Neth. J. Pl. Path. 93 (1987) 61-71

Temperature requirements for germination, germ tube growth and appressorium formation of urediospores of Hemileia vastatrix

E.J. DE JONG[1], A.B. ESKES[2], J.G.J. HOOGSTRATEN[3] and J.C. ZADOKS[1]

[1] Laboratory of Phytopathology, Binnenhaven 9, 6709 PD Wageningen, The Netherlands
[2] EMBRAPA/CNPDA, C.P. 69, CEP 13820, Jaguariuna, SP, Brazil
[3] 136 Rue Edith Piaf, Aubord, 30620 Uchaud, France

Accepted 14 August 1986

Abstract

Requirements for germination, germ tube growth and appressorium formation of urediospores of *Hemileia vastatrix*, the causal organism of coffee leaf rust, were investigated by applying treatments with constant temperatures, ranging from 10 to 31 °C, and with variable temperatures. Observations were made after 4, 6, 8 and 24 h of incubation of the spores in distilled water on glass slides. The lower and upper limits for germination were estimated to be 13 and about 30 °C, respectively. Germination was quickest at 22 to 28 °C, whereas appressoria formed more rapidly at 13 to 16 °C. After 24 h of incubation, a broad optimum, from 16 to 28 °C, was observed for germination and total appressorium formation. The shape of the appressoria was torpedo-like or roundish at 13 to 19 °C, whereas at higher temperatures their shape was predominantly irregular. Germ tube length increased linearly with time, with an optimum at 19 to 22 °C. The degree of branching of the germ tube was positively correlated with germ tube length. Germination percentage and appressorium formation were higher on leaf disks than on glass slides. With variable temperatures, significant correlation were found between temperature sum and percentage of germination, appressorium formation and germ tube length.

The results show that germination and appressorium formation can occur at lower temperatures than reported in literature. Penetration by *H. vastatrix* seems te be realized most rapidly in nature, if, after wetting of the spores, a decline in temperature occurs from initially ca. 23 to 17 °C within a few hours. Such conditions prevail during evening and early night in many areas where Arabica coffee is grown.

Additional keywords: coffee leaf rust, temperature sum, penetration.

Introduction

Coffee leaf rust, caused by *Hemileia vastatrix* Berk. et Br. is the most important coffee disease. In 1970 it spread to the American continent, first invading Brazil, and in 1986 it has reached nearly all areas in South and Central America where coffee is grown. Studies on the biology of *H. vastatrix* are the base for understanding the epidemiology of coffee leaf rust and its geographic or topographic distribution, and for forecasting rust occurrence (Zadoks and Schein, 1979; Kushalappa et al., 1983). The effect of temperature on germination of *H. vastatrix* has been studied earlier by Nutman and

Roberts (1963), Saccas and Charpentier (1971) and Montoyo and Chaves (1981). By these studies the optimum and maximum temperature limit for germination could be assessed, but the minimum limit was not well established. Appressorium formation and germ tube growth have not been studied into detail yet. The objective of the present study was to further determine temperature requirements for the germination process and appressorium formation of the coffee leaf rust fungus. Besides constant temperature treatments, also variation in temperature was tested to mimic natural conditions.

Materials and methods

Urediospores of race II of *H. vastatrix*, which is the common rust race in Brazil, were used. The spores were produced on coffee plants kept in the greenhouse and stored for a few weeks at ca 4 °C and 52% relative humidity. Spore suspensions in distilled water were prepared by manual stirring for 5 min. A density of 0.2 mg ml^{-1} was used in all experiments. Droplets of 25 μl, each containing about 650 urediospores were pipetted onto slightly wetted microscope slides. Subsequently, the slides were placed on a wetted plastic foam layer of 1 cm thickness inside sealed plastic or stainless steel boxes, excluding light and preventing evaporation of the germination droplet. Previously, the boxes were conditioned for some hours to the desired testing temperature. Two Percival growth chambers were used with temperature fluctuations less than 0.5 °C. For Experiment 1, a laboratory space with temperature control of 22 ± 1.0 °C was also used. After treatment, the spores were immediately killed by placing slides for 15 min in a dessiccator containing an aqueous solution of 40% formaldehyde. The droplets were then air dried and the slides placed in the refrigerator, until observations were made. When necessary, the formaldehyde treatment was repeated to avoid growth of the hyperparasite *Verticillium lecanii*.

