Proceedings of the Iowa Academy of Science

Volume 67 | Annual Issue Article 16

1960

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Self-inhibition of Germination of the Conidia of *Peronospora manshurica*[1]

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Abstract. Percentage germination of the conidia of *Peronospora manshurica* (Naum.) Syd. ex Gäum. was inversely proportional to their concentration on the surface of water agar. It appeared that the adverse effect of high concentration on germination was due to an inhibitor associated with the conidia. The inhibitor diffused readily in water, was lost after exposure to air for a few days, but accumulated when the conidia were stored anaerobically.

Conidia of the fungus *Peronospora manshurica* germinate erratically on the surface of water agar. Factors affecting germination are of interest because of the possible relationship these factors may have to the development of downy mildew of soybeans. The age of the lesion on which conidia are produced, the period of time conidia remain on a leaf before removal, and the period and temperature of storage of the conidia all contribute to germination irregularities. The present investigation arose from attempts to determine the nature of these factors and to relate germination potential of conidia under artificial conditions on water agar to disease development.

The concentration of conidia on the surface of water agar appeared to be the most influential factor in controlling germination. Spores in high concentrations germinated poorly, if at all, whereas germination was improved markedly if the concentration of conidia was decreased. This fact suggested that a germination inhibitor associated with the conidia might be responsible for low germination.

The association between high concentration and low germination of spores of some of the saprophytic fungi has been noted by Duggar (1901) and Doran (1922). This phenomenon has been described by the general term self-inhibition. It has been most clearly demonstrated in germination of the uredospores of rusts. Allen (1955) and Forsyth (1955) have determined several characteristics of the self-inhibitor of germination of the uredospores of *Puccinia graminis* var. *tritici* Erikss. and E. Henn. Allen (1955) has shown that heavy concentrations of spores floated on water would not germinate. They would germinate, however, after they were transferred to fresh water.

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[1]Journal Paper No. J-3862 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1179, in cooperation with the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

Very low concentrations of spores would also germinate on water. It was considered that germination inhibition was caused by a water soluble metabolite produced by the spores, for the presence of the inhibitor could be detected in the water on which heavy masses of spores had floated. Evidence was also obtained that the inhibitor was a volatile acid. Forsyth (1955) considered the volatile inhibitor released by the spores might be trimethylethylene.

Similarities between self-inhibition of germination of uredospores and of conidia have prompted further investigations of some of the major aspects of the phenomenon associated with germination of conidia of the downy mildew fungus.

Materials and Methods

Conidia used for these investigations were obtained from infected leaves of Illini soybean plants. A conidial suspension was prepared by washing the conidia off leaves into a small quantity of water. The conidial suspension was diluted in geometric progression to make a dilution series of 6 concentrations. One drop of the conidial suspension from each of the concentrations was placed on the surface of individual water agar disks and incubated 12 hours for the germination tests. Each concentration was replicated 4 times. The percentage germination on each disk was determined from 4 separate and random counts of 100 spores each. A uniform volume of agar in each disk was obtained by pouring 25 ml. of 2 percent water agar into flat-bottomed peri dishes and by cutting the agar with a cork borer 7 mm. in diameter.

Results

Conidia removed within 12 hours after being produced on the leaves of infected soybean plants germinated from 85 percent or more at the lowest concentration to 10 percent or less at the highest concentration of the dilution series. Adverse effects of high concentrations of conidia on germination suggested that an inhibitor, diffusible in water agar, was being produced by the conidia. This hypothesis was tested in the following manner: High, medium, and low concentrations of conidia were placed on the surface of water agar disks. The disks were turned upside down in a petri dish, and a water agar disk was placed over the top of each. A drop of a conidial suspension of the lowest concentration (approximately 12 conidia per cubic mm.) was placed on top of the double disk. The percentage germination of the conidia on the bottom of the 2 disks and of the conidia on the top of the disks was determined after 12 hours incubation at 10° C. Germination of conidia on the bottom of the lower disk was 7, 31, and 58 percent for the high, medium

and low concentrations, respectively. This compared with germination of 4, 29, and 57 percent on the top of the double disk. The control, consisting of the dilute conidial suspension not in association with conidia on the bottom of the disk, germinated 85 percent. These results indicated that the inhibitor associated with the conidia on the bottom of the disks diffused through the water agar and controlled the germination of conidia on the top of the disks, regardless of their low concentration.

