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I. E. Melhus

*Iowa State College*

M. K. Patel

*Iowa State College*

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## STUDY OF PERONOSPORA TRIFOLIORUM DEBARY ON SPECIES OF LEGUMINOSAE

I. E. MELHUS, AND M. K. PATEL

The downy mildew, *Peronospora trifoliorum* deBy. of alfalfa, *Medicago sativa* L., is a widespread disease occurring in almost all countries of the world where alfalfa is grown. This disease assumes importance in the middle west during wet seasons, particularly in the spring and fall. In Iowa it is most destructive on fall seedlings and on the first cutting in the spring. The present studies were undertaken to throw light on the relation of moisture and temperature to spore germination and infection, the possible host-range of the downy mildew, and its method of overwintering.

### INFECTION STUDIES

#### *Temperature in Relation to Germination of Conidia*

Temperature has been found to play an important role in the germination of the conidia of several species of Peronosporaceae. The following studies also indicate that the response of the conidia of *Peronospora trifoliorum* are markedly influenced by temperature. The low temperatures used were obtained in an electric refrigerator. Well regulated ovens were used for temperatures ranging from 20°C. to 43°C. Drops of spore suspension were placed on clean glass slides, resting upon pieces of moist filter paper in petri dishes and held for 16 hours before the percentage of germination was determined. The results are presented in figure 1. It is obvious that the conidia of *P. trifoliorum* germinate very well between 4° and 25°C. and that no germination takes place at and above 30°C. Likewise, the spores did not germinate at 0° and 3°C. That they were still alive, however, after being subjected to this low temperature (0° and 3°C.) for 12 hours is apparent in that they did germinate later when the temperature was raised to 18°C. Although the temperature range for the germination of conidia is rather wide, 18°C. seemed to be the most favorable temperature.

Attempts were also made to determine the length of time that spores of *P. trifoliorum* would live at or below freezing tempera-

tures. Spores were obtained from infected alfalfa plants growing in the greenhouse. Spore suspensions were made on clean glass slides. The slides with the cultures were then transferred to the refrigerator at 0°C., and the cultures allowed to freeze. This pro-

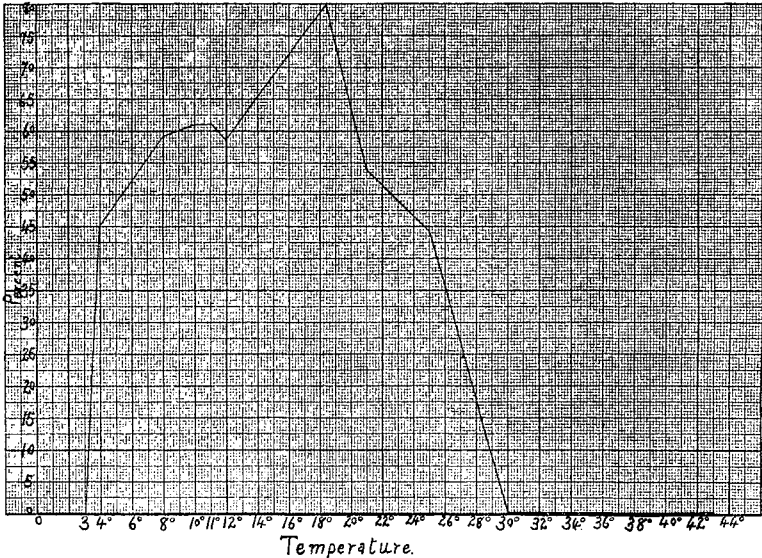


Fig. 1. This graph shows the germination of the conidia of *Peronospora trifoliorum* deBy. in relation to temperature

cess of freezing requires less than 30 minutes. Following this a number of the cultures were then placed in the refrigerator at temperatures of 12°C. to 14°C., for 16 to 20 hours. The remaining cultures were held frozen for 167 hours. The evidence of germination was carefully noted. The percentage of germination decreased in proportion to the length of time the water containing the spores was frozen. After the spores had been frozen in the ice for 167 hours only nine percent germinated.

