The Effect of Temperature on the Pathogen and on the Development of Blue Mold Disease in Tobacco Inoculated with Peronospora tabacina

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ABSTRACT

The highest degree of sporulation and colonization of *Peronospora tabacina* was found at 15 to 20 C. At constant temperatures, the optimum development of disease occurred at 25 C, and under 12-hr periods of 20-C night and 20 to 40-C day temperatures, at 20-25 C. Shortening the length of high temperature periods during the daytime resulted in a shift of the optimal disease development to 30-35 C in plants exposed to those temperatures for 2 hr/day. Exposure of previously infected but still symptomless plants to temperatures lethal for the fungus, i.e., up to 45 C, induced development of sterile lesions within 24 hr, with 35 C leading to the most accentuated lesions.

It was concluded that the optimal development of disease is affected by much higher temperatures than the development of the pathogen; but, for the high temperatures to exert their effect, a certain extent of previous colonization at a temperature favorable for the pathogen is needed. Under these conditions, the disease develops whether the pathogen remains alive or dies. Phytopathology 60:54-57.

Blue mold epidemics of tobacco in Israel start in winter in the plant bed and continue in the field until the end of June, when they are finally checked by high temperatures. However, even during the climatically favorable months, periods of extremely hot wheather occur with temperatures often exceeding 35 C. Field surveys showed that during these periods, leaves which previously did not show signs of *Peronospora tabacina* Adam suddenly exhibit typical lesions of blue mold.

As high temperatures are unfavorable for *P. tabacina* (1, 2, 3, 5, 6), two hypotheses were tested in an effort to explain the above-mentioned phenomenon; (i) occurrence of a thermophilic strain of *P. tabacina,* or (ii) high temperature induction of disease in leaves previously infected by the fungus, but not exhibiting any symptoms.

MATERIALS AND METHODS.—Some experiments were performed on the Michal (Oriental) tobacco, and some on the Yellow Special (Virginia) tobacco. The plants were cultivated in an air-conditioned greenhouse at 25 ± 2 C for the first 4 weeks in a seed bed, and for an additional 6 weeks in 0.3-kg pots filled with a 2:2:1 mixture of sandy loam, sand, and peat. Plants selected for inoculations had five to six leaves, a uniform height of 20-25 cm, and showed normal, healthy growth.

Plants were inoculated with an isolate from a commercial field of Michal plants, and maintained on Michal plants at 20 C. This isolate was found to be typical in frequent comparisons with others. All inoculations were performed by means of Schein's inoculator (7) with 1,000 ± 100 spores sprayed on a 4-cm^2 target on the lower side of the leaf. The inoculated plants were kept in moist chambers at 20 C for 6 to 24 hr, then transferred to Shirrer's growth chambers for 12-hr photoperiods at various temperatures.

The average disease value from five or six leaves of one plant was considered as one replication, and at least four replications were used for each treatment.

In different experiments, all or some of the following methods were used for evalution. (i) Disease severity was evaluated according to the following index: 0 = no lesion; 1 = light-yellowish-green-lesions; 2 = yellow lesions; 3 = yellow lesions with traces of necrosis; and 4 = entirely necrotic lesions. Intermediate categories were used as necessary. (ii) The area of diseased tissue was measured by tracing the outlines of lesions on cellophane, recopying the outlines on Bristol board, cutting out the area, and weighing it. (iii) Colonization, namely, the area occupied by mycelium, was considered as the area which was covered by spores after the plants had been held for an additional 24 hr in moist chambers at 20 C. These areas were then traced on cellophane and evaluated as previously described. It had been found that 1 day at 20 C, in addition to 13 days of exposure to different temperatures, did not lead to marked changes in results. (iv) Sporulating capacity of the infected areas was determined by counting the removed spores with a cytometer.

Data obtained from different evaluations were analyzed by the Tukey test (9).

RESULTS.—*The effect of constant temperatures on disease phenomena.*—Inoculated Michal plants were kept for 24 hr in moist chambers at 20 C, then transferred for 13 days to growth chambers at either 10, 15, 20, 25, or 30 C. Disease development was evaluated on different plants after 5, 8, or 13 days' incubation. Eight plants were tested in every combination of temperature and incubation period.

Disease development was significantly greater at 25 C (0.01) in each period of incubation. After 5 days' incubation, the disease was apparent only at 25 C. After incubation of 8 and 13 days, the range at which lesions appeared was 15 to 30 C and 10 to 30 C, respectively. In contrast to the 25 C optimum for lesion intensity, the largest area of lesions (13 days only) and of colonization was found at 20 C, and the highest degree of sporulation at 15 C. However, no significant differences were found between either of these effects at 20 and 15 C (Fig. 1-A, B, C, D).

