STUDIES ON THE PHYSIOLOGY OF PRIMARY INFECTION BY ERYSIPHE GRAMINIS TRITICI EL. MARCHAL, THE CAUSE OF POWDERY MILDEW OF WHEAT

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ABSTRACT

STUDIES ON THE PHYSIOLOGY OF PRIMARY INFECTION BY ERYSIPHE GRAMINIS TRITICI EL. MARCHAL, THE CAUSE OF POWDERY MILDEW OF WHEAT

by K. R. Sadasivan Nair

The purpose of this research was to determine the optimum conditions of temperature, light and relative humidity (RH) for each of the component phases of primary infection by powdery mildew of wheat.

The "rolling method" of inoculation devised during this study eliminated most disadvantages of previous methods. It utilized only fresh, young, separate conidia of fairly uniform age, and provided for their even distribution on the host leaf. This method together with the precise control of environmental conditions gave uniform and highly reproducible results.

Primary infection consisted of four separate stages: 1) germination of conidia, 2) formation of appressoria, 3) penetration of host, and 4) development of secondary hyphae. Each stage had a different set of optimum conditions. For germination, a relative humidity of 100% was more favorable than 65% at either 17 or 22°C, and maximum germination of 60% occurred in 4 hours. However, when the relative humidity was changed from 100 to 65% after the 4th hour, 93% germination was obtained after 5 more hours at 22°C. The same high level of germination was obtained in 4 hours by incubating inoculated plants for 1 hour at 17°C and 100% RH and 3 hours at 22°C and 65% RH. Since germination could be increased by altering relative humidity and temperature during the germination period the conidial population

probably was not physiologically uniform. Light (14-30 foot-candles or 120-290 foot-candles) or darkness did not influence germination. Appressorial initials were formed equally well after the 4th hour on plants exposed to 22 or 28°C, 32 or 65% RH, and in low light (14-30 foot-candles), high light (120-290 foot candles) or darkness. However, the largest number of appressoria matured at 22°C and 32% RH in low light, beginning with the 6th hour. By the 20th hour every germinated conidium had produced an appressorium. Penetration of host by the pathogen took place 6-8 hours after development of appressorium. This was followed by development of secondary hypha marking the end of primary infection. The rate of development of secondary hyphae was essentially the same at 32% and 65% RH, both conditions were more favorable than 100% RH. At 22°C and 65% RH in low light, secondary hyphal development began by the 18th hour after inoculation, and by the 32nd hour, 77% of the conidia had produced secondary hyphae.

A difference of one week in the age of the host plants did not influence germination and appressorial development. However, a slower rate of secondary hyphal development occurred on the leaves of older plants, probably due to a thicker cuticle on the older leaf.

Though the ideal conditions of temperature, relative humidity and light revealed in this study for different phases of primary infection apply to maximum mildew development in minimum time, varying degrees of mildew development were observed at all conditions studied. Thus, these conditions are not obligatory.

The optimum conditions for each phase of infection correspond to the conditions the fungus would normally encounter in a wheat field. In summer, conditions are ideal for conidial germination beginning at about 3:00 AM. Appressoria would mature by noon, penetration of host would take place during the afternoon, and development of secondary hyphae would begin at about 8:00 PM the same day.

STUDIES ON THE PHYSIOLOGY OF PRIMARY INFECTION BY *ERYSIPHE GRAMINIS TRITICI* EL. MARCHAL, THE CAUSE OF POWDERY MILDEW OF WHEAT

By

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Dedicated To My

Father and Mother

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INTRODUCTION

Powdery mildew of wheat, caused by Erysiphe graminis tritici El. Marchal is one of the very early recorded diseases of this crop. This disease has been reported from almost all wheat-growing countries of the world (1, 10, 19, 22, 23, 27, 37, 54, 59, 60). Powdery mildew is ranked second only to rust, if not equal to it, in its economic importance (9, 22, 23, 26, 27, 29, 47, 60). Considerable attention has been given to this disease since 1900, especially with regard to its symptomatology and epidemiology, and to its control by chemical means and through breeding for resistance. Yarwood (70) has made an excellent review of studies on this pathogen up to 1957.

Though E. graminis tritici can infect the host by means of ascospores and conidia, the present study is restricted to the primary infection of the host by conidia. Primary infection as used here refers to the complex process from germination of the conidium on the host until the establishment of a successful host-parasite interaction. This process involves several steps.

Previous workers have considered the entire process of infection as one step and have reported the effect of temperature, relative humidity, light, and other factors on the overall process of infection (10, 14, 22, 23, 40, 41).

Studies on the infection process by the uredospores of stem rust of wheat (Puccinia graminis tritici Ericks. and E. Henn.) (45, 53), and preliminary studies on the process of infection by powdery mildew indicated that primary infection is not a single step, but consists of at least 4 separate stages: 1) Germination of the conidium and the formation of the germ tube, 2) Formation of the primary appressorium, 3) Penetration

and formation of the primary haustorium, and 4) Development of the secondary hypha, which marks the establishment of a successful host-parasite interaction.

These different stages have been studied with the object of determining the optimum conditions for each stage of infection. With this information it should be possible to (a) better understand the host-parasite relationship as influenced by the environment, (b) increase the efficiency of infection, i.e., to obtain maximum infection by artificial inoculation in minimum time, and (c) predict with a high degree of accuracy the stage of infection and interaction between the host and parasite at any time in a known set of environmental conditions.

LITERATURE REVIEW

A diurnal periodicity in the production, dissemination and germination of conidia of some powdery mildews has been observed. There are reports of only one (11, 66) or several (24) conidia of Erysiphe polygoni DC. produced per conidiophore per day. However, no diurnal cycle was observed for the production of conidia of Erysiphe graminis DC. (10, 66). Dissemination of conidia of E. polygoni and E. cichoracearum DC. takes place mostly between noon and 4:00 PM. (49, 66), while in Spherotheca humuli (DC) Burr. dissemination occurs principally at night (31). Highest per cent germination of conidia of E. polygoni occurred when they were collected at mid-day (7) or from mid-day to 4:00 PM. (66). Germination decreased at night and reached a minimum in the early morning (66). Cherewick (10), however, could find no diurnal cycle of germinability of the conidia of E. graminis from wheat or barley.

Childs (11) classified powdery mildews into non-chain forming and chain-forming types. The former type, e.g., E. polygoni, has one or two conidia, and the latter type, e.g., E. cichoracearum, has 2-8 or more conidia maturing at a time. E. graminis belongs to the chain-forming type.

There have been reports of decreased germination percentage when the conidia remain in chains. Thus total germination decreased as the length of the chain increased (5, 6, 16), since it is usually the terminal rather than the intercallary conidia that germinate (6). This was explained by Brodie (5, 6) on the basis of facility for oxygen and carbon dioxide exchange through the permeable papillae which are located at both ends of each detached conidium. Delp (16) suggested that a germination inhibitor may be supplied to the conidial chain through the

conidiophore from the parent fungus mycelium which inhibits the germination of the terminal conidium attached to the parent conidiophore which has the papillae exposed at one end. Cherewick (10), however, found that germination in situ does occur, particularly when the infected tissues are kept at approximately 3°C and 100% RH.

Poor germination has been observed when the conidia are crowded (18, 37). This self-inhibition has been variously explained as either due to an exudation of a toxic substance by the conidia themselves, to limited oxygen supply (18), or to high carbonic acid or carbon dioxide content (18, 37).

Conidia germinated readily on leaves of plants other than the host (14, 55, 62), as well as on glass slides or on gelatin (17). In most studies a lower germination percentage was obtained on glass slides than on host leaves under identical conditions. Delp (16) obtained similar percentage of germination on a glass slide and on a host leaf under comparable environmental conditions, although others (30, 50, 66) claimed that host contact favors germination. Weinhold (61) has shown that under similar conditions there was a 6-12 fold increase in germination of peach mildew (Sphaerotheca pannosa (Walls.) Lev.) conidia on the host leaf as compared with that on a glass slide. He also obtained essentially the same per cent germination on host leaf and on parlodion membrane under favorable conditions. Evidently, water relations on parlodion membrane approach those of a leaf. Conidia of wheat powdery mildew germinated when inoculated onto four species of Triticum, two species of Hordeum, one species each of Bromus and Agropyron, Sonchus arvensis, and Capsella brusa-pastoralis (55). Conidia of Erysiphe graminis hordei El. Marchal germinated on barley, oats, rye, wheat and Agropyron; conidia of Erysiphe graminis tritici El. Marchal germinated on wheat, barley and Agropyron (14, 62, 66). It seems that germination of conidia on non-host leaves can be essentially the same as that on host leaves, if other conditions are favorable.

The overall process of primary infection by several powdery mildews seems to follow the same general scheme. Upon germination, the conidium puts out a germ tube from one 'corner' but not from the ends or the sides (70). The number of germ tubes produced may be one (7, 55) or more than one (46). Cherewick (10) found that 1-2 germ tubes are produced at temperatures below 35°C whereas 3-9 germ tubes are produced at higher temperatures. The germ tube becomes septate and swollen near the apex (55) and finally a much convoluted appressorium is formed (70). According to Corner (14), penetration of the host cell begins 24 hours after inoculation at about 20°C and 100% RH. There have been reports of appressorial formation and penetration of non-hosts by several powdery mildews (55, 62). Penetration of the host is effected by a stylar process proceeding from the center of the appressorium. The cuticle is penetrated mechanically, and a haustorium is produced inside the host epidermal cell (14). After 48 hours the first haustorium is more or less fully grown; after 72 hours all the secondary germ tubes have developed and a fairly extensive mycelium has formed. Conidia are produced in 4-6 days (14, 55).

Among the different factors influencing the development of powdery mildew, temperature, relative humidity (RH) and light have been most thoroughly investigated. Most studies were limited to one stage of development, i.e., germination, or to the general development of the mildew. Many of the conflicting or incomplete reports seem to be due to the lack of understanding that infection consists of separate component phases which may not have similar optima.

Temperature has been usually regarded as more important than relative humidity in controlling the development and distribution of powdery mildew (16, 50). Reports that mildew thrived during the hot summer in England when other fungi suffered seriously (14) and that

powdery mildew cultures survived greenhouse temperatures up to 43°C when there were alternating cooler periods (10) suggest the great tolerance of these fungi to heat.

Conidia of powdery mildew germinated over a wide range of temperature; the minimum reported is 0°C (10) and the maximum is in the range of 30-36°C (1, 10, 16). The optimum temperature is in the range of 12-25°C for several powdery mildews (16, 21, 22, 40, 50, 61). By contrast, the minimum, optimum and maximum temperatures (15.5, 21 and 26.5°C respectively) (28, 53) for infection with uredospores of wheat stem rust, show a much narrower range for this fungus. Powdery mildews seem to have an unusually great tolerance to low and high temperatures. With barley and wheat mildew, Cherewick (10) obtained a shift in the optimum temperature for germination of conidia from 10°C, when the natural light conditions were dull, to 15-20°C when the conditions were bright. Maximum development of E. graminis was delayed by 5-6 days when the temperature was reduced from 25° to 15°C (36).

No other aspect of the study of powdery mildew is more controversial than the effect of relative humidity. The predominance of powdery mildew in the arid areas and the progressive decrease of powdery mildew in humid or wet areas is attributed to the adverse effect of high relative humidity or to rainfall (3, 38, 67). Yarwood (68) suggested a control of powdery mildew by sprinkling water. While there are reports that free water reduces germination or produces abnormal germination (14, 16, 22, 23, 37), Weinhold (61) found that the primary requirement for germination of conidia of peach mildew (Sphaerotheca pannosa (Walls.) Lev.) on peach leaves is a suitable source of free water. Since, under identical conditions, percentages of germination on the host leaves and on parlodion floating on water were similar and greatly exceeded that on glass slide or parlodion-coated glass slide, he concluded

that the conidia on the leaves obtain the required moisture for germination through the cuticle and therefore the relative humidity of the air would not be a major factor influencing germination.

It appears that the humidity requirements for the germination of the conidia of different powdery mildews are widely different. While there are reports that high relative humidity reduced germination (16, 50), there are other reports that the conidia of several powdery mildews germinated better at 85-100% RH, and that very little germination took place below that level (1, 2, 10, 13, 23, 30, 37, 50). A very high degree of tolerance to a wide range of relative humidity is exhibited by powdery mildew of rose (Spherotheca pannosa), sunflower (Erysiphe cichoracearum), clover, cabbage, evening primrose (E. polygoni), poa (E. graminis) and Leveillula taurica (Lev.) Arn. (7, 13, 37, 67), the conidia of which germinated from 0 to 100% RH. Even among those conidia which germinated in very low relative humidity, many shrivelled and died in the dry air, and the tolerance to low humidity decreased with increase in temperature (67).

The effect of relative humidity on the development of mildew is also reported differently by different workers. Grainger (23) found a direct correlation between the number of hours of 100% RH per week and the per cent of oats leaf area covered by mildew. Reid et al. (44) also found that high relative humidity favored wheat mildew infection. Other reports claim a favorable effect of low relative humidity (38, 50, 52) or only a slight effect of relative humidity on mildew development (67). Nour (37) obtained similar development of barley mildew under saturated conditions and under conditions of normal climatic fluctuations.

The occurrence of greater mildew infection on the lower leaves of plants is believed (23) to be due to the higher relative humidity near the soil and to the lower leaves being exposed to higher saturation for a longer period than are the higher leaves. Tapke (57), however, observed

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more mildew on leaves near the soil than the terminal ones even when the mildewed barley plants were kept inverted. In this experiment the relative humidity was well below 100% and did not differ from top to bottom of the plants. He concluded that the mildew gradient is due to factors other than humidity of the atmosphere. The greenhouse conditions which gave the above results are entirely different from the field conditions where the stands are thick, and the leaves form a canopy above the soil reducing the air currents. Field conditions would maintain the higher relative humidity produced by transpiration of the overcrowding leaves near the soil. In lettuce powdery mildew (E. cichoracearum DC.) Schnathorst (51) also found no appreciable difference in relative humidity at different levels from top to bottom of the plant. He believes that the higher incidence of mildew on the lower leaves of lettuce is due to physiological differences rather than relative humidity, but indicated that "microclimate may be a major factor affecting the development of foliar pathogens in dense stands."

