophores. Based on data by VAN DOORN (1959).

Peronospora destructor. *Verloop van* K *in onverzadigde lucht. Lijnen* p *en* q *vertegenwoordigen elk een jonge conidiëngeneratie. R.v.* $< 95\%$*; conidiën* aan *conidiëndragers. Naar gegevens van* VAN DOORN *(1959).*

Age and radiation

We have seen that *light* is directly important to sporulation and, therefore, indirectly to maturation. Comparative storage tests in light and in darkness have shown that the direct effect of visible radiation on the germinative power of conidia is nil or negligible.

The effect of u.v. radiation upon *attached* conidia is under investigation. The effect upon *detached* mature conidia shows quantitative differences in different cases, since there is some relation with the age. Newly matured conidia are more sensitive to u.v. than fully ripened older ones. Senescent sporal material is hyper-sensitive to the fungicidal action of u.v. but rather unresponsive to stimulation by small doses. If we study fig. 7 again, now taking into account the specific susceptibility associated with the age of the sporal population, which also seems to hold good for air humidity effects (DORAN, 1922), we come to the conclusion that in the rhythm of natural nights and days the influence of adverse factors in the field will be particularly strong before 1 p.m. if a new conidial generation has just been formed and extremely strong when the sporal material present is one day older.

This implies that, if it is desired to judge the effect of weather factors upon K in the field, the age of the inoculum must be known. The dependence of sporulation on the occurrence of dark periods renders this possible.

Age and temperature

The influence of temperature (T) upon K is mainly secondary. The part of the graphs, given in fig. 3 and fig. 7, tending downwards (decline of K) will occur earlier according as T is higher, so that the error due to periodic harvesting of conidia increases with T. This effect is particularly noticeable at $T > 20°C$.

Since an increase in the temperature increases the dosage-effect of irradiations (OSTER, 1934; HOUTERMANS, 1954), inconstant T may constitute a possible source of errors.

In the faulty experiment of fig. 1 only the germination circumstances were correct. With regard to all other factors here discussed the method of working was erroneous.

SUMMARY AND CONCLUSIONS

Investigations were carried out into the causes of the great *variability* of the germinative power (K) of conidia in the Peronosporales. A great part of this variability can be eliminated if the phenomena underlying it are realized. Optimum working methods are discussed.

In investigating the influence of environmental factors upon the germinative power high demands have to be made on the material and the storage medium involved in the storage tests and the circumstances of germination after the storage period.

The sporal material under investigation should 1. be examined in samples of $\geqslant 500$ conidia; 2. be harvested from a uniform, not putrefactive substratum; 3. be harvested all at once at the beginning of the experiment; 4. statistically constitute a homogeneous population, which implies that all conidia must be of the same age. Such samples can be obtained by making use of the *periodicity* resulting from the effect of *light* (see fig. 7). In the storage medium the intensity of u.v. radiation, the humidity of the atmosphere and the temperature should be kept constant.

For all samples the period of germination should be of equal duration. If the temperature cannot be kept constant its variation should be the same for all samples. Obviously the drop size spectrum and the composition of the water in which the conidia are germinated should also be the same for all samples.

SAMENVATTING

Ter verkrijging van meer inzicht in de invloed van het weer op de mate waarin *Phytophthora infestans* en *Peronospora destructor* voorkomen, werd laboratoriumonderzoek verricht over de reacties van de conidiën van deze schimmels op het ecoklimaat.

Het onderzoek werd aanvankelijk erg bemoeilijkt door de variabiliteit van de kiemkracht (K) van het onderzochte sporenmateriaal. Deze leidt tot onregelmatige uitkomsten (fig. 1). Aangetoond wordt dat zulke onregelmatigheden veelal te wijten zijn aan systematische fouten.

Statistisch bezien moeten sporenmonsters minstens 500 conidiën omvatten, terwijl reeksen te vergelijken monsters preparaten dienen te omvatten, die een kiemkracht van aanzienlijk meer dan 5% vertonen (fig. 2). Ten dele kan de variabiliteit wellicht worden toegeschreven aan interactie van sporen binnen dezelfde druppel.

De in een laboratoriumproef betrokken conidiën dienen een statistisch homogene populatie te vormen, wat betekent dat de voorgeschiedenis van alle elementen dezelfde moet zijn: alle moeten ineens worden geoogst van een gelijkmatig substraat en daarbij nog van dezelfde leeftijd zijn. Een methode, waarmee zulke populaties worden verkregen, wordt besproken. De biologische grondslag daarvan is het rijpingsproces (fig. 3 en 9), gevolgd door een verval van de kiemkracht, het verouderingsproces (fig. 7). Dit verloop wordt beheerst door de natuurlijke afwisseling van dag en nacht. Zolang de lucht verzadigd blijft met waterdamp wordt dagelijks een nieuwe leeftijdsgroep conidiën aan de bestaande populaties toegevoegd, waardoor K een duidelijke *periodiciteit* vertoont. Licht verhindert conidiënvorming, maar stimuleert die in de daarop volgende donkere periode.

Fig. 4 toont de fout die ontstaat door geleidelijk oogsten uit een rijpende populatie; fig. 5 en 6 laten een poging zien om die fout, althans kwalitatief, uit de resultaten te verwijderen. Fig. 10 toont de fouten die ontstaan door geleidelijk oogsten uit een inhomogene, deels rijpende, deels verouderende populatie. In fig. 11 geeft i de daaruit voortvloeiende extra-veranderlijkheid van K. Pogingen om deze fouten, die mede het verloop van de lijn j (fig. 11) hebben bepaald, daaruit te verwijderen, worden uitgebeeld in fig. 11 en 12. Een kwantitatieve beoordeling van de onderzochte u.v.-stralingswerking is niet meer mogelijk.

Een onverzadigde atmosfeer is voor het kiemvermogen van nog aan de dragers zittende conidiën bijzonder ongunstig (fig. 13). Een verzadigingsdeficit voorkomt rijping of doet dat proces snel in dat der veroudering verkeren, waardoor de periodiciteit van K wordt doorbroken.

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