In an additional experiment, disease development at

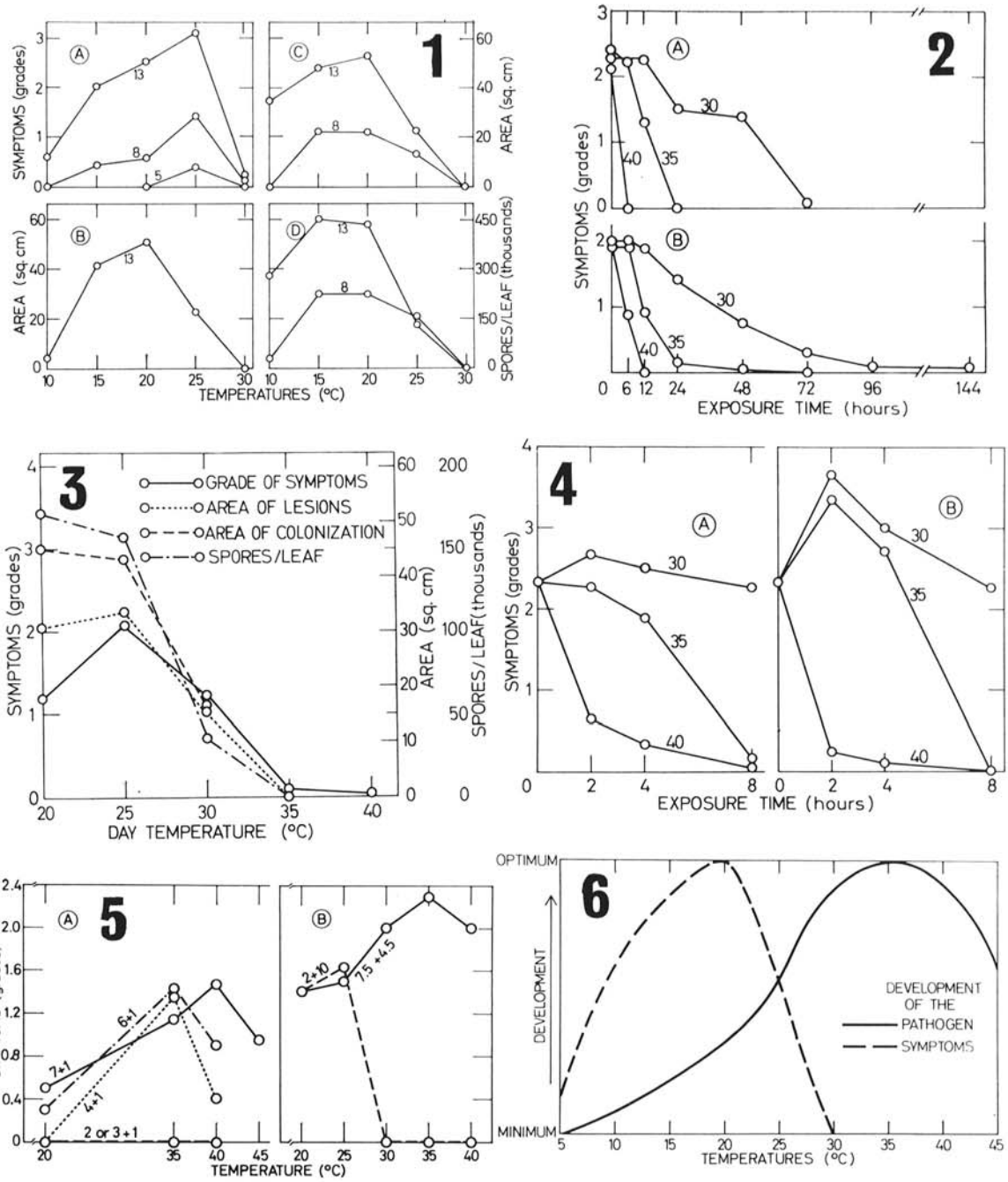

Fig. 1-6. **1)** The effect of constant temperatures on disease phenomena in tobacco plants infected by *Peronospora tabacina*. **A)** Grade of symptom development. **B)** Area of lesions. **C)** Area of colonization. **D)** Sporulating capacity. The numbers above the curves indicate the incubation period in days after which assessments were made. **2)** Effect of high temperatures on survival of *Peronospora tabacina* inside infected leaves of tobacco. **A)** After 6 hr. **B)** After 24-hr previous incubation in moist chambers at 20 C. The numbers above the curves indicate temperatures. **3)** Disease phenomena caused by *Peronospora tabacina* in tobacco exposed to 20 C night and 20 to 40 C day temperatures in 12-hr periods. **4)** Symptoms development in plants inoculated with *Peronospora tabacina* and exposed to 20 C night and 30 to 40 C day temperatures for up to 8 hr/day. **A)** Plants exposed for the above conditions for 5 days. **B)** Plants exposed for 13 days. **5)** Effect of length of incubation period at 20 C and subsequent exposure to high temperatures on the development of the blue mold symptoms in tobacco previously inoculated with *Peronospora tabacina*. **A)** Plants kept for 2, 3, 4, 6, and 7 days at 20 C, then transferred for 1 day to 35-45 C. **B)** Plants kept for 2 and 7.5 days at 20 C, then for 10 and 4.5 days, respectively, at 25-40 C. **6)** Schematic representation of the effect of temperatures on the development of the pathogen and of disease symptoms of the blue mold disease of tobacco.