When coffee leaf disks were used as a substrate for germination, leaf surface replicas were made with transparant fingernail polish to study spores and germ tubes. A light microscope was used for the observations. At least 150 spores were counted per droplet to determine germination, sixty germ tubes for appressoria counts and five for estimations of tube length and degree of branching. Per treatment ten droplets were examined. A spore was considered to be germinated when the germ tube reached the size of the diameter of the spore (ca 30 μm). An appressorium was considered to be formed when the thickening at the end of the germ tube reached a diameter of at least 1.5 times the germ tube width. For estimations of the length of the germ tube a scale was used with units of 37.5 μm. Branching of germ tubes was assessed by a four-point scale, indicating no branching to maximum branching.

In experiment 1, eight temperatures were compared. The experiment was divided into four sub-experiments, performed on different days, which consisted of the following treatments: 10/13/22 °C, 16/22 °C, 19/31/22 °C and 25/28/22 °C. For comparison between the sub-experiments, the results were transformed into values relative to those of the 22 °C treatment. Transformation of percentage of germination (PG) into transformed percentage of germination (TPG) was done by the following formula:

$$TPG = \frac{PG\ (x\ °C,\ y\ h)}{PG\ (22\ °C,\ y\ h)} \cdot \overline{PG}\ (22\ °C,\ y\ h)$$

where x is the temperature, y the duration of incubation and $\overline{PG}$ the average percentage of germination obtained at 22 °C in the four sub-experiments. A similar transformation was applied for the germ tube length (GTL) into the transformed germ tube length (TGTL). The percentage of appressoria (PA) was transformed in relation to the values of the 22 °C treatment, at 24 h incubation by the following formula:

$$TPA = \frac{PA\ (x\ °C,\ y\ h)}{PA\ (x\ °C,\ 24\ h)} \cdot \overline{PA}\ (22\ °C,\ 24\ h)$$

This was done because of the low values obtained at 4, 6 and 8 h of incubation which, when divided by each other, would lead to errors. The percentage of appressoria was expressed in relation to the number of germinated spores (PA) and to the total number of spores counted (PAT), using the symbols TPA and TPAT, respectively, for the transformed percentages.

For experiments 2 and 3, the temperature sum was calculated by the following formula:

$$TS = \int_0^t (T - T_{min})\ dt$$

where TS = temperature sum (°C h), t = time (h), T = temperature (°C) and T_{min} = minimum temperature for activity.

For statistical analysis, the percentage data were transformed into arcsine $\sqrt{x}$, unless indicated otherwise. Individual droplets were considered as replications. To compare mean percentages of germination and appressorium formation, Duncan's multiple range test was applied and to compare germ tube lengths Student's t-test, both at 5% level of significance. For computer analyses the Statistical Package for the Social Sciences was used.

Results

Preliminary tests. In the first test the percentage of germination (PG) of freshly harvested spores was compared to that of spores kept in the refrigerator for two days. Temperatures applied were 16 and 22 °C and observations were made after 14 h of incubation. PG values were, respectively, 18.6 and 19.9, at 16 °C, at 29.0 and 29.9, at 22 °C. The differences between the spore batches were not significant, indicating that storage of spores for a few days at ca 4 °C does not affect germination.

The second test estimated the experimental variation of the adopted methodology. Germination was observed of spores placed in two growth chambers, both set at 22 °C. No significant difference between the growth chambers was observed at any duration of incubation. Standard deviations for the untransformed data increased with increasing germination percentages (Table 1). The magnitude of variation observed in this test was representative for the other experiments.

In the third test the effects of droplet size and substrate for germination were studied. No effect of droplet size on germination and appressorium formation was observed (Table 2). Saccas and Charpentier (1971) had found a stimulative effect of small droplets on germination in Van Tieghem cells. They suggested that their results might be explained by differences in oxygen availability in the droplets. We suspended

Table 1. Average percentage of germination (PG) and its standard deviation (SD) of urediospores of *Hemileia vastatrix* observed after 3, 4, 6 and 8 hours of incubation at 22 °C.