Germination of various concentrations of conidia placed on the surface of single or double agar disks is presented in Figure 1. Germination was 12 percent on a single disk and 37 percent on a double disk at the highest concentration of conidia. This indicated that the volume of water agar available for dilution of the inhibitor was also an important factor in determining germination, particularly at the higher concentrations of conidia. The increase in volume of water agar was therefore similar in its effect on germination to the decrease in concentration of conidia per unit volume of water.

The hypothesis that the inhibitor was a product of the metabolism of conidia, and therefore should be influenced by an increase in

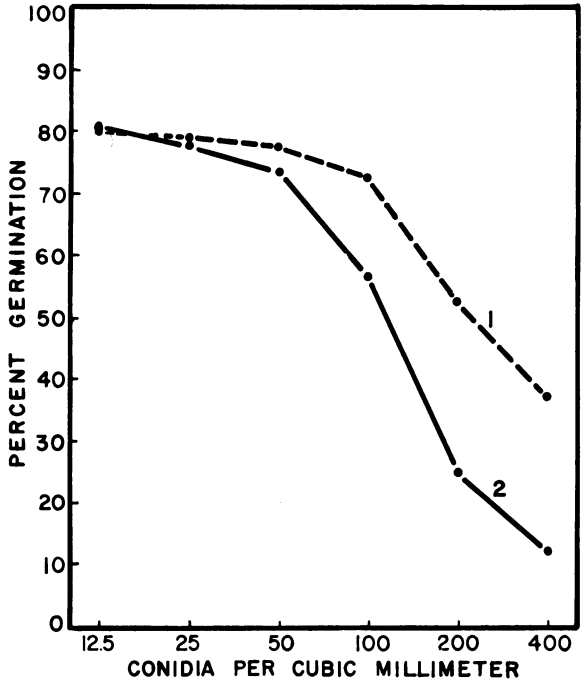

Figure 1. Percent germination of the conidia of *Peronospora manshurica* at various concentrations at 10° C. (1) Germination on the surface of a double agar disk. (2) Germination on the surface of a single agar disk.

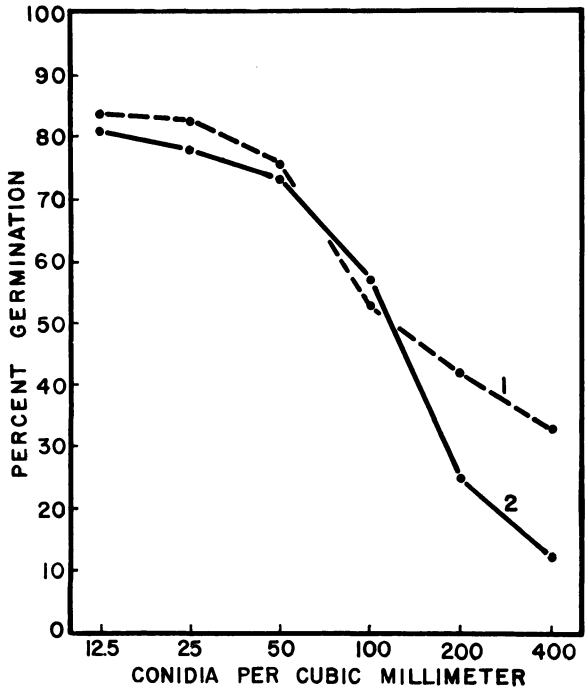

Figure 2. Percent germination of the conidia of *Peronospora manshurica* at various concentrations.

temperature, was tested by incubating conidia on the surface of water agar disks at 10° C and 20° C (Figure 2). [At the highest concentration] conidia germinated 12 percent at 10° C but 33 percent at 20° C. High temperature and dilution of inhibitor were similar, in that germination was increased by both factors. It appeared, therefore, that increased germination at 20° C was due to reduced concentration of the inhibitor, possibly by volatilization or inactivation during the incubation period. This hypothesis was tested by allowing a conidial suspension to stand in an open beaker or in a corked test tube for 3 days at 5° C (Figure 3). The effectiveness of the self-inhibitor was reduced by exposure to air for 72 hours in an open beaker, but the effectiveness was increased upon standing in corked test tubes. In subsequent tests, germination of the conidia stored in corked test tubes could not be obtained at the end of 12 days, but conidia stored in an open beaker still germinated to some extent. Conidia stored in corked test tubes were not killed, however, because infection of soybean seedlings could readily be obtained with them.