"Conflicting ideas of the relation of water to infection with powdery mildew have led to contrasting methods for making artificial inoculations" (70). The usual method is by dusting the dry conidia onto the dry leaves of the host (7, 10, 13, 23, 32, 57, 66), or slight modifications of this method such as blowing the conidia onto the host leaf (42), using a camel hair brush or an inoculating needle (17, 36), by drawing a conidium-bearing specimen over the leaf to be inoculated (36), or placing single conidium on a host leaf (65). Others have used conidial suspensions in water (22, 48).

A stimulatory effect of light on germination of mildew conidia and the development of mildew are reported by several investigators (48, 58, 63). Conidia of several powdery mildews gave higher germination percentage in light than in darkness (63). Increased percentage of germination of conidia collected during the light portions of the day or

under normal light rather than under reduced light or darkness has also been reported (10, 24, 66, 67). Mildew development was reduced in plants kept in the dark after inoculation (66). Development of mildew was favored by high carbohydrate level of the host (58, 64). Wheat and barley seedlings kept in the dark for 4 days before inoculation became quite resistant to mildew (10), whereas exposure of wheat plants to light rendered them susceptible (58). Increasing day length produced vigorous host plants and better development of mildew (48). Although light is essential for the normal development of mildew, slight shade gave better development than full daylight or stronger shading (15, 24, 58).

In many instances of host-parasite relations the thinner the cuticle of the host, the better the development of the pathogen. Succulence of the host and thinner host cuticle also favored development of powdery mildew (4, 67). Toughened barley plants grown outdoors developed only sparse mildew while tender plants grown under greenhouse conditions developed a heavy infection of powdery mildew regardless of age (56, 57). Graf-marin (22) showed that the cuticle of leaves from young barley plants was 0.4-1.5 μ thick while those from adult plants were 2.5-5.0 μ. By removing the cuticle, he obtained abundant infection of mildew on adult leaves which were normally almost resistant. A rougher surface or a heavier layer of wax on the epidermis is characteristic of cucumber plants resistant to powdery mildew (43). Little or no infection on an older leaf as compared to a young leaf was reported with Puccinia graminis Pers. on barberry and Rhyncosporium secalis (Oud.) J. J. Davis. on Brome grass (8, 12).

Studies on the process of infection by Puccinia graminis on wheat by Rowell et al. (45) showed that at 21°C under conditions of dew and little or no light maximum germination of uredospores took place during the first 4 hours. During the second 4 hours 52% of the appressoria developed under the same conditions. The appressoria remained quiescent

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when the free moisture on the leaf surface was dried slowly at 18.5°C under low light intensities and were viable even after 3 days at 18.5°C in the dark, irrespective of humidity (45). Sharp et al. (53) also found that germination and appressorial development of uredospores of wheat stem rust occurred equally over a range of 15.5-24°C and at light intensities below 300 foot-candles.

Post-appressorial development was favored by a higher temperature and light intensity than that which favored germination and appressorial formation (45, 53). When the appressoria were exposed to natural light of 1500 foot-candles or more at about 29.5°C they produced the substomatal vesicle and completed the infection process. On the other hand, Emge (20) reported that on artificial media, vesicle formation and development of infection hyphae occurred only in the darkness for 16-18 hours. In Puccinia sorghi Schw. (39) germination of uredospores on artificial substrates was best after 2 hours at 17°C in total darkness. Vesicle development was favored in light intensities under 200 foot-candles at 20-24°C in 4 hours. When either time period was prolonged or at temperatures above 35°C or in light intensities above 500 foot-candles, vesicle formation was retarded.

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MATERIALS AND METHODS

The present study was designed to investigate the relationship between the host, parasite, and environment under conditions as natural as possible. Therefore, all the experiments were done directly on the leaf of the intact, living host. Most other workers have used glass slides, gelatin, or excised leaves.

Maintenance of Stock Culture

The host plant used was the Little Club variety of wheat (Triticum compactum Host). Stock cultures were maintained on plants grown in 4 inch pots in the greenhouse or in a growth chamber. In the greenhouse, humidity was not regulated, no artificial lights were used, and the night temperatures were 21-24°C.. Inoculations were made during the late afternoon or early evening by shaking dry conidia onto the leaves of 10-day-old plants. Mildew was visible 4 days after inoculation and fresh conidia for inoculations were available at 5-7 days depending on the season (Figure 1).

During late spring and summer, when the temperatures in the greenhouse were high, stock cultures were maintained in a growth chamber at 20°C (± 2°). These plants were provided with artificial light from both incandescent bulbs and fluorescent tubes with a total light intensity of 450-650 foot-candles over a 15 hour day. Fluorescent lights alone did not give satisfactory results. Relative humidity in the chamber was 50-70% (± 5%) during the light hours and almost 100% during the dark hours. Under these conditions an abundant supply of conidia was available on the sixth day. Conidia were collected only once from each plant which was then discarded.

Figure 1. Growth and development of powdery mildew (Erysiphe graminis tritici) on wheat plants (var. Little Club) 5 to 8 days after inocubation under greenhouse conditions. Left to right: plants 5, 6, 7 and 8 days after inoculation.

Methods of Inoculation

Three inoculation methods were used in this study. In an early experiment, inoculations were made by the usual "dusting method" (7, 10, 13, 23, 32, 57, 66). The pot of plants to be inoculated was laid on its side and slowly rotated while the mildewed plants which furnished the inoculum were held about 2 feet above and shaken vigorously. This method resulted in the deposition of most conidia in clumps and in chains. Their distribution was not uniform. Since only single conidia were counted, the per cent of germination was based only on a fraction of the conidia actually present on the leaves.

Later, a new method of inoculation, "rolling method (unmodified)," was developed. Mildewed plants 6-7 days after inoculation were inverted and shaken to remove the old conidia. About 6 hours later, a swab of soft, sterile, absorbent cotton was gently rolled over selected young pustules to pick up the conidia. The conidia were transferred to the host leaf by gently rolling the cotton swab onto the upper surface of the leaf to be inoculated. This method had important advantages over the dusting method. Clumps of conidia were practically eliminated and the number of chains of conidia was reduced. In addition, the conidia were more uniformly distributed on the host leaf surface.

The disadvantage of this method was the removal by the cotton swab of some immature conidia, a few collapsed conidia, and a few chains of conidia some of which were still attached to the intact conidiophores. Thus, the conidia used for inoculation were not perfectly uniform in age and maturity.

The inoculation method was further improved and called the "rolling method" (34). In this method, used in most experiments, the conidia were harvested from young, developing pustules on the host 6-7 days after inoculation. Old conidia present on the pustules were removed and discarded by gently shaking the plants and blowing away

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the conidia. Four to six hours later, conidia from fresh young pustules were collected by gently tapping the leaf with a glass rod over a 1 x 3 inch glass slide. These conidia were examined under a microscope to determine the uniformity of distribution and the number that had germinated. When fresh conidia were collected 6 hours after old conidia were shaken off and discarded, 5-8% conidial germination in situ was noted if the relative humidity of the air was about 70% or more. At a lower relative humidity, the value was 0-4%. When the time between removal of old conidia and collection of fresh conidia was reduced from 6 hours to 4 hours, the average percentage of conidial germination in situ was less than 2%. As single conidia tended to adhere to the glass surface, most clumps of conidia could be removed by gently blowing on the slide. Thus a thin layer of single conidia remained. The conidia were picked up by gently rolling a soft, sterile, absorbent cotton swab over a small portion of the glass slide. The conidia were then transferred to the host leaf by one gentle roll forward and backward over an inch of the leaf surface near the tip. About 15 plants could be inoculated from the conidia collected on one slide, but a new cotton swab was used for each inoculation. Extreme care was taken to use minimum pressure with the cotton swab at all times, since the thin-walled conidia could be easily crushed. By adjusting the quantity of conidia on the glass slide, a relatively uniform number was obtained on a replicated set of host plants. A 2-inch section of the leaf on each of 12 plants could be inoculated in 2 minutes.

This method of inoculation virtually eliminated conidia in clumps and chains and gave conidia of reasonably uniform age, maturity, and germination. The conidia were examined under a microscope before inoculation to ensure the suitability of the inoculum. Any remaining conidial chains were broken up when the conidia from the glass slide were picked up by the cotton swab and rolled onto the host leaf.

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Breaking the conidial chains afforded each conidium the opportunity of germination (5, 6, 16). Although a few conidia were crushed during inoculation, with careful technique the number was negligible. The conidia were distributed uniformly on the host leaf and thus the possibility of self-inhibition as a result of crowding (18, 37) was avoided. This method was very economical as it used little inoculum. Any specific portion of the plant could be inoculated.

Host plants used for the experiments were 5-6 days old and at a stage when the first leaf was fully formed and the second leaf was emerging. For preliminary studies the host plants were grown in groups of 10-30 in 4 inch pots. In all subsequent studies, plants grown singly in 1 inch pots were used for inoculation. Inoculations were performed during early morning, forenoon, afternoon, or early evening. No difference in the production or germinability of the conidia was observed at any time.

Control of Environmental Conditions

Inoculated plants were incubated at various temperatures, relative humidities, and light intensities. Temperatures of 13, 17, 22, and 28°C; relative humidities of 32, 65, and 100%; and light intensities of 14-30 foot-candles (low light), 120-290 foot-candles (high light), and darkness were used. During fall and winter, experiments were done at night in diffused light in greenhouses regulated at 13, 17, and 22°C (± 2°) or in a growth chamber at 17°C (± 2°). During spring and summer when the experiments were done in the laboratory in different light conditions, temperatures of 17, 22, and 28°C (± 2°) were used. The laboratory itself was maintained at 22°C and thus required the use of only two incubators, at 17 and 28°C. Experiments in the laboratory were also done at night so that comparisons could be made with the previous experiments.

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Relative humidity experiments were performed either in commercial incubators or in hand-constructed boxes. The latter were made of wooden frames 30 x 25 x 18 inches and covered with clear 4 mil polyethylene film. All edges and joints were secured and made moisture-tight with plastic tape. Two 8 x 10 inch flaps of polyethylene film on the front of each box served as convenient doors for inserting and removing plants. The doors were taped shut when the boxes were in use. For complete darkness, the boxes were covered with black 4 mil polyethylene film. For low and high light, fluorescent tubes were placed above the boxes or in front of the incubators. The incubators were made moisture-tight by sealing the entry with polyethylene film; flaps of the film served as small doors.

Relative humidities of 32 and 65% were maintained by placing calcium nitrate or sulphuric acid solutions (25) in a number of small beakers which were arranged along the walls inside the boxes. No toxic effect of sulphuric acid was noted on the plants or the fungus. For maintaining a saturated atmosphere, a layer of distilled water about 2 inches deep was placed in the bottom of the polyethylene boxes or in deep, rectangular, aluminum baking pans placed along the sides. In either case, there was heavy condensation of water on the walls of the box which assured a saturated atmosphere.

In the two lower humidities, the pots and the surface of soil were covered with inverted polyethylene freezer bags to prevent evaporation of water. The free edges were sealed with masking tape and the plant projected through a slit in the bag (Figure 2).

One hygro-thermograph calibrated every week or 10 days was installed inside each box. Six representative hygro-thermograph charts selected at random illustrate the degree of control of the humidity and temperature (Figure 3).

Figure 2. Wheat plant (var. Little Club) in the first leaf stage (6 days old) used for inoculation. The pot and soil surface are covered with a polyethylene bag.

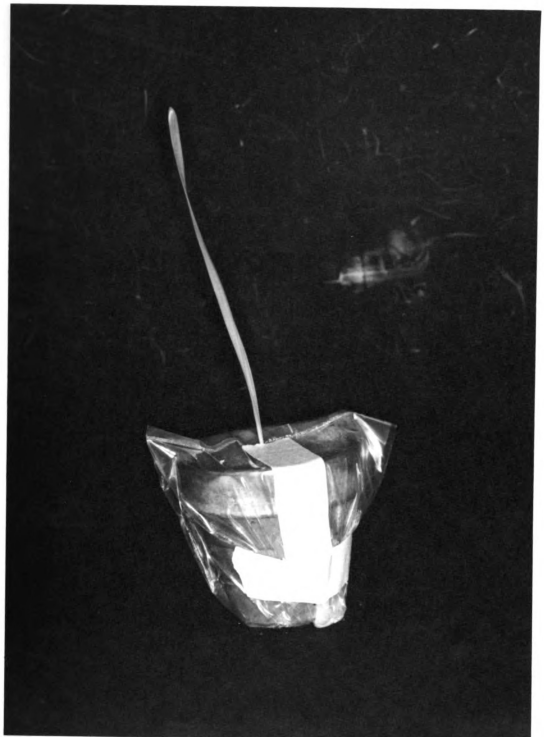
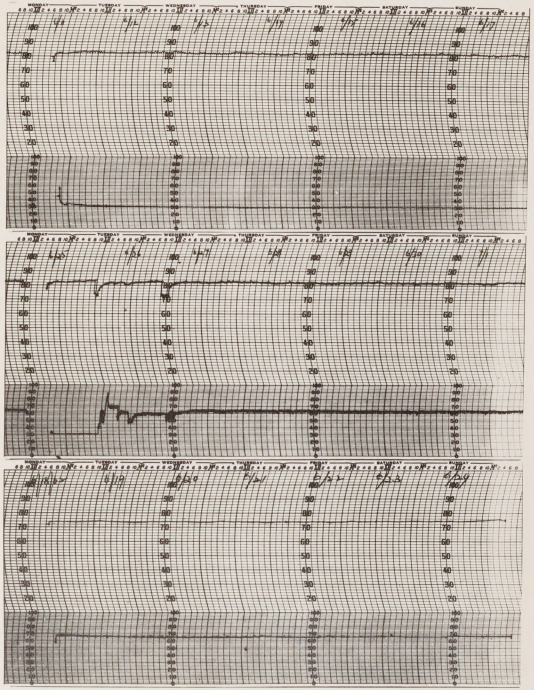
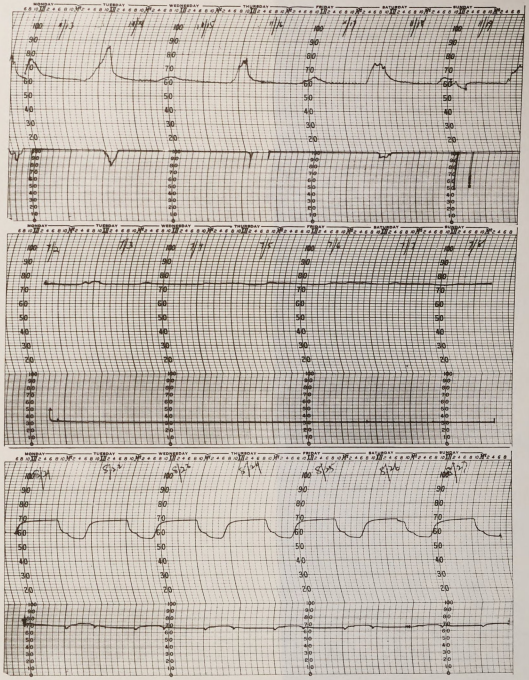


Figure 3. Six representative hygro-thermograph charts illustrating the precision of control of humidity and temperature inside the incubation chambers.