Duration of incubation (h)	PG	SD
3	11.1	2.3
4	22.2	4.1
6	28.3	4.4
8	34.5	5.4

Table 2. Percentage of germination (PG) and appressorium formation (PA) of *Hemileia vastatrix* on glass slides and leaf disks, determined after 6, 12 and 24 h of incubation.

Substrate	Droplet size[2]	PG				PA		
		Incubation (h)				Incubation (h)		
		6	12	24	Mean	12	24	Mean
Glass slide	large	18a[1]	23a	21a	21a	11a	43a	27a
Leaf disk	large	26b	27a	25ab	26b	19b	59b	39b
Leaf disk	small	24b	26a	28b	26b	20b	56b	38b

[1] Different letters indicate significant differences between treatments, within columns, according to Student's t-test at 0.05 level of significance.

[2] Spores were applied in small droplets, by spraying, or in large droplets (25 µl), by pipetting.

the spores by manual stirring for 5 min, thus introducing extra air into the water, which may have been sufficient to allow for equal germination in both types of droplets.

As substrates, glass slides were compared to leaf disks. Table 2 shows a positive effect of leaf disks on germination and on appressorium formation. This result agrees with the experience reported by Rayner (1972). Statistical analysis did not indicate interaction between treatments and duration of incubation. Therefore, the relative speed of germination and appressorium formation is expected to be similar on both substrates, although total germination and appressorium formation may be higher on leaf disks.

Experiment 1. Eight temperatures were tested in four sub-experiments, each including a 22 °C reference treatment. The germination capacity of the spore batches used in the sub-experiments differed slightly, with PG and PA values of the 22 °C/24 h observations varying from 36.1 to 40.4 and from 39.8 to 62.6, respectively. Therefore, comparison between temperatures was done by transforming values relative to those of the 22 °C treatments of each sub-experiment (see Materials and Methods).

The results show that germination was nearly zero at 10 and 31 °C (Fig. 1). At 13 °C germination was low, reaching 2.5% after 24 h. Germination was abundant at 16 to 28 °C, with 22 °C being the optimum. The proportion of germinated spores after 4 h