5 C was tested. No lesions were formed at this temperature, although traces of colonization and sporulation did occur.

Similar results were obtained with Virginia Yellow Special plants.

Effect of high temperatures on survival.—The effects of high temperatures on the survival of *P. tabacina* inside the infected leaves were tested in two instances. In the first, inoculated Michal plants were kept for 6 and 24 hr in moist chambers at 20 C. Those kept for 6 hr were immediately transferred to 30, 35, or 40 C for varying periods from 2 to 144 hr, after which they were returned to 20 C. Plants kept for 24 hr in the moist chambers were transferred immediately, after 24 hr, or after 48 hr at 20 C to either 30, 35, or 40 C. After exposure for 2 to 144 hr to high temperatures, all groups were returned to 20 C until the lesions were indexed and sporulation was assessed on the 14th day after inoculation. Four plants were tested in each treatment.

The effect of high temperatures on development and viability of *P. tabacina* was more pronounced in plants kept in a moist chamber for 6 hr than in those of all subtreatments kept there for 24 hr (Fig. 2-A, B). However, differences were highly significant in relation to plants exposed thereafter to 30 and 35 C, but not to 40 C. The differences between various periods of exposure were significant for periods of up to 12 hr, but not for longer periods. Sporulation tests revealed that whenever the lesions appeared, spores were also produced.

In the second experiment of this series, Michal plants were inoculated in four replications 6 days before exposure to 30, 35, and 40 C for 24, 48, and 72 hr, after which they were returned for 24 hr to a moist chamber at 20 C. Viability of the fungus was assessed by amount of sporulation. In plants thus exposed to 30 C for 24, 48, or 72 hr, 864×10^3, 50×10^3, and no spores/lesion were produced, respectively. No spores were produced in plants exposed for 24, 48, or 72 hr at temperatures of 35 and 40 C.

Effect of thermoperiods.—The reaction of blue mold to high temperatures during only part of the diurnal cycle was tested on Michal plants. In the first experiment, the night temperature (12 hr) was kept at 20 C, and the day temperatures at 20, 25, 30, 35, or 40 C for a period of 13 days (Fig. 3). The most rapid disease development and the largest lesions developed at 25-C day temperatures, while the largest area of colonization and the largest amount of spores were produced at a constant temperature of 20 C. Day temperature of 35 C was lethal, or almost so, to all of the measured processes. The same experiment repeated with 10 C at night and 20, 25, 30, 35, or 40 C during the day yielded similar but always lower values.

In the next experiment, the basic temperature was 20 C with 2, 4, and 8 hr of 30, 35, and 40 C thermoperiods, respectively. The inoculated plants, first kept for 20 hr in moist chambers at 20 C, were then divided into three groups. The first group was exposed for 13 days to the above mentioned thermoperiods. The second and third groups were exposed to thermoperiods for 9 and 5 days, respectively, after which they were transferred to constant 20 C for the remainder of the 13-day period.

The reactions of plants exposed for 13 and for 9 days to high temperature periods were similar, but significantly higher than those of plants exposed to the same conditions for 5 days (Fig. 4-A, B). In plants exposed to high temperature periods for 9 and 13 days for 2 and 4 hr/day, the intensity of disease was significantly higher than in plants kept at a constant temperature of 20 C.

Inductive effect of high temperatures on the appearance of lesions.—The following experiments were designed to determine whether blue mold can develop at temperatures lethal to the fungus, provided colonization was previously initiated. In preliminary tests, hundreds of inoculated plants were kept for various periods at temperatures conducive to colonization, and were then exposed to various temperature levels. Some of the experiments, two of which are described here, were repeated under carefully controlled conditions. Fig. 5-A refers to inoculated Michal plants first kept for 2, 3, 4, 6, or 7 days at 20 C, then exposed for 24 hr to 35, 40, or 45 C and evaluated according to the intensity of disease. The data, which are averages of four similar tests with four plants/treatment, indicate that a minimum period of 4 days' incubation at 20 C was required for subsequent high temperatures to exert their effect. The most pronounced induction of disease took place at 35 C for plants previously incubated at 20 C for 4 and 6 days—and at 40 C for plants previously incubated for 7 days. At 45 C, most of the plants died, but those that survived this temperature were less diseased than plants maintained at 35 and 40 C.