Examination of Conidia

One plant was removed from the box or incubator at the end of each hour or at other required times during the period of study, and the inoculated portion of the leaf was cut into sections approximately 1 cm. in length on a glass slide. These fresh sections were immediately examined under a microscope. The number of conidia on the 1 cm. leaf section varied in the different experiments from 30-120. The time required for examining each section was 12-15 minutes. Since the conidia were living, some development must have taken place during this period, but since this short time was involved in every case it was not considered to be a serious objection. This method was preferable to killing the conidia and clearing the leaf in hot lactophenol or other chemicals, since this resulted in washing the conidia into the furrows of the leaf, and produced a crowded condition which rendered accurate counting impossible. Some conidia were washed off the leaf which prohibited quantitative studies. The total number of conidia as well as the number of conidia at different stages of development were counted and recorded. The plants were then discarded. Therefore, a different population of conidia on a different plant was examined at each time interval. Each experiment was replicated 4-12 (usually 6-8) times, on successive days. Usually 40-60 conidia were present on each 1 cm. section of the leaf. A total of at least 400 conidia were examined for each time interval. The total number of conidia examined for the entire research was 146,047.

Statistical Analysis

Comparison of results of different treatments and determination of statistical significance were done by a modified chi square test, applying the formula $\frac{(p_1 - p_2)^2}{s_1^2 + s_2^2}$ where, p_1 and p_2 are the proportions of

conidia in a particular stage of development under two different conditions, and s_1 and s_2 are corresponding standard deviations. This test was suggested by Dr. Herman Rubin and Dr. Esther Seiden, taking into consideration the special characteristics of this study; namely, 1) incomplete blocks in the treatments in some experiments, 2) necessity for a 2x2 comparison of the different treatments to determine the most favorable among them, and 3) necessity to compare a hypothetical per cent of germination with the actual per cent germination in some experiments. The significant difference between the two, and thus an estimation of the more favorable set of conditions, was determined with the aid of tables of percentiles of chi square distribution using one degree of freedom. Differences at the 5% level were considered statistically significant for the purpose of this study. Since the populations studied were fairly large (normally 500-800) and the variations between replications of the same experiment were very small, this test assured no loss of precision as compared with the analysis of variance. The level of significance of variation between replications of different treatments from various experiments at different hours of study and stages of development were calculated using chi square tests.

Precision in Methodology

The methods developed in this study gave consistent results with excellent reproducibility between repetitions of the same experiment. Chi square tests were made to determine the amount of variation between repetitions of 21 different treatments from various experiments. Random selection of treatments were made at different hours for germination, formation of appressoria and formation of secondary hyphae. The dusting method or the unmodified rolling method of inoculation gave much larger variation between repetitions than the rolling method (Table 1).

Table 1. Results of Chi Square tests for variability between replications of 21 different treatments from various experiments, both selected at random.

Stage of Study	Method of Inoculation	P
Germination	Dusting	.05
Germination	Unmodified Rolling	.01
Germination	Rolling	.1
Germination	Rolling	.9
Germination	Rolling	.2
Germination	Rolling	.01
Germination	Rolling	.9
Germination	Rolling	.2
Germination	Rolling	.6
Germination	Rolling	.9
Germination	Rolling	.9
Germination	Rolling	.7
Germination	Rolling	.9
Appressorial Development	Rolling	.4
Appressorial Development	Rolling	.4
Appressorial Development	Rolling	.05
Appressorial Development	Rolling	.1
Appressorial Development	Rolling	.9
Secondary Hyphal Development	Rolling	.8
Secondary Hyphal Development	Rolling	.6
Secondary Hyphal Development	Rolling	.2

P = probability of obtaining greater variability due to chance alone.

In the analysis of 19 treatments where the rolling method was used, variation between replications was very low except in two treatments (Table 2). This showed that by the use of the rolling method where fresh, viable, single conidia of reasonably uniform age and maturity were evenly distributed on the host leaf, remarkably uniform results would be obtained. Other factors contributing to the consistency of the results were the precise control of temperature (± 2°) and relative humidity (± 3%).

Table 2. Variation in formation of germ tubes, appressoria or secondary hyphae among replications of 7 treatments selected at random when plants were inoculated by the rolling method.

Stage of Study	Hour	Replications	Per Cent	P
Germination	5	1	54.8	
		2	52.2	
		3	53.6	.1
		4	80.5	
		5	64.1	
Germination	10	1	85.8	
		2	82.5	
		3	84.7	.6
		4	83.3	
		5	90.0	
		6	85.0	
		7	75.0	
Germination	6	1	96.3	
		2	95.0	
		3	95.3	
		4	100.0	.9
		5	93.9	
		6	94.7	
		7	94.9	
		8	94.9	
Appressoria	12	1	81.5	
		2	78.5	
		3	76.2	
		4	84.4	.4
		5	81.5	
		6	68.3	
		7	88.0	

Continued

Table 2 - Continued

Stage of Study	Hour	Replications	Per Cent	P
Appressoria	9	1	0.0	
		2	7.8	
		3	8.3	
		4	5.6	
		5	8.5	
		6	10.0	.9
		7	7.1	
		8	10.6	
		9	4.0	
		10	10.5	
Secondary Hyphae	32	1	61.5	
		2	56.2	
		3	54.0	
		4	62.5	
		5	63.1	.8
		6	55.7	
		7	63.0	
		8	53.8	
Secondary Hyphae	28	1	20.6	
		2	30.5	
		3	26.0	
		4	26.4	
		5	34.7	.6
		6	31.2	
		7	34.2	
		8	29.3	

P = probability of obtaining greater variability due to chance alone.

RESULTS

Germination of Conidia

Germination of the conidia began shortly after inoculation and appeared as small budlike projections which rapidly grew out into germ tubes. Although the term tubes are usually produced from the corners, it was not uncommon for them to emerge from the centre or even from the very end of the conidium, where the adjacent conidium was attached. The usual number of germ tubes per conidium was 1-4 and very rarely more.

Effect of Temperature and Relative Humidity on the Germination of Conidia

In a preliminary study to determine the optimum temperature for the germination of the conidia, groups of host plants, 10-30 per pot, were inoculated by the dusting method and placed under benches in three temperature-controlled rooms in the greenhouse at 13.5, 17 and 22°C, respectively. More conidia germinated at 17°C than at 13.5 or 22°C (Figure 4). A maximum germination of approximately 50% was obtained 5-6 hours after inoculation at 17°C.

In a preliminary study of the effect of relative humidity on conidial germination, plants were inoculated by the rolling method (unmodified) and incubated at 65 or 100% RH in diffused light under a bench in a 17°C growth chamber.

At 100% RH, 70% germination was obtained in 6 hours whereas at 65% RH, the germination was only 60% in the same period (Figure 5).

The effect of various combinations of temperature and relative humidities on the germination of conidia were studied using 13, 17 or 22°C and 65 and 100% RH. All possible combinations were used except

Figure 4. Effect of incubation temperatures of 13, 17 and 22°C on germination of Erysiphe graminis tritici conidia on wheat plants.

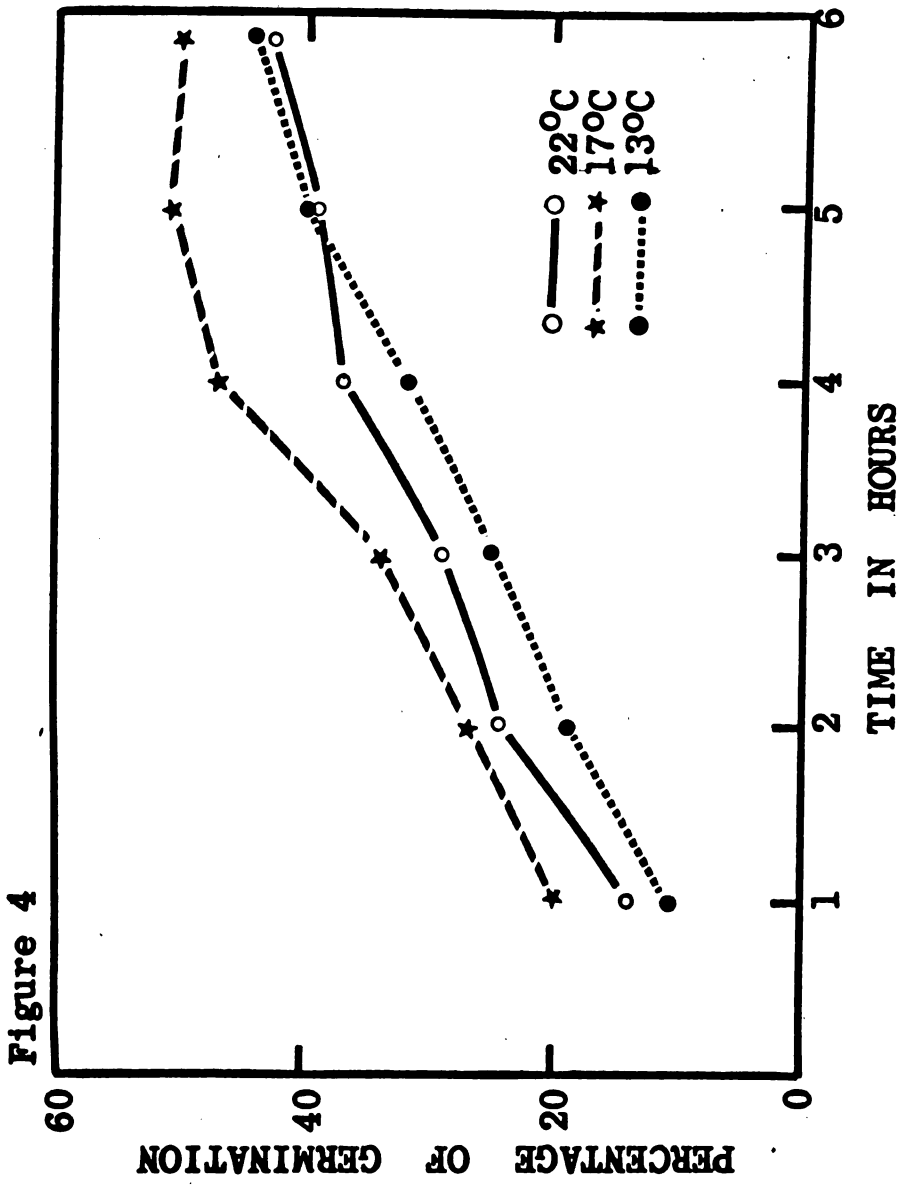

PERCENTAGE OF GERMINATION
0
20
40
60
TIME IN HOURS
1
2
3
4
5
6
22°C
17°C
13°C

Figure 4

Figure 4. Effect of incubation temperatures of 13, 17 and 22°C on germination of Erysiphe graminis tritici conidia on wheat plants.

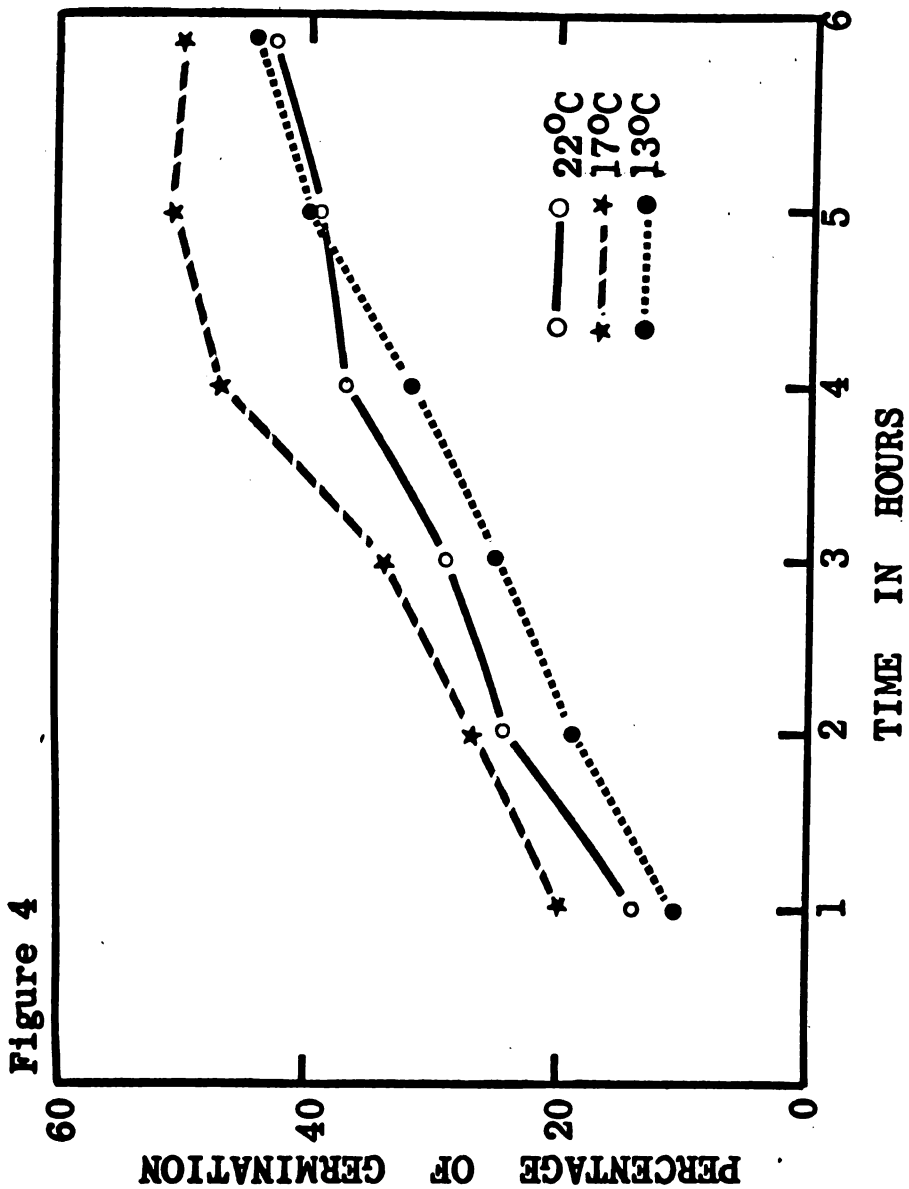

PERCENTAGE OF GERMINATION
0
20
40
60
TIME IN HOURS
1
2
3
4
5
6
22°C
17°C
13°C

Figure 4

Figure 5. Effect of relative humidities of 65 and 100% on the germination of Erysiphe graminis tritici conidia on wheat leaves at 17°C. Each value at 100% RH differed statistically from that at 65% RH for the same hour.