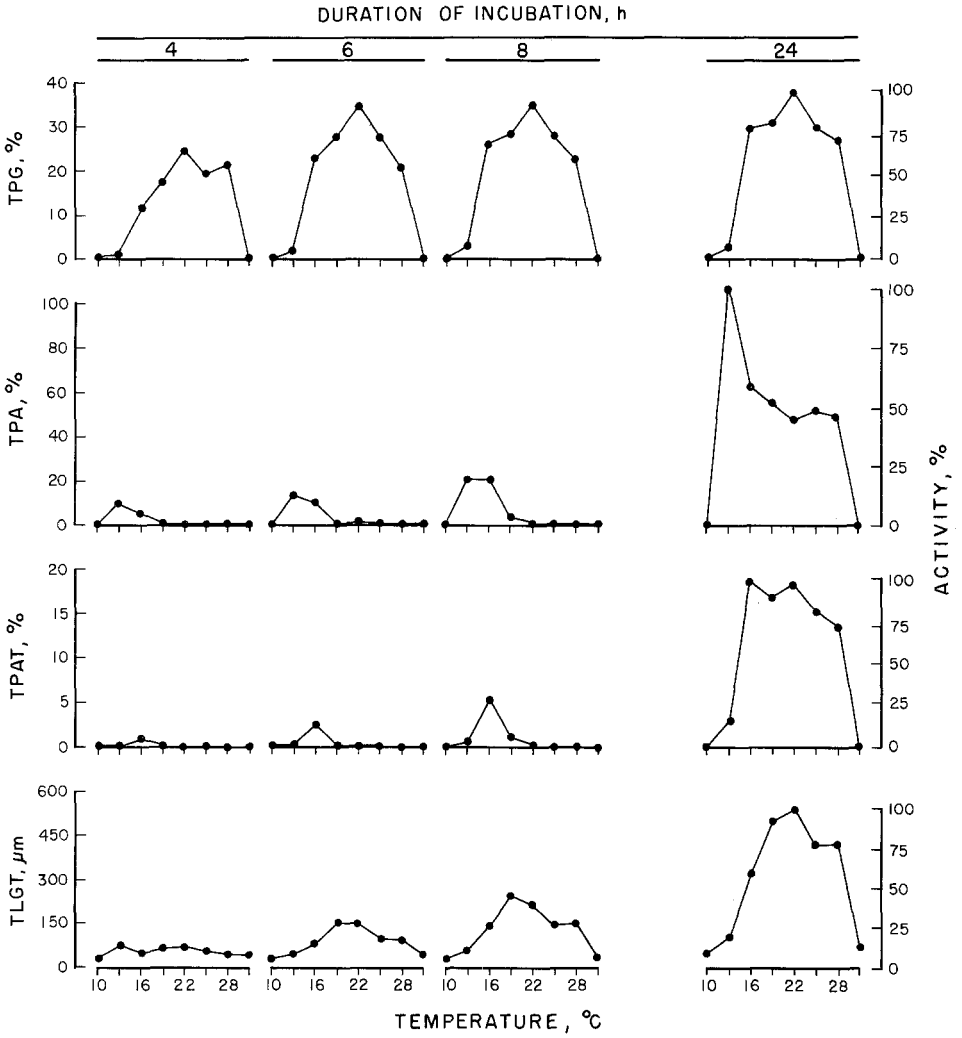


Fig. 1. Effect of constant temperatures on the transformed percentages of germination (TPG), of appressorium formation in relation to the number of germinated spores (TPA) and of appressorium formation in relation to the total number of spores (TPAT), and on the transformed length of the germ tube (TLGT) of urediospores of *Hemileia vastatrix* after 4, 6, 8 and 24 h of incubation.

relative to the number of germinated spores after 24 h was 0.08, 0.40, 0.57, 0.65 and 0.77 for the 13, 16, 19, 22, 25 and 28 °C treatments, respectively. This indicates that the rate of germination was highest at 22 to 28 °C. TGP increased significantly between 4 and 6 h of incubation at 13, 16, 19, 22 and 25 °C, after which no further increase occurred.

Appressorium fromation was fastest at 13 and 16 °C. After 8 h, TPA reached about 20% at 13 and 16 °C, whereas at higher temperatures TPA was nearly zero. After 24 h, TPA was 100% at 13 °C, being 50 to 60% at higher temperatures. The transformed

percentage of appressoria relative to the total numbers of spores counted (TPAT) showed a wide optimum after 24 h with 16, 19 and 22 °C being most favourable. The shape of the appressoria varies with temperature, being roundish or torpedo-like at 13, 16 and 19 °C, whereas at higher temperatures it was predominantly irregular.

The growth of the germ tubes increased linearly with time. The optimum was at 19 and 22 °C, with growth being nearly absent at 10, 13 and 31 °C. Branching of the germ tube started when the tube reached a length of 150 to 200 μm. The degree of branching of the germ tube was correlated with the average germ tube length, the coefficient of linear correlation (r) being 0.87.