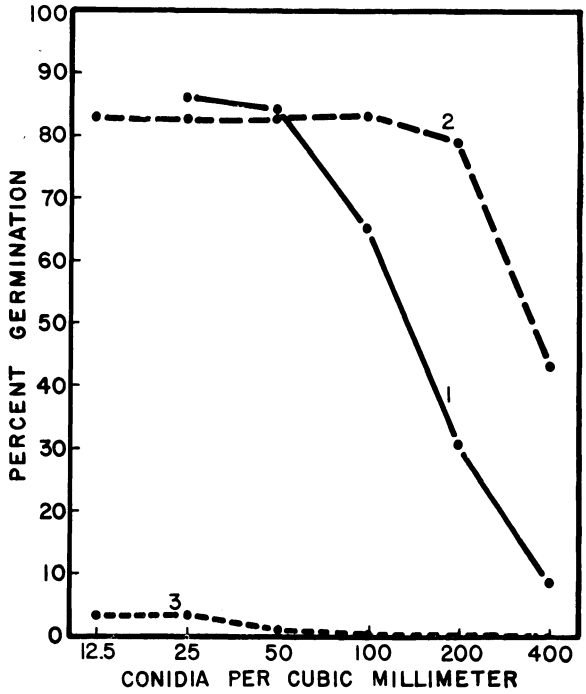

Figure 3. Percent germination of the conidia of *Peronospora manshurica* at various concentrations at 10° C. (1) Initial germination. (2) Germination after storage 72 hours at 5° C. in an open beaker. (3) Germination after storage 72 hours at 5° C. in a corked test tube.

Discussion

The phenomenon of self-inhibition of germination of the conidia of *Peronospora manshurica* appears similar in several respects to that associated with the uredospores of *Puccinia graminis* var. *tritici*. The conidia are extremely sensitive to changes in concentration of the inhibitor or of the spores themselves. The inhibitor diffuses readily in water and is lost from suspensions exposed to the air for a few days, but can accumulate in suspensions stored in corked test tubes.

Inhibition is more pronounced when conidia are present. A spore-free filtrate is much less effective in inhibiting germination of a low concentration of fresh spores. It appears, therefore, that the self-inhibitor is a product of the metabolism of the conidia and is produced during the early stages of germination. There is also some indication, however, that the inhibitor may be present in spores on the conidiophores, because conidia harvested soon after sporulation exhibit a stronger self-inhibitory effect than conidia which have been left on the leaves for a few days. Conidia germinate occasionally *in*

situ only when they are sparsely populated on the surface of detached leaves stored more than 24 hours in an atmosphere of 100 percent relative humidity. It is not known whether the inhibitor is volatile and escapes from conidia by diffusion or if it is lost or inactivated by some other process.

The most significant aspect of the self-inhibition phenomenon is that conidia may be prevented from germinating until they are scattered. The conidiphores undoubtedly serve to lift and keep the conidia free from contact with the moist surface of a leaf and thus prevent germination in place, but the additional factor of a self-inhibitor associated with conidia in high concentration also serves to prevent germination before the conidia become adequately disseminated.

Literature Cited

Allen, Paul J. 1955. The role of a self-inhibitor in the germination of rust uredospores. Phytopathology 45: 259-266.

Doran, W. L. 1922. Effect of external and internal factors on the germination of fungous spores. Bul. Torr. Bot. Club 49: 313-340.

Duggar, B. M. 1901. Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. 31: 38-66.

Forsyth, F. R. 1955. The nature of the inhibiting substance emitted by germinating uredospores of Puccinia graminis var. tritici. Can. J. Botany 33: 363-373.