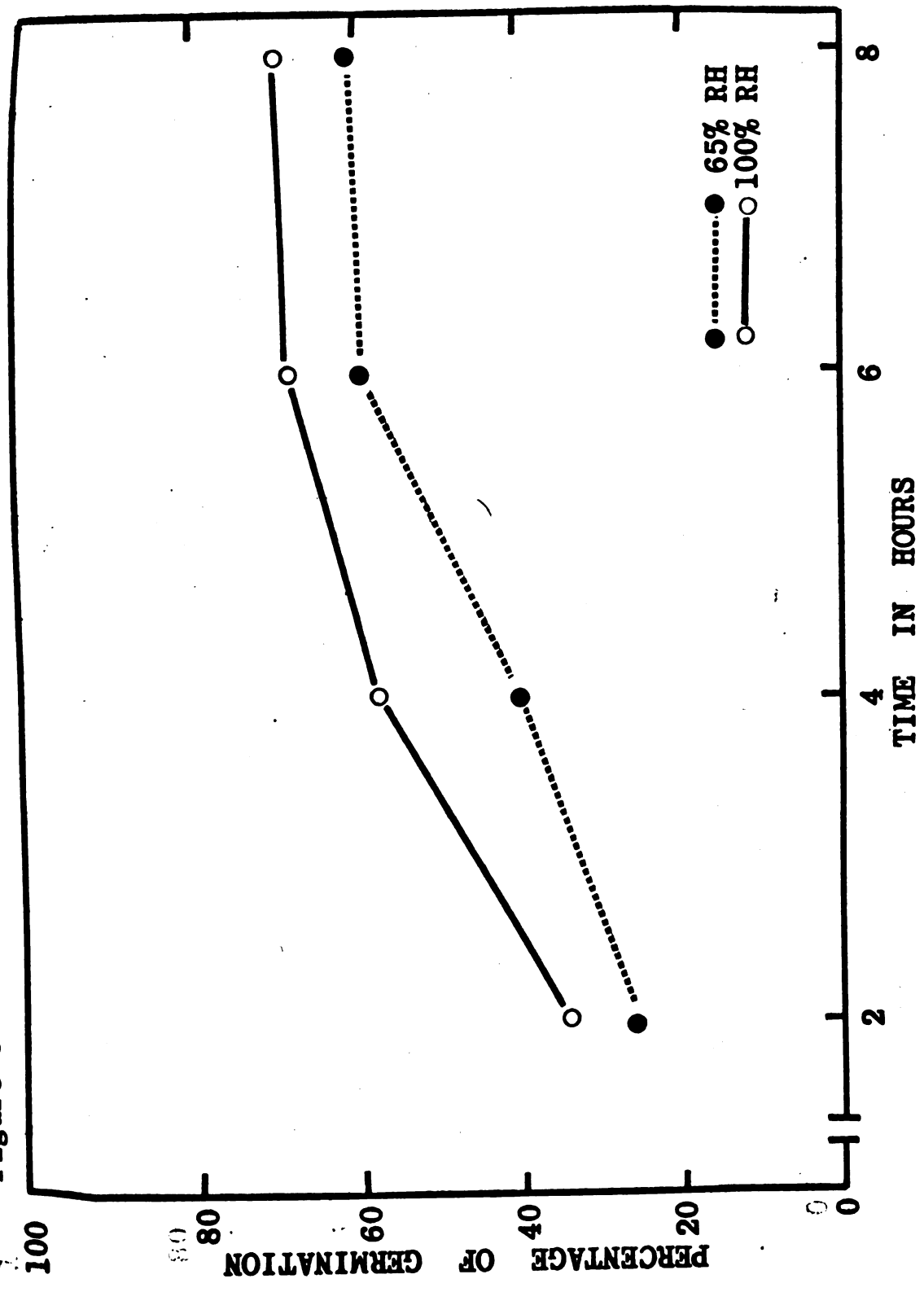

PERCENTAGE OF GERMINATION
100
80
60
40
20
0
0
2
4
6
8
TIME IN HOURS
65% RH
100% RH

Figure 5

13°C and 65% RH. In this and all subsequent work reported in this study the rolling method of inoculation was used.

At all three temperatures, more conidia germinated at 100% RH than at 65%. At 100% RH, a higher percentage of germination occurred at 17 or 22°C than at 13°C. Even though germination was higher at 17°C and 100% RH than at 22°C and 100% RH at the third hour after inoculation, by the fifth hour germination in both these environments was the same. Since the combination 17°C and 100% RH gave maximum per cent germination (65%) at a faster rate, it was considered more favorable than 22°C and 100% RH (Figure 6).

The difference between the numbers of germinated conidia at the 2 relative humidities was expressed within 1 hour following inoculation, when germination at 100% RH exceeded that at 65% at both 17 and 22°C. Thereafter, the difference in the numbers of germinated conidia at the 2 relative humidities remained approximately the same, even though the percentage of germination increased with continuous exposure. Under these conditions, when germination was determined at 15 minute intervals during the first hour, a higher rate of germination was observed at 100% RH than at 65% RH, confirming the previous finding. This rate difference occurred irrespective of temperature.

Effect of exposure to variable temperatures and relative humidities or an increased incubation time, on conidial germination was studied. Inoculated plants were incubated initially at 17°C and 100% RH for 4 hours and then placed at 13, 17 and 22°C and 100% RH for an additional 8 hour period. Essentially no additional germination took place at any of the 3 temperatures after the initial incubation period. All the conidia which germinated during the first 4 hours were viable during the entire additional 8 hour period. Thus, the maximum per cent germination (60%) was obtained in 4 hours and remained at this level throughout the additional incubation period (Figure 7).

Figure 6. Effect of temperatures of 13, 17 and 22°C combined with relative humidities of 65 and 100% on Erysiphe graminis tritici conidial germination on wheat leaves. At 100% RH, 22 and 17° differed statistically at the 3rd hour, but not at the 5th hour; 22 and 13° did not differ at the 3rd hour but differed at the 5th hour.

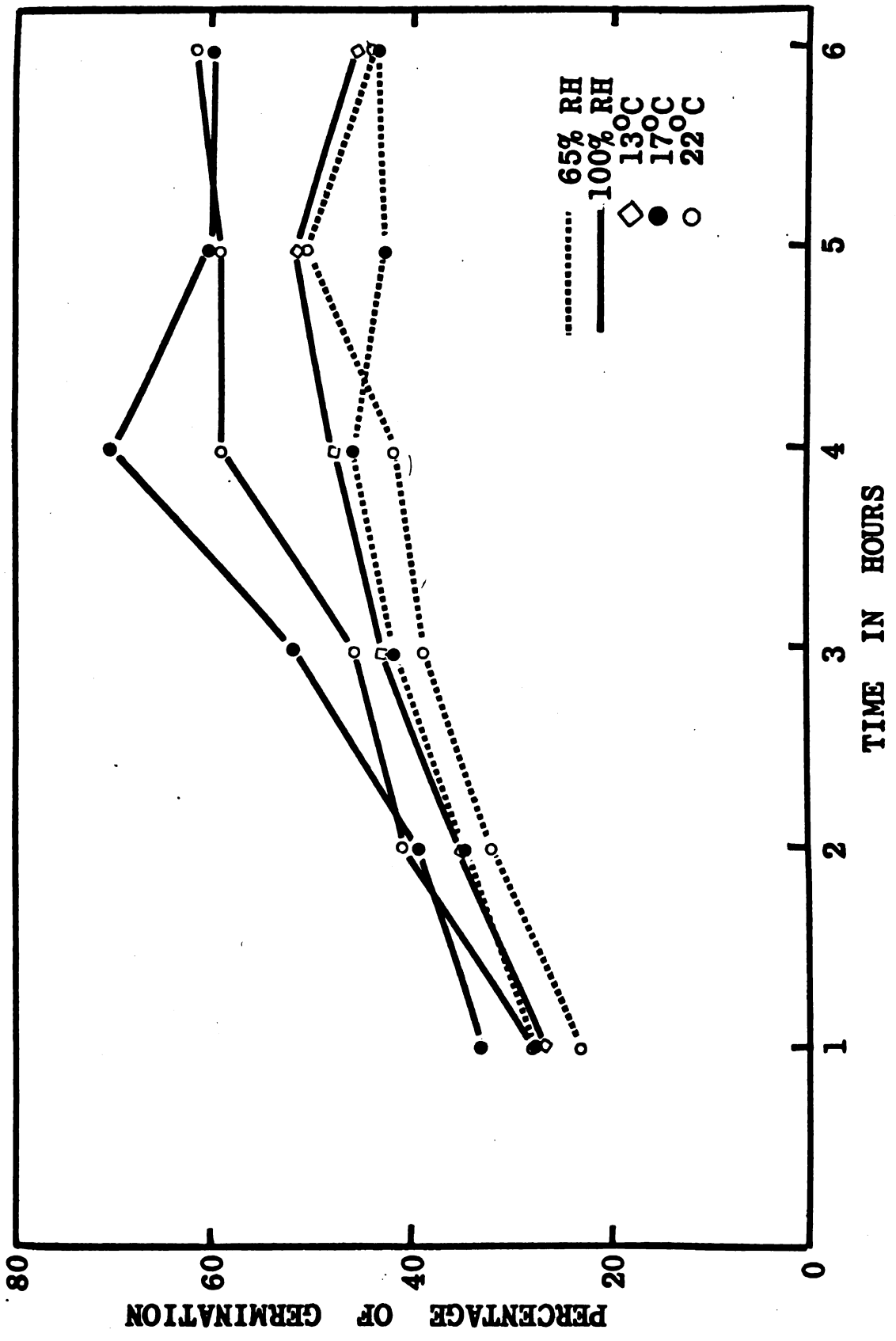

PERCENTAGE OF GERMINATION
0
20
40
60
80
1
2
3
4
5
6
TIME IN HOURS
65% RH
100% RH
13°C
17°C
22°C

Figure 6

Figure 7. Effect of temperatures of 13, 17 and 22°C at 100% RH on germination and appressorial formation of conidia of Erysiphe graminis tritici on wheat leaves. Appressorial formation: 8th hour, 13, 17 and 22°C differed statistically; 10th hour, 13° differed from 17 and 22°; 17 and 22° did not differ; 12th hour, 13, 17 and 22° did not differ.

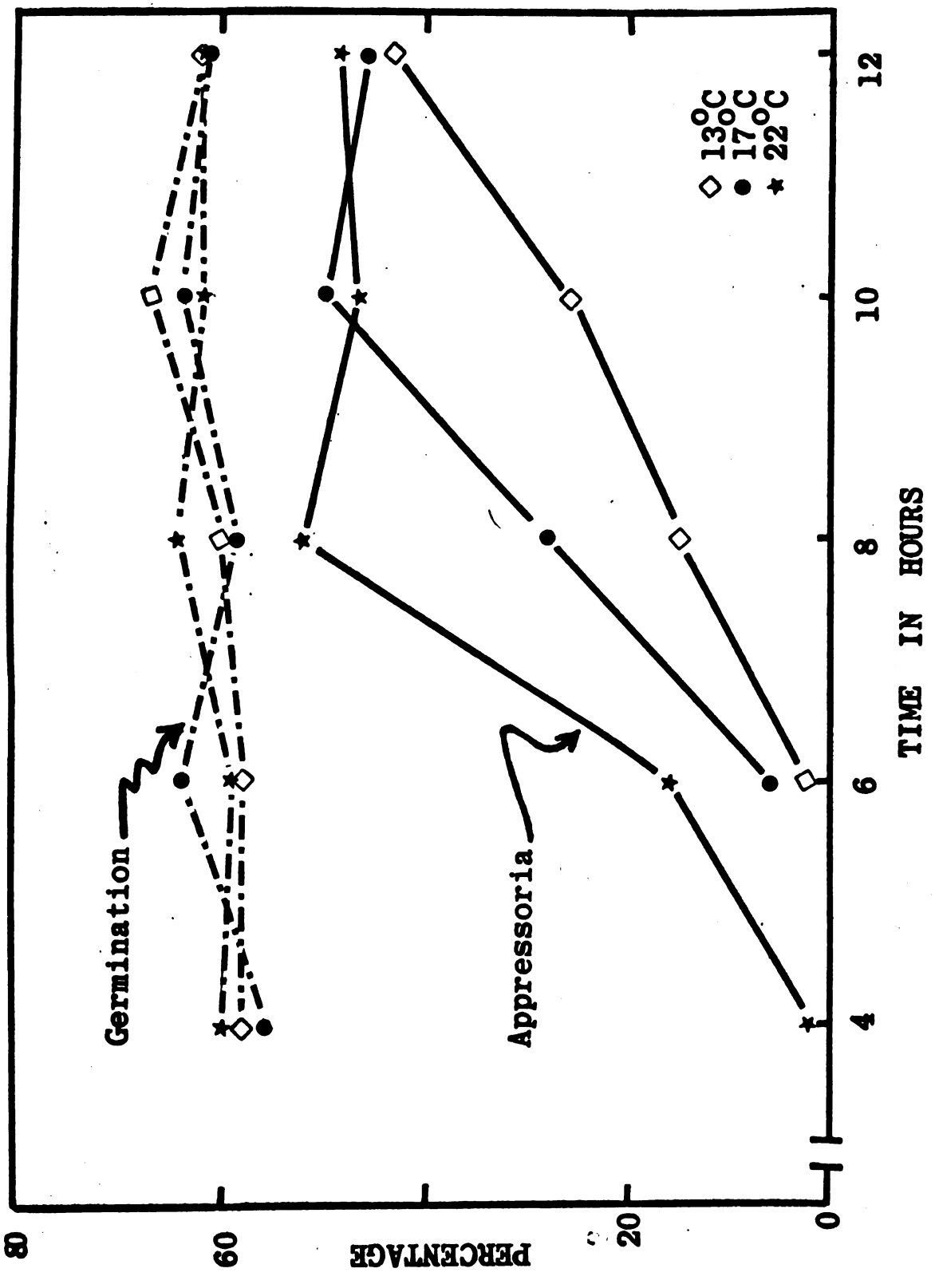

80
60
20
0
PERCENTAGE
4
6
8
10
12
TIME IN HOURS
13°C
17°C
22°C
Germination
Appressoria

Figure 7

The effect of concurrent changes in temperature and relative humidity following 4 hours incubation at 17°C and 100% RH was next examined. After the initial incubation, inoculated plants were transferred to 65% RH, at 13, 17 and 22°C, respectively. The usual percentage of germination (60%), was obtained on all plants 4 hours after inoculation. A remarkable increase in germination was observed following the transfer of plants to any of the new conditions. In 9 hours 93% germination was obtained on plants transferred to 65% RH and 22°C, and 80% germination was obtained on plants transferred to 65% RH at either 17 or 13°C. After the ninth hour additional conidia germinated at 17°C but not at 13 or 22°C; at 12 hours, germination at 17 and 22°C was essentially the same. Since it had already been shown that germination was not increased by changes in temperature after an initial exposure for 4 hours at 17°C and 100% RH, the larger numbers of conidia which germinated in this experiment were attributed to the decrease in relative humidity or to the combined effect of a change in temperature and relative humidity (Figure 8).