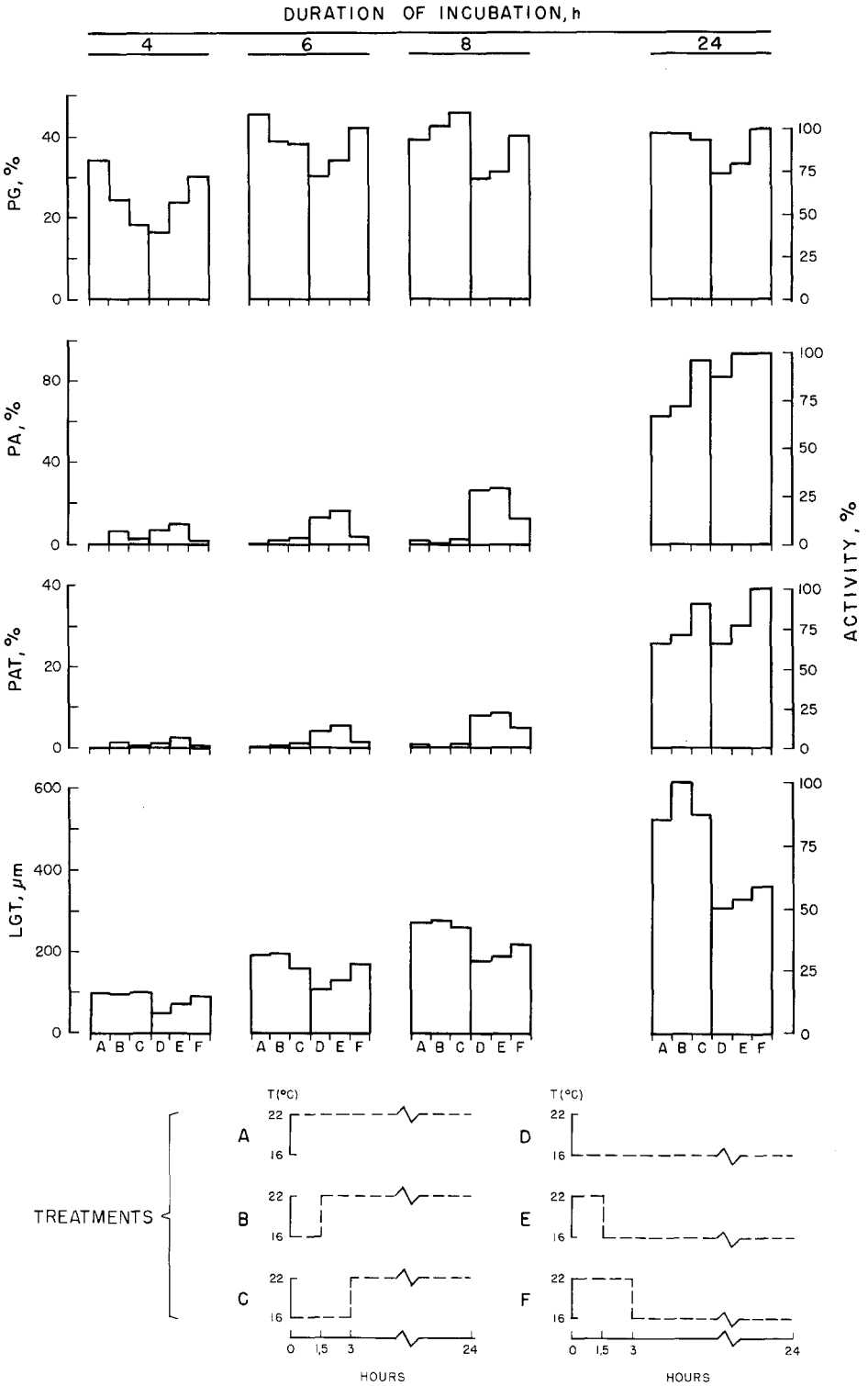
Experiment 2. The objective was to compare germination and appressorium formation at fluctuating and at constant temperatures. As constant temperatures 16 and 22 °C were chosen, being optimal for appressorium formation and germination, respectively. Fluctuations from 16 to 22 °C and vice versa were applied after 1.5 and 3.0 h by transferring the slides with spores from one growth chamber to the other. Fig. 2 shows that initial exposure to 16 °C (treatments B and C) delayed germination to some degree, but after 6 to 8 h of incubation PG values were similar to that in the treatment with constant 22 °C (A). Appressorium formation and germ tube length were barely affected by initial exposures to 16 °C, except for PA values after 24 h, which were higher with treatment C than with A or B. The degree of branching of the germ tube was correlated with germ tube length ($r = 0.97$).

Comparison between treatments A, D, E and F shows that an initial exposure of 3 h to 22 °C (F) stimulates germination as much as the constant 22 °C treatment (A). Appressorium formation was fastest at the lower temperature treatments (D and E), as in Experiment 1.

For calculation of the correlation between temperature sum (TS) and PG or PA values, 12 °C was taken as the temperature of minimum activity (see Materials and Methods). TS and PG values were linearly related for the observations after 4 and 6 h, with correlation coefficients of 0.97 and 0.91, respectively. Maximum PG values were obtained at TS values of 40 to 50 °C h. TS was negatively correlated to PA for the observations after 6 and 8 h, obtaining r values of -0.86 and -0.83. The correlation between TS and LGT was significant at all durations of incubation ($r = 0.96$). These results suggest that the effect of varying temperatures can be largely deducted from the summation of the effects of constant temperatures.

Experiment 3. In this experiment a constant 22 °C temperature treatment (A) was compared to a treatment with varying temperature (B), representative for the average variation of winter nights in Campinas, SP, Brazil. Fig. 3 shows higher PG values for treatment A at all observation times, but PA values were much higher for B than for A. PAT values for A and B were similar at 6 and 9 h of incubation, but B exceeded A at 12 and 15 h. As expected, growth of the germ tube was slower with treatment B than with A. Branching of the germ tube was again correlated with the germ tube length ($r = 0.87$).