Results from a variation of the above experiment are presented in Fig. 5-B. When the inoculated Michal plants were kept for only 2 days at 20 C and thereafter exposed for 10 days to temperatures from 20 to 40 C, the disease appeared in plants left at 20 and 25 C, i.e., temperatures conducive to further colonization, but not in plants exposed to 30 to 40 C. Plants incubated at 20 C for 7.5 days, which at that time still did not show disease symptoms, showed symptoms at 30 to 40 C, with 35 C again being optimal.

The above results and those of tests not detailed here showed that 35 C was optimal for expression of symptoms. In a few cases, however, the best results were obtained at 30 or 40 C. Although in the majority of tests an initial 4 days' incubation at 20 C was needed for the subsequent high temperatures to induce symptoms, in a few cases initial incubation of 3 or 5 days was needed. In comparative tests, the high-temperature effect on disease development was always more pronounced on Yellow Special than on Michal.

DISCUSSION.—The optimum and maximum temperatures for colonization of *P. tabacina* in tobacco leaves were found to be 15-20 and 30 C, respectively (Fig. 1). These values correspond fairly well with the values reported in the literature (1, 2, 3, 4, 5, 8). The lack of full agreement between our data and those cited above, as well as among themselves, probably derives from the difference in the methods of evaluation.

Temperatures above 30 C, when applied in short thermoperiods, decreased the disease development (Fig. 3, 4), and when applied constantly were lethal to the pathogen after a comparatively short time (Fig. 2). These results disprove the assumption that a thermophilic strain of *P. tabacina* was involved in the appearance of blue mold symptoms during hot weather. The alternative assumption, that high temperatures induce disease in leaves previously infected by the fungus, seems to be correct. Thus, at temperatures favorable for the pathogen, the highest grade of symptoms occurred at 25 C (Fig. 1). However, when the infected plants were exposed to short periods of high temperatures, those exposed to 30-35 C developed the most marked symptoms (Fig. 4-B). This shift of peak of disease expression from 25 C at constant temperatures to 30-35 C in 2-4 hr periods is apparently due to the fact that a certain extent of previous colonization at a convenient temperature (e.g., 20 C) is needed if high temperatures are to exert their effect. The last point was further developed in the final series of experiments, which proved that in most cases 4 days' incubation at 20 C is needed if high temperatures are to exert their effect, with 35 C being optimal in spite of its lethal effect on the pathogen.

In view of these findings, it appears that colonization and disease development are processes which respond to different environmental conditions. This is supported by the fact that almost no lesions appeared in inoculated plants incubated at 10 C (Fig. 1), and by observations showing that in infected seed beds grown in cold weather, no lesions are seen in the germinating plants in which sporulation is often the sole sign of disease. On the other hand, lesions appearing at high temperatures do not often yield spores because these temperatures are lethal to the fungus. A schematic representation of the effect of temperatures on the development of the pathogen and of disease symptoms is outlined in Fig. 6.

LITERATURE CITED

1. Armstrong, G. M., & C. D. Summer. 1935. Investigations of downy mildew of tobacco. S.C. Agr. Exp. Sta. Bull. 303:5-23.
2. Clayton, E. E., & J. G. Gaines. 1945. Temperature relation to development and control of blue mold (*Peronospora tabacina*) of tobacco. J. Agr. Res. 71: 171-182.
3. Hill, A. V. 1965. The role of temperature in the development of blue mold (*Peronospora tabacina* Adam) disease in tobacco seedlings. II. Effect on plant growth. Australian J. Agr. Res. 16:609-615.
4. Hill, A. V., & Sheila Green. 1965. The role of temperature in the development of blue mold (*Peronospora tabacina* Adam) disease in tobacco seedlings. I. In leaves. Australian J. Agr. Res. 16:597-607.
5. Jadot, R. 1966. Effets de la temperature sur l'incubation de *Peronospora tabacina* Adam en milieu conditionne. Parasitica 22:208-215.
6. McGrath, Hilde, & P. R. Miller. 1958. Blue mold of tobacco. Plant Dis. Reptr. Suppl. 250:3-35.
7. Schein, R. D. 1964. Design, performance, and use of a quantitative inoculator. Phytopathology 54:509-513.
8. Shepherd, C. J, &. M. Mandryk. 1967. A necrotrophic reaction in Nicotiana species induced by *Peronospora tabacina* Adam. Australian J. Biol. Sci. 20: 87-102.
9. Tukey, J. W. 1953. The problem of multiple comparisons. Dittoed MS of 396 pages, Princeton Univ., Princeton, N.J. (After Scheffe, H. The analysis of variance. John Wiley & Sons, Inc., N.Y., 1959, p. 477).