To determine whether the increase in conidial germination was due to the germination of a portion of the conidial population which would not germinate at 17°C and 100% RH, a series of experiments were performed to verify whether conidia with different optima for germination were present in the conidial population.

At first, inoculated plants were incubated in each of the following conditions: 17°C and 100% RH, 22°C and 100% RH, 17°C and 65% RH and 22°C and 65% RH. Results at the end of 4 hours substantiated the previous finding that more conidia germinated at 100% RH than at 65% RH irrespective of temperature. A maximum of 63% of the conidia germinated at 100% RH, while 45% germinated at 65% RH. No difference due to temperature was evident at this stage. Some specificity in response to relative humidity was therefore indicated by the results; there also

Figure 8. Effect of a decrease in relative humidity to 65% at 13, 17 and 22°C on conidial germination and appressorial formation of Erysiphe graminis tritici on wheat leaves, after obtaining 60% germination in 17°C and 100% RH for 4 hours. Germination: 9th hour, 17 and 22°C differed statistically, 13 and 17°C did not; 12th hour, 17 and 22°C did not differ, 13 and 17°C differed. Appressorial formation: 11th hour, all values differed statistically: 12th hour, 17 and 22°C did not differ; 13 and 17°C differed.

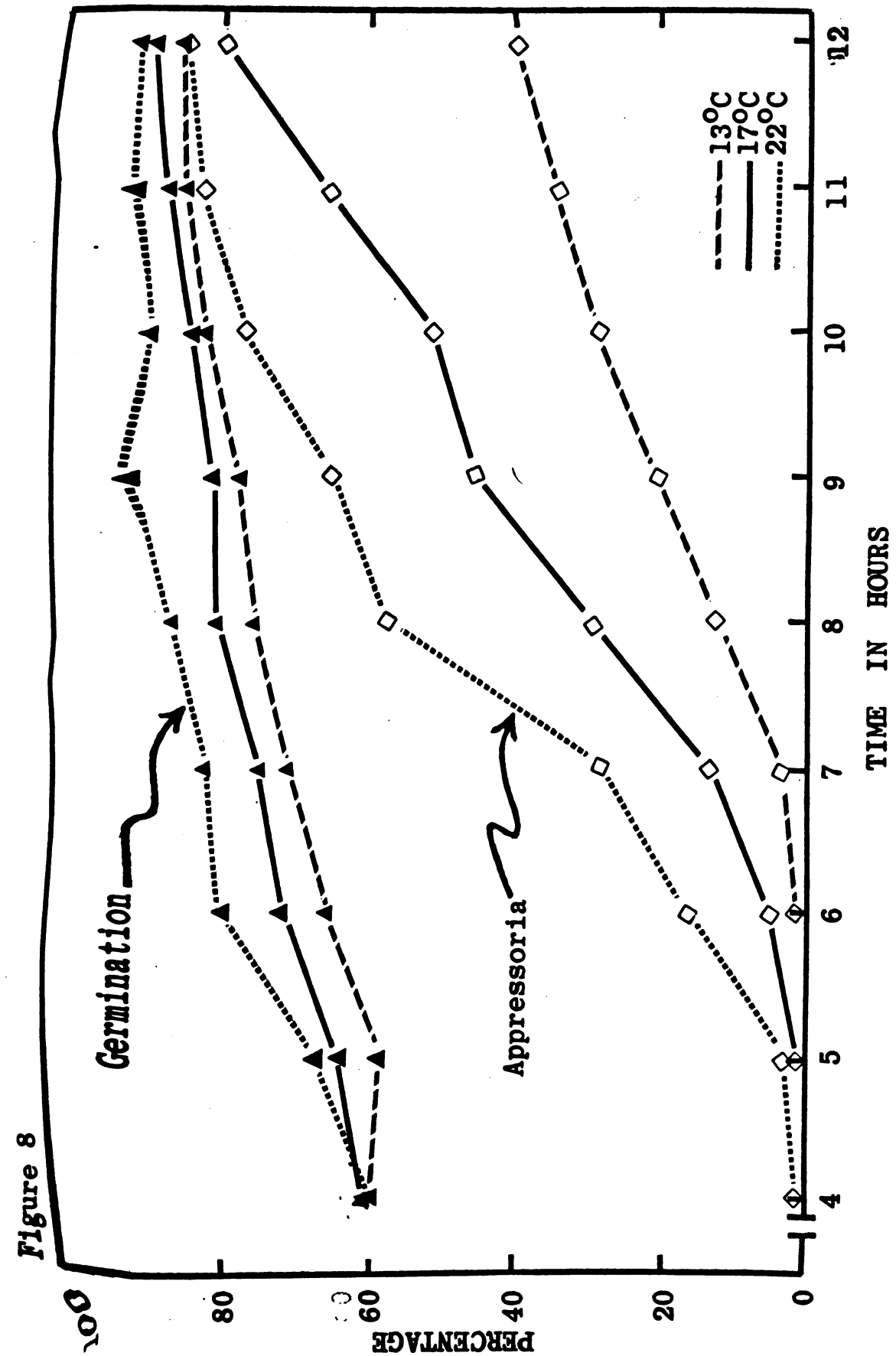

Germination
Appressoria
13°C
17°C
22°C
PERCENTAGE
TIME IN HOURS
0
20
40
60
80
100
4
5
6
7
8
9
10
11
12

Figure 8

might be some portion of the conidia which would germinate at either relative humidity (Figure 9).

In a second experiment, inoculated plants were incubated for 1 hour at 65% RH and 17 or 22°C followed by 3 hours at 100% RH and at the same temperatures. The reverse sequence of 1 hour in 100% RH and 3 hours in 65% RH was also tested at the same 2 temperatures. The germination obtained in each treatment was compared with a hypothetical value, calculated by summing the per cent of germination obtained at the appropriate hours in control treatments at corresponding constant temperatures and relative humidities (Figure 9). The mean germination obtained in these treatments was 72%, which was higher than that obtained at constant temperatures and relative humidities, and similar to the hypothetical values except for plants at 17°C and 65% RH for 1 hour followed by 17°C and 100% RH for 3 hours; this result barely differed statistically at the 5% level. This increase in per cent germination, obtained by exposing conidia to alternating relative humidities, indicated that an additional portion of the conidial population was stimulated equally by a change in relative humidity in either direction.

When 100% RH and 65% RH were alternated every hour or 2 hours at 17°C, germination (72%) was similar to that obtained when conidia were exposed for 1 hour in 100% RH and 3 hours at 65% RH. It was inferred from this, that although a humidity change gave higher percentage of germination, one such change was sufficient to induce the maximum germination (Figure 9).

Although 72% conidial germination could be obtained in 4 hours by a treatment of 65% followed by 100% RH, or the reverse, it was less than the 93% obtained when plants first incubated for 4 hours in 17°C and 100% RH were changed to 22°C and 65% RH for an additional 5 hour period. Since this combination of treatments involved a change in temperature as well as relative humidity, this possibility was investigated

Figure 9. Effect of various combinations of temperatures of 17 and 22°C and relative humidities of 65 and 100% on conidial germination of Erysiphe graminis tritici on wheat leaves.

A_1 - A_4 17° and 100% RH

B_1 - B_4 22° and 100% RH

C_1 - C_4 17° and 65% RH

D_1 - D_4 22° and 65% RH

E 1 hr. at 17° and 65% RH + 3 hrs. at 17° and 100% RH. Actual

E^1 1 hr. at 17° and 65% RH + 3 hrs. at 17° and 100% RH. Expected

F 1 hr. at 17° and 100% RH + 3 hrs. at 17° and 65% RH. Actual

F^1 1 hr. at 17° and 100% RH + 3 hrs. at 17° and 65% RH. Expected

G 1 hr. at 22° and 65% RH + 3 hrs. at 22° and 100% RH. Actual

G^1 1 hr. at 22° and 65% RH + 3 Hrs. at 22° and 100% RH. Expected

H 1 hr. at 22° and 100% RH + 3 hrs. at 65% RH. Actual

H^1 1 hr. at 22° and 100% RH + 3 hrs. at 65% RH. Expected

I 17° and 100% RH and 17° and 65% RH alternated every hr. Actual

I^1 17° and 100% RH and 17° and 65% RH alternated every hr. Expected

J 17° and 100% RH and 17° and 65% RH alternated every 2 hrs. Actual

J^1 17° and 100% RH and 17° and 65% RH alternated every 2 hrs. Expected

K 1 hr. at 17° and 100% RH + 3 hrs. at 22° and 65% RH.

L 1 hr. at 22° and 100% RH + 3 hrs. at 17° and 65% RH.

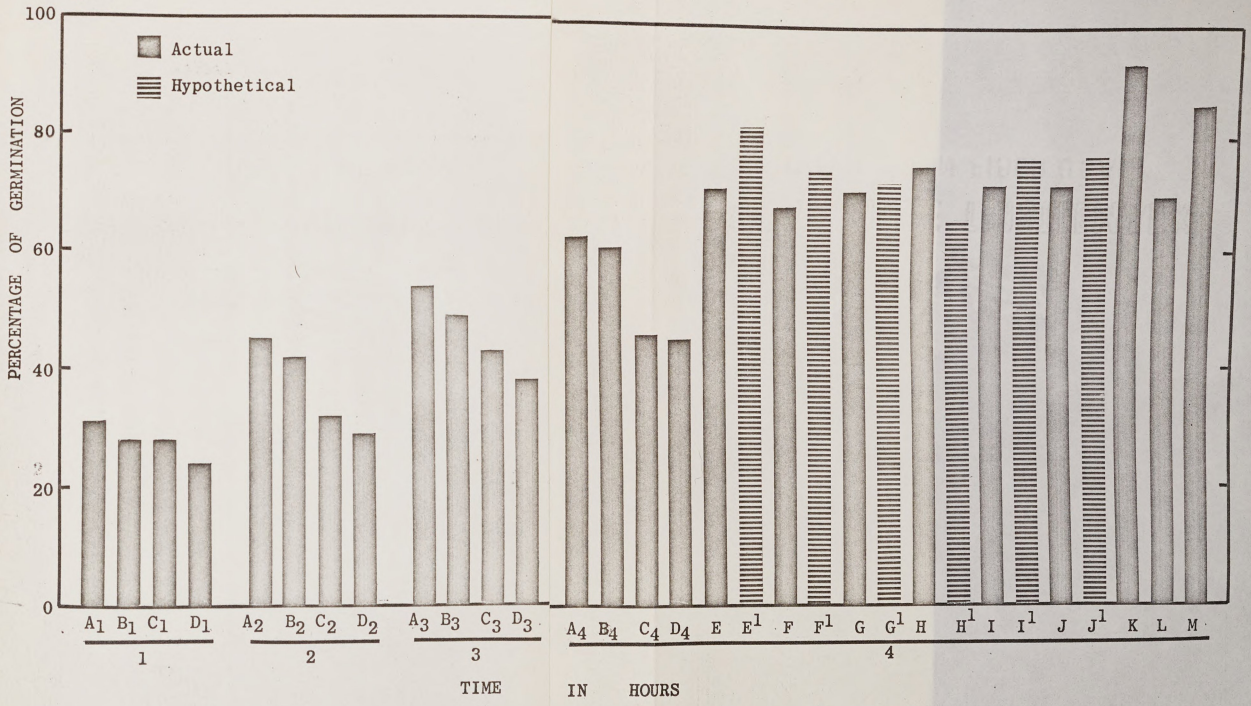
M 17° and 100% RH and 22° and 65% RH alternated every hr.

Expected values calculated by summing the germination per cent at the appropriate hour in control treatments at corresponding constant temperatures and relative humidities.

A_4 differed statistically from E, F, G, H, I, J, K, L, and M.

Comparisons of actual values and expected values (E through J): E and E^1 differed; K, L, and M all differed.

Figure 9


100
80
60
40
20
0
PERCENTAGE OF GERMINATION
Actual
Hypothetical
A1 B1 C1 D1
A2 B2 C2 D2
A3 B3 C3 D3
A4 B4 C4 D4 E E1 F F1 G G1 H H1 I I1 J J1 K L M
1
2
3
4
TIME IN HOURS

further by incubating inoculated plants at 17°C and 100% RH for 1 hour and then at 22°C and 65% RH for 3 more hours. In this experiment, 93% germination was again obtained, but in 4 hours (Figure 9). Since a change in relative humidity alone at a constant temperature gave only 72% germination, it was inferred that the additional increase of 21% in conidial germination was due to the temperature change or due to a combined effect of a change in temperature and relative humidity.

Reversing the temperature sequence so that inoculated plants were exposed to conditions of 22°C and 100% RH for 1 hour followed by 17°C and 65% RH for 3 hours gave 70% germination, a value similar to that given by the humidity change alone (Figure 9). Thus, more conidia responded when the change in relative humidity was accompanied by a change from lower to higher temperature rather than to the reverse temperature sequence.

Conidia subjected to alternating conditions of 17°C and 100% RH with 22°C and 65% RH every hour for 4 hours gave 87% germination, less than that obtained when the same conditions were changed only once (Figure 9). It appeared that only one change from lower to higher temperature was necessary to stimulate the conidia to germinate, but that a minimum period of 3 hours in the higher temperature and lower humidity after the change was required to insure maximum percentage of germination in minimum time.