Fig. 2. Effect of six temperature regimes on germination (PG), appressorium formation relative to the number of germinated spores (PA) and total number of spores (PAT), and length of the germ tube (LGT) of *Hemileia vastatrix* urediospores after 4, 6, 8 and 24 h of incubation.


DURATION OF INCUBATION, h
4
6
8
24
PG, %
PA, %
PAT, %
LGT, µm
ACTIVITY, %
A B C D E F
TREATMENTS
T(°C)
22
16
HOURS
0 1,5 3 24

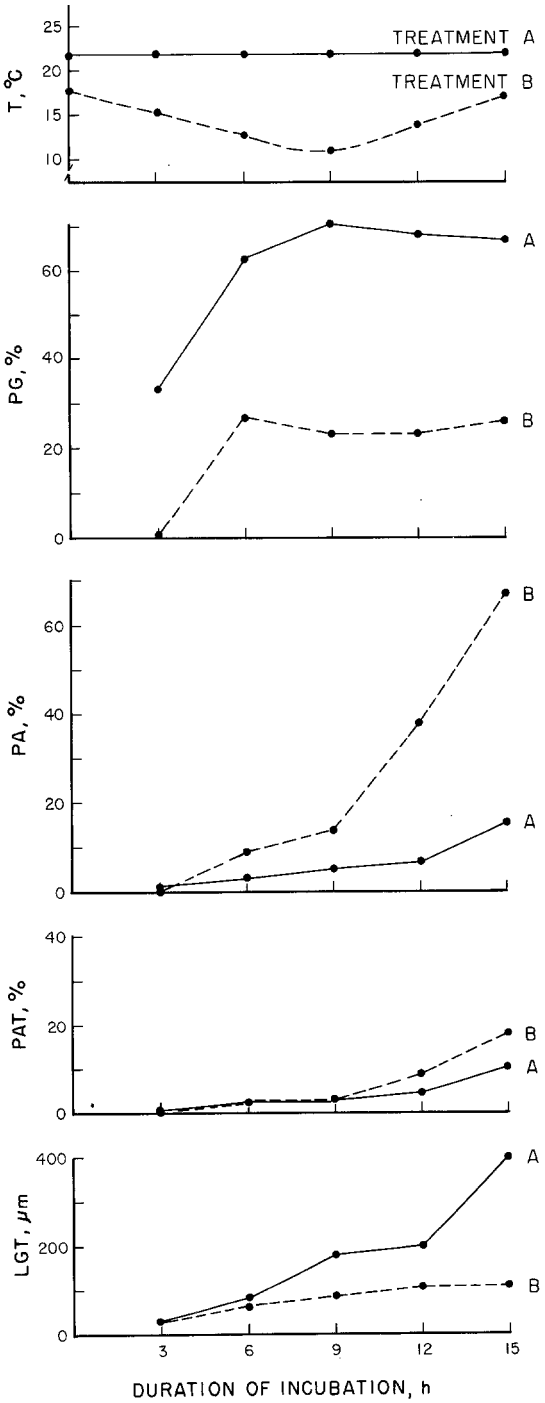


Fig. 3. Effect of two temperature regimes on germination (PG), appressorium formation relative to the number of germinated spores (PA) and the total number of spores (PAT), and length of the germ tube (LGT) of *Hemileia vastatrix* urediospores after 3, 6, 9, 12 and 15 h of incubation.

Discussion

The temperature ranges reported in other publications varied from 18 to 28 °C (Nutman and Roberts, 1963), 20 to 40 °C (Saccas and Charpentier, 1971) and 18 to 26 °C (Montoyo and Chaves, 1981). The maximum temperature for germination was found to be near 28 °C (Nutman and Roberts, 1963) or between 28 to 30 °C (Saccas and Charpentier, 1971), which is in agreement with our results. However, in the present study the minimum temperature was 2 to 3 °C lower than calculated by Nutman and Roberts (1963).

Within the range of favourable temperatures, the speed of germination varied, being lower at lower temperatures (16 and 19 °C). This behaviour is in agreement with the data presented by Saccas and Charpentier (1971).