This series of tests suggested that although 100% RH was more favorable for conidial germination than 65% RH, maximum per cent germination was obtained only by subjecting the conidia to an amphidirectional change of the above 2 relative humidities concurrent with unidirectional temperature change from 17 to 22°C.

When the temperature was increased to 28°C or the relative humidity decreased to 32% for an additional 8 hours, germination percentage did not increase beyond the 93% obtained in 4 hours at the

favorable conditions. Viability of germinated conidia also was not decreased by this treatment.

Effect of Light on Conidial Germination

The effect of darkness, low light, and high light on conidial germination was studied under the optimum temperature and relative humidity conditions (1 hour at 17°C and 100%, 3 hours at 22°C and 65% RH). Ninety-five per cent germination occurred in 4 hours irrespective of light conditions. After this period of time, a change in temperature or relative humidity accompanied by a change in light did not increase percentage of germination.

Resumé

Germination of the conidia began shortly after inoculation. High percentage of conidial germination was consistently obtained with fresh, single conidia uniformly distributed on the leaf. Beginning with about 50% germination in 6 hours with the dusting method of inoculation and relatively uncontrolled conditions, germination was increased to 60% in 4 hours with the rolling method of inoculation and by incubating inoculated plants at 17°C and 100% RH. Successive incubation for 1 hour at 17°C and 100% RH followed by 3 hours at 22°C and 65% RH gave 93% germination in 4 hours. Germination of conidia was insensitive to light, but was dependent on temperature and relative humidity. Maximum germination was obtained by an amphidirectional change in relative humidity accompanied by a unidirectional change in temperature from 17 to 22°C.

Development of Appressoria

Appressorial formation follows germination and begins with the conidium producing a short germ tube which develops a slight

enlargement at its tip at about the fourth hour. This swelling can be termed the "appressorial initial" (Figure 10). This structure enlarges rapidly becoming a conspicuously swollen appressorium which when fully mature is cut off by a septum from the rest of the term tube and is characteristically bent to one side (Figure 10). This bent condition was taken as the criterion of a mature appressorium.

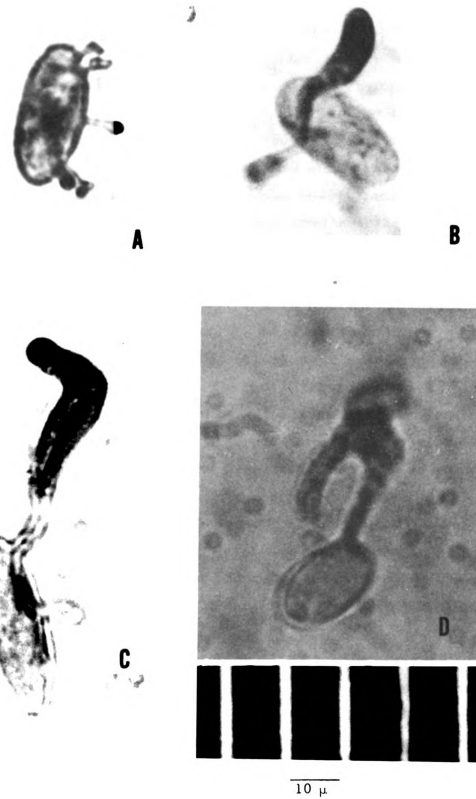
Effect of Temperature and Relative Humidity and Light on the Development of Appressoria

In a preliminary experiment, it was found that appressorial formation began from the fourth hour after inoculation and that more appressoria were formed at 22°C than at 13 or 17°C.

Since a relative humidity of 100% was more favorable for germination than 65%, this relative humidity was used to study the effect of temperature on appressorial formation. Inoculated plants were incubated at 17°C and 100% RH in diffused light to induce germination (60%), then placed at 13, 17 and 22°C in diffused light for 8 more hours. At 22°C, 52% of the conidia produced appressoria in 8 hours as compared with 28% at 17°C, and 15% at 13°C. No appreciable increase in percentage of appressorial formation occurred at 22°C after the eighth hour, whereas at 17°C, a maximum of 50% of the conidia produced appressoria in 10 hours after which no further increase was observed. At 13°C, a maximum of 43% of the conidia produced appressoria in 12 hours. Thus, the number of conidia producing appressoria at all 3 temperatures was essentially the same by the twelfth hour when most of the germinated conidia had produced the appressoria. For every 5° decrease in temperature, there was a delay of almost 2 hours for maximum appressorial formation (Figure 7).

Appressorial formation was studied at the same 3 temperatures under diffused light but at 65% RH in another test. Inoculated plants were

Figure 10. Stages in primary infection by conidia of powdery mildew of wheat (Erysiphe graminis tritici). (Penetration of host was not studied). A) Conidium with germ tubes, B) Germ tube enlarged to form appressorial initial, C) Mature appressorium, D) Beginning of secondary hypha.


A
B
C
D
10 μ

placed at 17°C and 100% RH for 4 hours for conidial germination, and then transferred to 13, 17 and 22°C at 65% RH. Following transfer of plants to conditions of decreased relative humidity at all 3 temperatures, additional conidia germinated, and appressorial formation also increased proportionately. A maximum of 84% of the conidia produced appressoria at 22°C and 65% RH, as compared with 67% at 17°C and 35% at 13°C. Since, germination continued for a period of 4-5 hours beyond the usual 4 hour period under the new set of conditions, the highest per cent of appressorial formation at 22°C was delayed until the eleventh hour. At 17°C, appressoria continued to be formed until the twelfth hour and by the end of this period the percentage of appressorial formation was essentially the same in both 17 and 22°C. A temperature of 13°C was less favorable than 17 or 22°C even at this time (Figure 8).

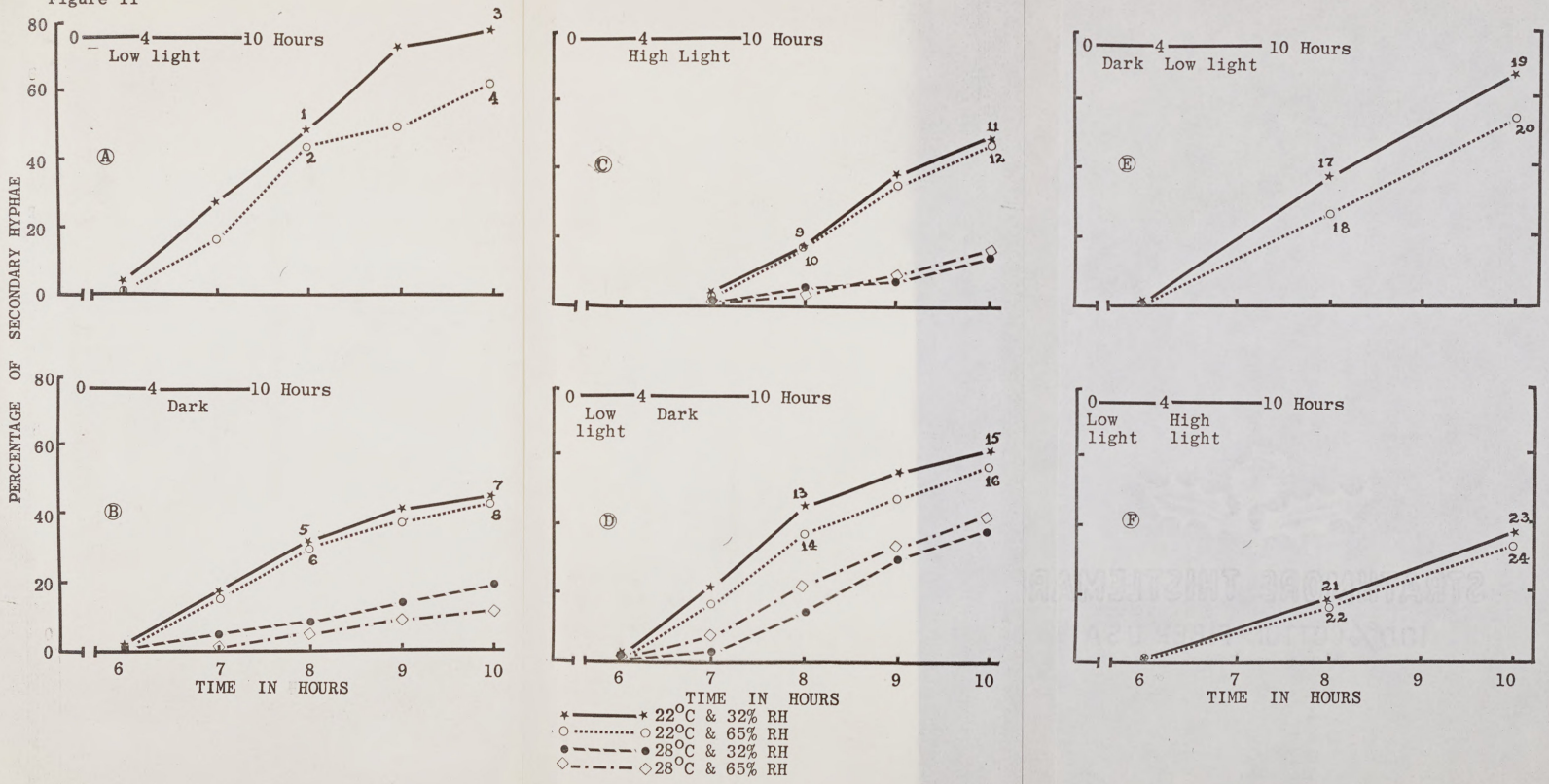
The results of these experiments indicated that most of the conidia are potentially able to form appressoria over a wide range of temperatures given sufficient time. Whereas 93% germination of conidia could be obtained only by incubating the inoculated plants at two different relative humidities and two different temperatures in appropriate sequence, 83% of the conidia formed appressoria at one relative humidity (65%) and at one temperature (22°C) (Figure 8).

Additional experiments were designed for a more detailed investigation of the conditions of temperature, relative humidity and light influencing appressorial development. Since the previous test indicated that 100% RH was not favorable for the development of appressoria, 65% and 32% RH were used in these experiments.

Initiation and formation of appressoria were studied after the conidia were germinated at the favorable conditions of temperature and relative humidity combined either with low light, high light or darkness. These conditions were followed by incubation at 22 or 28°C and 32 or 65% RH for 6 more hours in low light, high light or darkness. In all, 18 combinations of conditions were tested (Figure 11).

Figure 11. Effect of incubation temperatures of 22 and 28°C relative humidities of 32 and 65%, and low light, high light and darkness on appressorial maturation of Erysiphe graminis tritici on wheat leaves. Temperature: 22 and 28°C differed statistically in treatments B, C, and D; relative humidity: (at 22°) when comparisons were made within any treatment values at 32 and 65% RH, differed statistically except in the following; 5 and 6, 7 and 8, 9 and 10, 11 and 12, 21 and 22, 23 and 24; Light: (at 22°C and 32% RH), of all 2 x 2 comparisons made, all differed except the following: 1 and 13, 9 and 21.

Figure 11


0 4 10 Hours
Low light
A
B
Dark
C
High Light
D
Low light
Dark
E
Dark Low light
F
Low light
High light
PERCENTAGE OF SECONDARY HYPHAE
TIME IN HOURS
22°C & 32% RH
22°C & 65% RH
28°C & 32% RH
28°C & 65% RH

Appressorial initials began to appear at the fifth hour irrespective of temperature, relative humidity and light; 90% of the conidia had produced immature appressoria by the ninth hour (Figure 12). Evidently, the factor limiting formation of appressorial initials was conidial germination. Maturation of appressoria, however, was influenced by temperature, relative humidity and light.

Several important observations were made on appressorial formation: 1) Appressorial maturation began at the sixth hour after inoculation at all conditions except when high light was given for the entire 10-hour period, in which case formation of the first appressorium was delayed until the seventh hour (Figure 11). 2) More appressoria were formed at 22°C than 28°C at all conditions of light (or darkness) and at either relative humidity (Figure 11). 3) Continuous low light during the 10-hour period gave more appressoria than all other conditions of changing or continuous light conditions. 4) Darkness during germination followed by low light was next best. 5) Low light followed by darkness, or the reverse sequence, was better than darkness or high light for the entire period. 6) High light for 10 hours was more favorable than darkness for the same period. 7) With low light for 10 hours or during the second 6 hours, more appressoria were produced at 32% than at 65% RH. 8) Differences due to humidity were not expressed in darkness or in high light.

Other observations of significance include: 9) More appressorial production in low light followed by darkness than in low light followed by high light. 10) At the eighth hour the number of appressoria on plants changed to darkness was $2\frac{1}{2}$ times greater than those changed to high light, but the rate of appressorial maturation between the eighth and tenth hours increased in high light and decreased in darkness. 11) Although low light was more favorable than high light for appressorial maturation, low light followed by high light produced fewer appressoria

Figure 12. Germination, formation of appressorial initials and development of mature appressoria of Erysiphe graminis tritici on wheat leaves at 22°C, low light and either 32 or 65% relative humidity after 4 hours incubation to permit maximum germination. Values at the 10th hour did not differ statistically except for mature appressoria at either relative humidity. Both values for appressorial maturation differed statistically from those for germination and formation of appressorial initials.

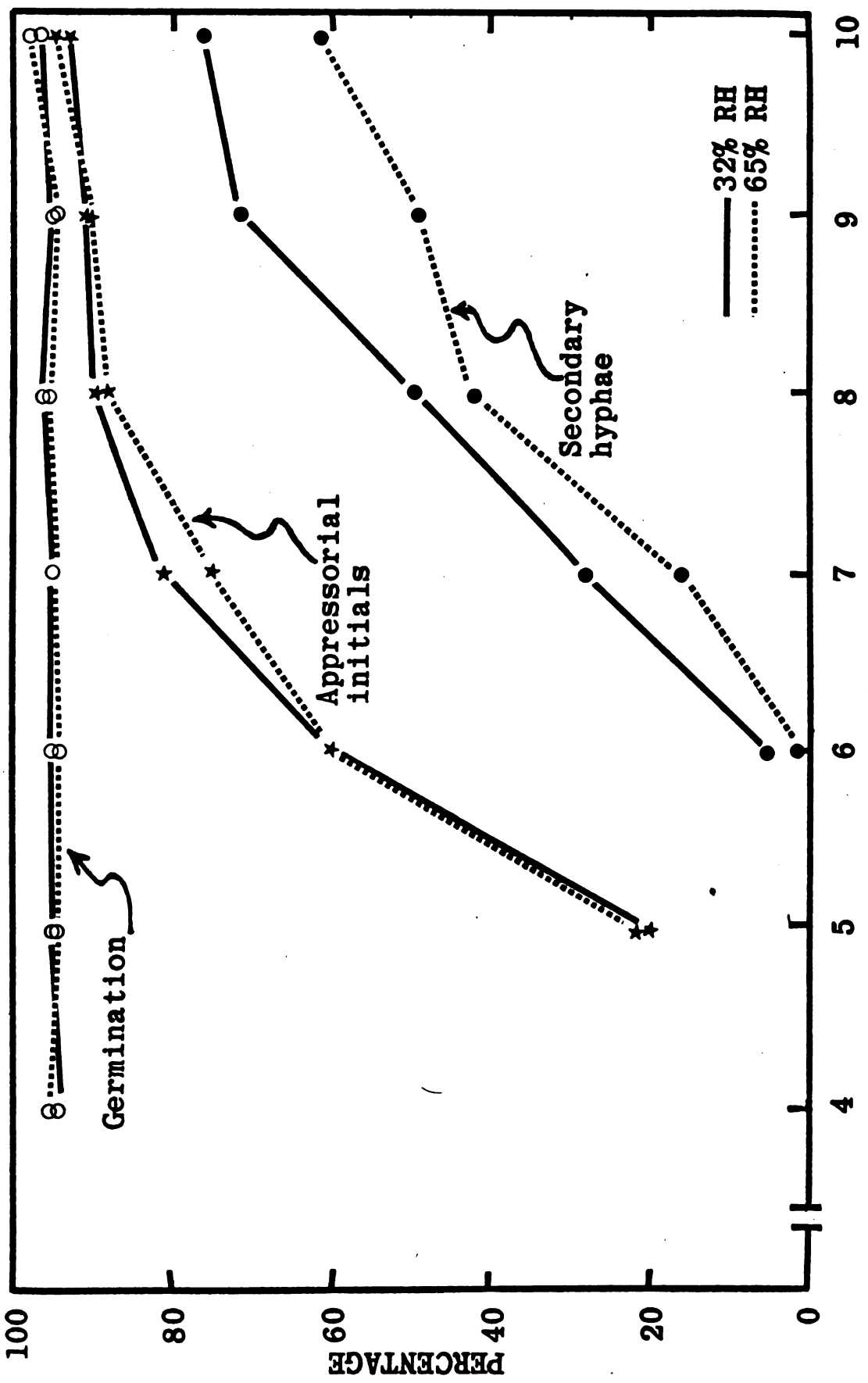

PERCENTAGE
100
80
60
40
20
0
4
5
6
7
8
9
10
TIME IN HOURS
Germination
Appressorial initials
Secondary hyphae
32% RH
65% RH

Figure 12

than continuous high light. 12) The unfavorable effect of high light was expressed by an immediate decrease followed by a gradual increase in rate of appressorial maturation, whereas the unfavorable effect of darkness was expressed by a gradual decrease in the rate of appressorial maturation as the time of exposure to darkness increased. Therefore, at the eighth hour more appressoria were formed in darkness, but at the tenth hour more were formed in high light.

It appeared that the light-sensitive phase may begin before the fourth hour, since more mature appressoria were formed in continuous low light than in darkness followed by low light. Possibly by reducing the dark period and providing low light before the fourth hour, the number of appressoria formed would be equal to that formed in low light for all 10 hours.

Since germination was independent of light, the mechanism whereby low light given only during germination resulted in higher per cent of mature appressoria than in darkness for the entire stage of development may involve some kind of preconditioning of the germinated conidia by light. Since at this stage of infection, the pathogen had not established any organic contact with the host, it can be assumed that the effect of light was directly on the pathogen rather than on the host.

At 28°C, the effect of relative humidity on appressorial maturation was inconsistent at all 3 light conditions studied and no conclusions could be drawn.

When additional time beyond 10 hours was given under the optimum conditions of 22°C and 32% RH and low light, every germinated conidium produced a mature appressorium by the 20th hour.

Resumé

Appressorial initials developed equally well irrespective of temperature, relative humidity and light conditions studied. Germination

of conidia was not influenced by light, but more mature appressoria were formed in low light than in darkness or high light. Appressorial maturation was favored by a lower (32%) relative humidity than that which was required for conidial germination. Whereas germination occurred equally at 17 or 22°C at either 65 or 100% RH, the most favorable temperature for appressorial matuation was 22°C. While appressorial maturation depended only on one temperature, relative humidity and light, maximum germination depended on two temperatures and relative humidities in the proper sequence.

Development of Secondary Hyphae

The mature appressorium produces a penetration process which penetrates the host and produces a haustorium in the host epidermal cell (14). The development of secondary hyphae begins as small buds from the outer side of the bend of the primary appressorium (Figure 10). In some cases, they were formed slightly lower down from the side of the appressorium, or rarely as a continuation of the appressorial tip. These buds grow out rapidly as hyphae and in about 14 hours after they were first seen some of them had already produced branches.

Effect of Relative Humidity and Light on the Development of Secondary Hyphae

Time required for the production of secondary hyphae and the effect of relative humidity on this phase of primary infection were studied in a preliminary experiment. Inoculated plants were incubated for 4 hours at 17°C and 100% RH for germination of conidia followed by 8 hours at 22°C and 100% RH in diffused light for the development of appressoria. They were then changed to 17°C and 100% RH or to 17°C and < 50% RH, (relative humidity of the greenhouse room). Development of secondary hyphae began between the 16th and 20th hours following inoculation.

At the end of this experiment, 28 hours after inoculation, 30% of the conidia had produced secondary hyphae at < 50% RH as compared with 12% at 100% RH. The effect of relative humidity was first evident at the 20th hour after inoculation; at this time, 10 times more secondary hyphae were formed in the lower humidity than in the saturated air (Figure 13).

The effect of different combinations of relative humidities and light (or darkness) on the formation of secondary hyphae was investigated. Plants inoculated and incubated in the optimum conditions for germination and appressorial formation, respectively, until the 12th hour were transferred to low light, high light or to darkness, either at 32 or 65% RH. These tests were done at 22°C.

The development of secondary hyphae was the same in all the light and relative humidity conditions studied until the 20th hour. After this period, development of secondary hyphae was influenced by light conditions, but not by differences in relative humidity. At either 32 or 65% RH, 72% of the conidia produced secondary hyphae in low light in 32 hours as compared with 55-58% in high light or in the dark in the same time (Figure 14). Between the 20th hour and 28th hour a higher percentage of secondary hyphae was produced in darkness than in high light, but at the 32nd hour the percentages were the same. The decrease in the rate of development of secondary hyphae on plants held in darkness continuously for more than 20 hours (10th thru the 32nd hour after inoculation) was probably due to starvation of the host.

Resumé

Whereas relative humidity was an important factor controlling the maximum conidial germination and appressorial maturation, development of secondary hyphae occurred equally well at 32 and 65% RH. This phase of infection was favored by low light as compared with

Figure 13. Effect of relative humidities of < 50% and 100% at 17°C on conidial germination, appressorial formation and development of secondary hyphae of Erysiphe graminis tritici on wheat leaves. Secondary hyphae at < 50% RH differed statistically from 100% RH at each hour.

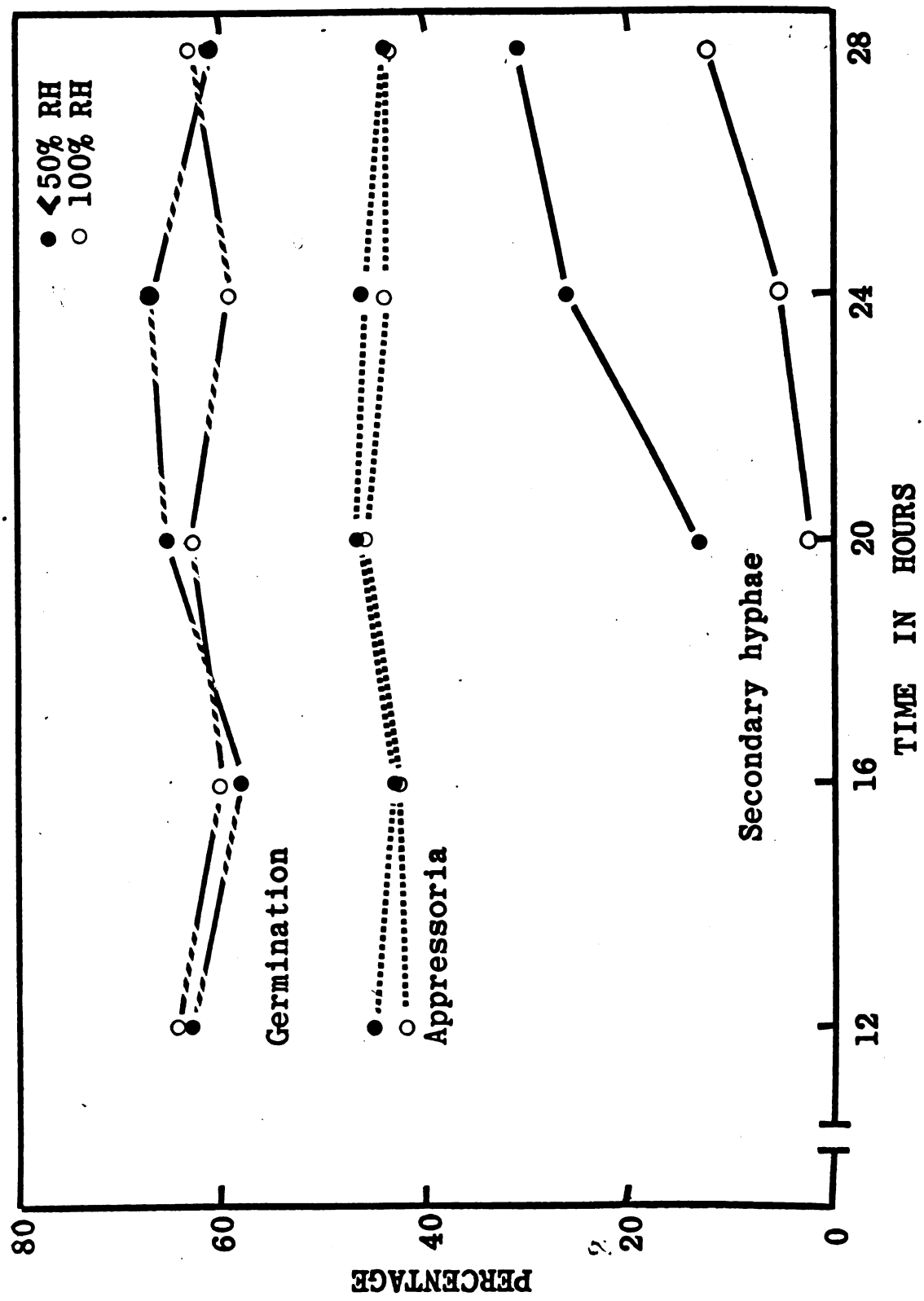

● <50% RH
○ 100% RH
Germination
Appressoria
Secondary hyphae
PERCENTAGE
TIME IN HOURS
0
20
40
60
80
12
16
20
24
28

Figure 13

Figure 14. Effect of relative humidities of 32 and 65% in low light, high light or darkness at 22°C on development of secondary hyphae of Erysiphe graminis tritici on wheat leaves. Relative humidity: 32 and 65% did not differ statistically at any hour in any light condition: Light (at 65% RH): at 24 and 28 hours all 3 light conditions differed; at 32 hours low light differed from high light and darkness.

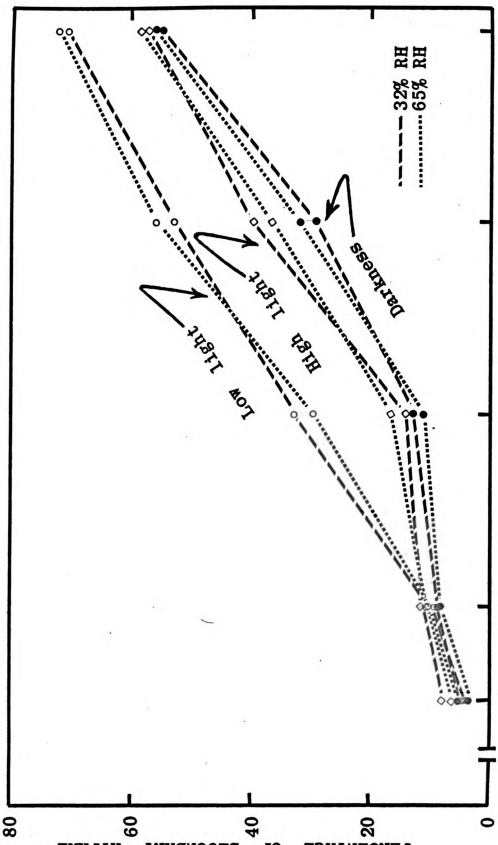

PERCENTAGE OF SECONDARY HYPHAE
0
20
40
60
80
18
20
24
28
32
TIME IN HOURS
Low light
High light
Darkness
32% RH
65% RH

Figure 14

darkness or high light. Darkness was more favorable than high light in the early stage of secondary hyphae development, but the rate of development decreased on continued exposure to darkness possibly due to depletion of carbohydrate reserves of the host.

Overall Process of Infection

Results obtained regarding the optimum conditions for the different stages of the process of primary infection were pooled and tested in one continuous experiment covering the entire period of 32 hours following inoculation.

Inoculated plants were incubated in the respective optimum conditions for each stage of primary infection as follows: 1 hour at 17°C and 100% RH, then 3 hours at 22°C and 65% RH in low light for germination; 8 hours at 22°C and 32% RH in low light for appressorial formation; and 22 hours at 22°C and 65% RH in low light for the development of secondary hyphae.