Our data indicate clearly that germination occurs fastest at 22 to 28 °C, whereas appressoria formed more rapidly at lower temperatures, viz. at 13 to 16 °C. In contrast, the results of Nutman and Roberts (1963) suggested similar optima for germination and appressorium formation. However, their data may not be comparable because temperatures below 18 °C were not considered and also the duration of incubation for appressorium formation was not indicated.

With respect to temperature fluctuations, Nutman and Roberts (1963) indicated that exposure to low temperatures (15 to 17 °C), followed by transfer to 22 °C after a few hours, increased germination speed and final number of germinated spores. Our results may confirm the increase in germination speed, but the final number of germinated spores was not increased by the cold treatments (Fig. 2, treatments B and C). The stimulating effect on germination speed might be explained by dilution of a germination inhibiting compound (Musumeci et al., 1974), when spores are incubated at lower temperatures, thus preparing the spores for rapid germination at more favourable temperatures.

Degree of branching of the germ tubes was linearly correlated with germ tube growth. This suggests that both processes are similarly affected by temperature. Stomata of Arabica coffee leaves are about 70 μm distant from each other. Therefore, one can expect the germ tube to need an average length of about 100 μm to have a reasonable chance to reach a stoma. At 19 to 22 °C this length is reached within 4 to 6 h.

Germination of *H. vastatrix* depends on the presence of liquid water (Nutman and Roberts, 1963; Rayner, 1972). From the data of Eskes (1982) it can be deduced that appressorium formation and penetration of *H. vastatrix* are water dependent, as is the case with *Puccinia graminis* (Zadoks, 1968). Because of the relative rareness of long leaf wetness periods in the field, lesion formation by *H. vastarix* in nature will greatly depend on the speed of germination and appressorium formation. Our results suggest that penetration will be most rapid if wetted spores are exposed to 22 to 26 °C for some hours and then to 15 to 18 °C. Under such conditions penetration may start after 4 to 6 h of wetting, whereas at constant 22 °C at least 9 to 10 h would be required. Therefore, rain or early dew occurring in the evening or beginning of the night, followed by a drop in temperature, is expected to favour mostly the initiation of new lesions of coffee leaf rust.

The observed differences in temperature optima for germination and appressorium formation of *H. vastatrix* might have an important survival value for the rust fungus

under natural conditions. Specific adaptation to environmental conditions has also been reported for germination and penetration of *P. graminis* (Zadoks, 1968), with temperature optima of ca 20 and 29 °C, respectively.

Acknowledgements

The hospitality of the Instituto Agronômico de Campinas (IAC), where the experimental phase was carried out, is acknowledged. Especially, the heads and personnel of the Genetics and Virology Sections are thanked for their kind co-operation. The contribution of T. Witmer for obtaining the data of Table 2 is also acknowledged.

Samenvatting

Temperatuurbehoeften voor kieming, groei van de kiembuis en appressoriumvorming van urodosporen van Hemileia vastatrix

De temperartuurbehoeften voor kieming en appressoriumvorming van uredosporen van *Hemileia vastatrix* werden onderzocht door incubatie bij constante temperaturen, 10 tot 31 °C, en variabele temperaturen. Waarnemingen werden gedaan na 4, 6, 8 en 24 uur incubatie van de sporen in gedestilleerd water op glasplaatjes. De onder- en bovengrens voor kieming bleek bij 13 en ca 30 °C te liggen. De kieming kwam het snelst op gang bij 22 tot 28 °C en de appressoriumvorming bij 13 tot 16 °C. Na 24 uur incubatie werd een breed optimum waargenomen van 16 tot 28 °C voor het kiempercentage en het totale aantal appressoria. De vorm van de appressoria was torpedoachtig of rond bij 13 tot 19 °C, terwijl bij hogere temperaturen de vorm meestal onregelmatig was. De lengte van de kiembuis nam vrijwel rechtlijnig toe met de tijd, met een optimum van 19 tot 22 °C. De vertakkingsgraad van de kiembuizen was positief gecorreleerd met de kiembuislengte.

Kieming en appressoriumvorming verliepen beter op de bladschijven dan op glasplaatjes. Bij de behandelingen met variabele temperaturen werden significante correlaties waargenomen tussen de temperatuursom en de kiembuislengte en ook tussen de temperatuursom en het kiem- en appressoriumpercentage.

De resultaten tonen aan dat kieming en vooral appressoriumvorming bij lagere temperaturen kan plaatsvinden dan verondersteld in de literatuur. Ze suggereren dat penetratie van *H. vastatrix* het snelst gerealiseerd kan worden in de natuur als, na bevochtiging van de sporen, een temperatuurdaling plaatsvindt van aanvankelijk ca. 23 naar ca. 17 °C binnen een paar uur. Deze conditie komt veelvuldig voor in de namiddag en het begin van de nacht in vele gebieden waar Arabica-koffie wordt verbouwd.

References

Eskes, A.B., 1982. The use of leaf disk inoculations in assessing resistance to coffee leaf rust (*Hemileia vastatrix*). Netherlands Journal of Plant Pathology 88: 127-141.

Kushalappa, A.C., Akutsu, M. & Ludwig, A., 1983. Application of survival ratio for monocyclic processes of *Hemileia vastatrix* in predicting coffee rust infection rates. Phytopathology 73: 96-103.

Montoyo, R. & Chaves, G.M., 1981. Influencia de la temperatura y la luz en la germinación, infectividad y periodo de generación de *Hemileia vastatrix* Berk & Br. Publicación Miscelánea

no. 278. Instituto Interamericano de Cooperación para la Agricultura. Coronado, San José, Costa Rica, 33 p.
Musumeci, M.R., Moreas, W.B.C. & Staples, R.C., 1974. A self-inhibitor in uredospores of the coffee rust fungus. Phytopathology 64: 71-73.
Nutman, F.J. & Roberts, F.M., 1963. Studies on the biology of *Hemileia vastatrix* Berk & Br. Transactions of the British Mycological Society 46: 27-48.
Rayner, R.W., 1972. Micologia, historia y biologia de la roya del cafeto. Publicación Miscelána no. 94. Instituto Interamericano de Ciencias Agricolas de la Organización de Estados Americanos, Centro Tropical de Enseñanza e Investigación, Turrialba, Costa Rica, 67 pp.
Saccas, A.M. & Charpentier, J. 1971. La rouille des caféiers due à *Hemileia vastatrix* Berk & Br. Bulletin no. 10 Institut Français du Café, du Cacao et autres Plantes Stimulantes, Paris, 123 pp.
Zadoks, J.C., 1968. Meteorological factors involved in the dispersal of cereal rusts. In: A.J.W. Borghorst (Ed.), Proceedings Regional Training Seminar on Agro-meterology. Wageningen, p. 179-194.
Zadoks, J.C. & Schein, R.D., 1979. Epidemiology and plant disease management. Oxford University Press, New York, 427 pp.

Book review

A.F. Sherf & A.A. Macnab, 1986. Vegetable diseases and their control, 2nd edition, John Wiley & Sons, New York, VII + 728 pp. £ 45.65.

The wide variety of vegetable crops makes the writing of a general book on their diseases a heavy task. New books in this field are therefore relatively rare, and not even those published in major languages such as English, French or German are often regularly updated. The present book is thus welcome.

The authors stress the practical orientation of their book, the audience being defined as ranging from commercial growers to university workers. The contents warrant this.

The first edition under the same title was published in 1960, by C. Chupp and A.F. Sherf. But for some minor modifications, the outline of the 1960 edition, presentation of the diseases by crops, has been maintained. However the original chapter on general diseases (like bacterial soft-rot and gray mold) has been omitted. These diseases are now treated separately with the crops concerned. Sometimes, however, especially for nematodes, repetitive descriptions are avoided by referring the reader to details discussed in chapters on other crops.

After an introductory chapter on 'Disease causes and controls', diseases are listed according to an impressive range of vegetables, each with its own chapter: asparagus, artichoke, and Jerusalem artichoke/snap and dry beans/Lima bean/beet/carrot/celery/corn/crucifers/cucurbits/eggplant/lettuce/onion, garlic, leeks, and shallots/pea/pepper/spinach/sweet potato/tomato/and minor crops (okra, parsley, parsnip, and salsify). The arrangement of the diseases under each crop makes identification easy. Symptoms, life-cycle of the causal organism, and control are described for major and minor diseases. A list of references immediately follows each disease description.