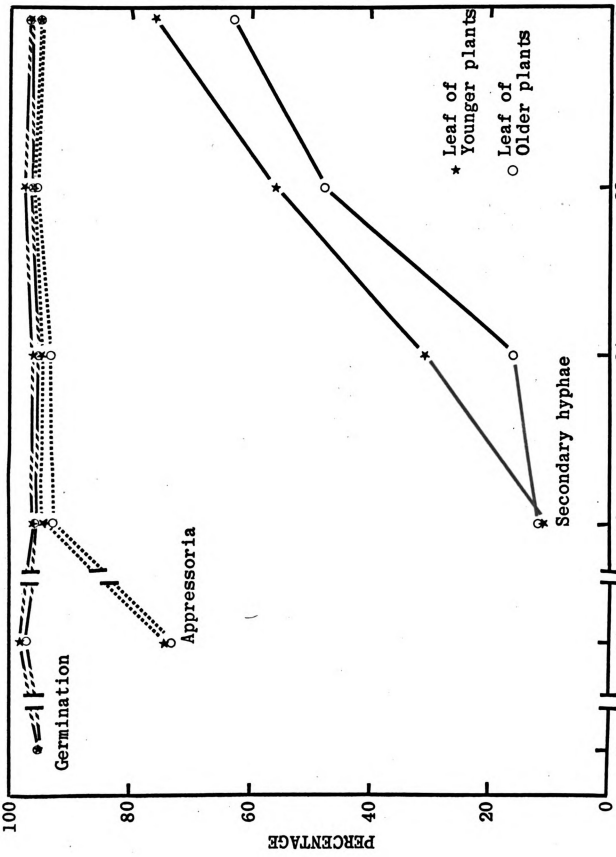
Germination of 95% of the conidia occurred in 4 hours and there was no increase after that time. At the 10th hour, 74% of the conidia had produced appressoria and by the 20th hour this had reached 94%. Thus, almost all the conidia which germinated had produced appressoria by 20 hours. Twelve per cent of the conidia produced secondary hyphae before the 20th hour and this reached 75% in 32 hours (Figure 15). This experiment confirmed the optimum conditions for the different phases of primary infection with respect to time and percentage.

Comparison of Host Leaves on Plants of Two Different Ages With Regards to Primary Infection

The overall process of infection was compared on leaves of host plants of two different ages. For this purpose, one set of plants used was of the usual age and development, i.e., 5-6 days old after sowing,

Figure 15. Effect of difference of 1 week in age of host plants on the process of primary infection by conidia of Erysiphe graminis tritici on wheat leaves at the optimum conditions. Germination: analysis made at 4, 10, 32 hours, values did not differ statistically. Appressorial formation: analysis made at 10, 20, 32 hours, values did not differ. Germination and appressorial formation compared at 20, and 32 hours, values did not differ. Secondary hyphae: analysis at 24 and 32 hours, values differed at each hour.

Figure 15


100
80
60
40
20
0
PERCENTAGE
4
10
20
24
28
32
TIME IN HOURS
Germination
Appressoria
Secondary hyphae
★ Leaf of Younger plants
○ Leaf of Older plants

with the first leaf fully formed and the second leaf just emerging. The other set consisted of plants 10-12 days old in their 3-leaf stage with the first 2 leaves fully formed and the 3rd leaf just emerging.

Plants inoculated as usual on the primary leaves were incubated in the optimum conditions for germination, appressorial formation, and secondary hyphal development. Percentages and rate of germination and appressorial formation were identical for conidia on the leaves of younger and older plants. All germinated conidia had already produced the appressoria by the 20th hour. On plants at both ages, the percentage of secondary hyphae formed was the same at the 20th hour. Thereafter, the rate of formation of secondary hyphae was more rapid in the leaves of younger plants than in those of older plants. At 32 hours, 78% of the conidia on the leaves of younger plants had produced secondary hyphae, thereby completing primary infection, whereas on the leaves of older plants the corresponding value was 63% (Figure 15). This showed that in leaves which differed in age only by a week, fewer secondary hyphae were produced on the leaves of older plants. Whether the same per cent of primary infection could be obtained on both these kinds of leaves on longer incubation was not determined.

Resumé

Under identical conditions, germination and appressorial maturation were not influenced by a week's difference in the age of the host plants. However, rate of secondary hyphae development on leaves of younger plants was greater than that on older plants.

DISCUSSION

The use of single host plants for inoculation enabled a better control of environmental conditions, than the use of host plants in groups. Furthermore, a more uniform microclimate surrounding the conidia was also provided by the use of individual host plants. The uniformity and excellent reproducibility of the results obtained in this research are attributed to four factors in the techniques employed:

1) the use of fresh, young conidia of a fairly uniform age,
2) the use of single conidia obtained by breaking of the conidial chains,
3) the uniform distribution of the conidia on the leaf, and
4) the precise control of environmental conditions.

A reasonably synchronous germination of conidia was achieved by using the conidia of fairly uniform age. The importance of using fresh, young conidia to give higher germination was demonstrated by Graf-Marin (22), who obtained 11.9% germination when all the conidia in the inoculum were used, as compared with 66.9% when only fresh, young conidia were used. The poor germination obtained using conidia in chains or overcrowded on the leaf (5, 6, 16, 18, 37) indicates the importance of using uniformly distributed single conidia for inoculation.

Germination of conidia in situ was frequently noted in this study, particularly when the relative humidity of the air was high. Similar observations were made by Cherewick (10), although others have reported to the contrary (5, 6, 16). When the old conidia were shaken off and discarded, and fresh conidia collected 6 hours later, 5-8% conidial germination in situ was noted if the relative humidity of the air was about 70% or more. At a lower relative humidity the value was 0-4%.

Abundant conidia were collected from plants in the early morning, forenoon, afternoon and evening with no evidence of a diurnal cycle in the maturation and germinability of the conidia. Although such a cycle has been reported in some powdery mildews (11, 24, 66), Yarwood (66) and Cherewick (10) also noted the absence of a diurnal cycle in Erysiphe graminis.

Yarwood (70) stated that conidia germinate only from the conners, usually by means of one germ tube. It was observed here that although this was usually true, germ tubes were frequently produced from the center or even from the very ends of the conidium where it was broken off from those adjacent to it. The number of germ tubes ranged from 1-4 per conidium, rarely more (Figure 10A). There was no relation between these observations and environmental conditions.

A relative humidity of 100% was more favorable than 65% for germination of conidia at either 17 or 22°C, and maximum germination of 60% occurred in 4 hours. However, when the relative humidity was changed from 100% to 65% after 4 hours, a remarkable increase in germination to 93% was obtained after 5 more hours at 22°C. Furthermore, the same higher level of germination was obtained in 4 hours by incubating inoculated plants for 1 hour at 17°C and 100% RH and 3 hours at 22°C and 65% RH. It was evident from these results that although most conidia germinated at 100% RH, the highest percentage of germination was obtained only by a change from 100% to 65% RH accompanied by a change from 17°C to 22°C. The most plausible explanation for this phenomenon is that the conidial population actually consists of more than one physiological group. It would seem that the groups differ physiologically rather than genetically because of: 1) germination of some conidia at either relative humidity, 2) germination of some conidia only following a change in temperature and relative humidity, and 3) probable difference in the age of the conidia (collected 4-6 hours after discarding old conidia), since conidia are formed in chains.

Of the environmental factors studied, light did not influence germination although Yarwood (63) reported a stimulation of conidial germination by light. Temperature and relative humidity influenced germination to a great extent; indeed a combination of suitable temperatures and relative humidities in proper sequence was essential for maximum germination. Poor germinability of conidia produced under low light conditions is evidently an indirect effect due to limitations of the host nutrition. Similar observations were made by other investigators (10, 58, 66, 67).

Although the same number of appressorial initials were formed at all temperatures, relative humidities, and light conditions studied, appressorial maturation was favored by low light, 32% RH, and a temperature of 22°C. This phase of infection thus differed from germination in its better development in lower relative humidity and its preference for low light as compared with the insensitivity to light of the germination phase.

Secondary hyphae developed equally well at 32 and 65% RH, indicating the adaptability of this phase of infection to a wide range of relative humidities below 100%. This phase of infection was similar to appressorial maturation in that both showed a preference for low light, but differed in decreased specificity of secondary hyphal development for relative humidity as compared with the best appressorial development at 32% RH.

The development of secondary hyphae was considered as marking the end of primary infection and the beginning of a successful host-parasite interaction. The pathogen was apparently quiescent for 8-10 hours following appressorial maturation. Although the actual penetration of the mildew into the host was not investigated directly in this research, penetration and development of a haustorium takes place in the host epidermal cell during this quiescent period. There are reports

that inoculation of several powdery mildews onto non-host plants have resulted in germination and even penetration of the plants, however, secondary hyphae developed only in the appropriate host plants (14, 55, 62, 66).

The great similarity between younger and older plants in the rate of conidial germination and appressorial development on their leaves indicates that age differences of the host did not influence these phases of primary infection. The striking difference between plants of different ages in the rate of secondary hyphal development on their leaves suggests that the nature of the cuticle of the host leaf is an important factor in penetration and establishment of the pathogen in the host. Since the cuticle is known to be thicker on older plants (22), and older leaves or thicker cuticle are reported to reduce infection by several other pathogens (8, 12, 43), presumably the thicker cuticle caused the slower rate of development of secondary hyphae in this study.

The process of primary infection consists of component parts, each with its own optimal requirements. Although 100% RH produced more germination than 65% RH at either 17 or 22°C, maximum germination was obtained only with a change from 17°C and 100% RH to 22°C and 65% RH. No other phase of primary infection investigated in this study required, for best results, a temperature as low as 17°C and relative humidity as high as 100%. As the process of primary infection advanced, a higher temperature (22°C) and lower relative humidity (32%) became more favorable. Finally, in secondary hyphal development, relative humidities of 32 and 65% gave similar results both of which were more favorable than 100% RH. In addition, an increasing preference for low light was observed during the course of primary infection.

The environmental requirements for the various stages of primary infection as revealed in this study are similar to the conditions the fungus would encounter in nature. Powdery mildews are widespread in

spring and summer. In wheat fields where the stands are thick, the leaves are crowded above the soil reducing the air currents and maintaining a high relative humidity close to the ground. Since lower temperature and high relative humidity were most favorable for germination, the requirements for germination of the largest portion of the conidial population would be met in the early morning shortly before sunrise. More conidia would germinate as the temperature increases and relative humidity decreases; within 2-3 hours after sunrise, germination would be completed. During the morning the humidity would decrease, but the shade of the overhanging leaves would provide the low light and low temperature conditions favorable for production of appressoria. Most of the appressoria would mature before noon and penetration of the host by the pathogen would take place during the afternoon. Development of secondary hyphae would soon follow. This stage would be completed in the low light of the late afternoon and in the shade of leaves, and would occur in a wide range of relative humidities below 100% RH. The first secondary hypha would be formed by early evening. According to the schedule of events detailed in this research, if conidial germination began by 3:00 AM, secondary hyphal development would begin by 8:00 PM the same day.

The occurrence of more powdery mildew on the lower leaves has been explained as due to higher humidity near the soil (23), or to factors other than humidity differences (57), including physiological differences in the leaves (51). The information gained in the present study suggests that in field conditions where foliage is dense, the microenvironment to which the lower leaves are exposed is more favorable for infection by powdery mildew than the upper leaves. These conditions which favor higher primary infection, together with the physiologic difference of leaves may offer a more complete explanation for the occurrence of the mildew gradient.

Though the ideal conditions of temperature, relative humidity and light demonstrated in this study for the various phases of primary infection apply to maximum mildew development in minimum time, varying degrees of mildew development have been observed in all conditions studied. Thus, these ideal conditions are not obligatory. This pathogen is adaptable to a wide range of temperature, relative humidity and light conditions making it a very versatile organism, capable of development in a variety of conditions and widely distributed throughout the wheat-growing countries of the world (1, 10, 19, 22, 23, 37, 59, 60).

The process of infection in Puccinia (39, 45, 53) broadly resembles the process of primary infection of wheat powdery mildew. In both these organisms germination was favored by darkness or by low light intensities below 300 foot-candles. Germination in both these pathogens was also favored by a lower temperature and higher relative humidity than the other phases of infection. In Puccinia, the conditions which favored germination of uredospores and appressorial formation were the same, but in wheat mildew, appressorial formation was favored by a lower relative humidity than that required for germination, and was also favored by low light. Post-appressorial development in both Puccinia and wheat powdery mildew was favored by increased light, lower relative humidity and higher temperature than were required for spore germination.

SUMMARY

The purpose of this research was to determine the optimum conditions of temperature, light and relative humidity (RH) for each of the component phases of primary infection of wheat by powdery mildew (Erysiphe graminis tritici).

The "rolling method" of inoculation devised during this study eliminated most disadvantages of previous methods. It utilized only fresh, young, separate conidia of fairly uniform age, and provided for their even distribution on the host leaf. This method together with the precise control of environmental conditions gave uniform and highly reproducible results.

Primary infection consisted of four separate phases: 1) germination of conidia, 2) formation of appressoria, 3) penetration of host, and 4) development of secondary hyphae. Each phase had a different set of optimum conditions. For germination, a relative humidity (RH) of 100% was more favorable than 65% at either 17 or 22°C, and maximum germination of 60% occurred in 4 hours. However, when the relative humidity was changed from 100% to 65% after the 4th hour, 93% germination was obtained after 5 more hours at 22°C. The same high level of germination was obtained in 4 hours by incubating inoculated plants for 1 hour at 17°C and 100% RH and 3 hours at 22°C and 65% RH. Since germination could be increased by altering relative humidity and temperature during the germination period the conidial population probably was not physiologically uniform. Light (14-30 foot-candles or 120-290 foot-candles) or darkness did not influence germination. Appressorial initials were formed equally well after the 4th hour on plants exposed to 22 or 28°C, 32 or 65% RH, and in low light (14-30 foot-candles), high light (120-290 foot-candles) or darkness. However, highest percentage of appressoria

matured at 22°C and 32% RH in low light, beginning with the 6th hour. By the 20th hour every germinated conidium had produced an appressorium. Penetration of host by the pathogen took place 6-8 hours after development of appressorium. This was followed by development of secondary hyphae marking the end of primary infection. The development of secondary hyphae was essentially the same at 32 and 65% RH, both conditions were more favorable than 100% RH. At 22°C and 65% RH in low light, secondary hyphal development began by the 18th hour after inoculation, and by the 32nd hour, 77% of the conidia had produced secondary hyphae.

A difference of one week in the age of the host plants did not influence germination and appressorial development. However, a slower rate of secondary hyphal development occurred on the leaves of older plants, and is probably due to a thicker cuticle on the older leaf.

Though the ideal conditions of temperature, relative humidity and light revealed in this study for different phases of primary infection apply to maximum mildew development in minimum time, varying degrees of mildew development were observed at all conditions studied. Thus, these conditions are not obligatory.

The optimum conditions for each phase of infection correspond to the conditions the fungus would normally encounter in a wheat field. During the summer, conditions are ideal for conidial germination beginning at about 3:00 AM. Appressoria would mature by noon, penetration of host would take place during the afternoon, and development of secondary hyphae would begin at about 8:00 PM the same day.

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