#### *Effect of Freezing Temperatures on the Conidia*

Another set of experiments was carried on, in the open during December, 1924, to determine whether or not the conidia might live over winter and infect the young foliage early the next spring. Fresh conidia were suspended in pure water in small test tubes. Approximately one and one-half cc. of spore suspension was placed in each of 23 tubes. These tubes were placed in the soil out-of-doors on December 21, 1924. A minimum temperature of -26°C. was observed on December 22, 1924, and -29.4°C. on December 28, 1924.

STUDY OF *PERONOSPORA TRIFOLIORUM*

115

After from 23 to 339 hours these cultures were brought into the laboratory and exposed to favorable conditions for germination. The results of these trials are presented in table I.

Table I—Effect of freezing on Germination of Conidia

NUMBER OF CULTURES	PERIOD OF FREEZING	PERCENT GERMINATION IMMEDIATELY AFTER THAWING	PERCENT GERMINATION AFTER 16-24 HOURS AT 18°C.
2 check	—	—	66
2	81 hours	0	70
3	176 hours	0	10
3	241 hours	0	0
3	339 hours	0	0
3	23 days	0	0
7	80 days	0	0

The trials in table I show that a low temperature for a period of seven days (168 hours) is not decidedly injurious to the germination of conidia. Periods of freezing longer than seven days (176 hours) caused the death of the conidia.

In another trial small test tubes were filled with water suspensions of fresh conidia obtained from plants infected in the greenhouse. These were set outside the window of the laboratory at 11:00 o'clock in the morning on December 18, 1924, and remained there until the following morning. During the entire day there was no sunshine. The maximum temperature for this day was 4.4°C. and the minimum was -14°C.

The contents of the tubes were frozen in a short time. The tubes were taken into the laboratory December 25 at nine o'clock, allowed to thaw out, and the spore suspensions poured into clean watch glasses. The watch glass cultures were placed at approximately 18°C. and in eight hours approximately 50 per cent of the spores had germinated. These trials indicate that the conidia of *Peronospora trifoliorum* may be alive after the water medium in which they are contained has been frozen for 23 hours. The significance of this low temperature tolerance in relation to overwintering was not studied.

## HOST RANGE

The host range of *Peronospora trifoliorum* is not well defined. Studies were, therefore, made to determine the leguminous host range of *P. trifoliorum* found growing on alfalfa in the vicinity of Ames, Iowa.

In this connection it is interesting to record the host range as reported in the literature. *Peronospora trifoliorum* was first de-

scribed by deBary<sup>1</sup> in 1863 occurring on *Trifolium medium* L., *T. alpestre* L., *T. incarnatum* L. and *Medicago sativa*.

Fischer<sup>2</sup> in Rabenhorst records *Peronospora trifoliorum* occurring on the following additional hosts: *Trifolium agrarium* L., *T. arvense* L., *T. minus* Koch, *T. pratense* L., *T. repens* L., *T. rubens* L., *T. spadicum* L., *T. striatum* L., *Medicago falcata* L., *M. lupulina* L., *Melilotus alba* Desr., *M. officinalis* (L) Lam., *Lotus corniculatus* L., *L. uliginosus* Schk., *Coronilla varia* L., *Ononis spinosa* L., and *O. repens* L.

Smith<sup>3</sup> noted its appearance on *Trifolium medium*, *T. pratense*, *T. incarnatum* and *Medicago sativa* in England.

Butler<sup>4</sup> found it on the Indo-gangetic plain on *Melilotus alba*, *M. indica* (L) All., *M. parviflora* and *Medicago lupulina*, *M. denticulata* Willd. and *M. sativa*, and on *Glycine hispida* L. in Kashmir, the extreme northern province of India. Nauomoff<sup>5</sup> mentions its occurrence on soybeans in Formosa.

Wolf and Lehman<sup>6</sup> reported a new downy mildew on soybeans (*Glycine sojae*), which they called *Peronospora sojae* Wolf,<sup>7</sup> distinguishing it from *P. trifoliorum* because it would not infect alfalfa. They also called attention to some differences in morphology.

Nauomoff<sup>5</sup> established a variety of *Peronospora trifoliorum* which he named *P. trifoliorum* var. *manshurica* on *Glycine hispida*. He found a difference of 400  $\mu$  in length of conidiophores and 18  $\mu$  difference in diameter of the oospores. Butler,<sup>4</sup> however, reported that *P. trifoliorum* on *Glycine hispida* resembles the normal type of *Peronospora trifoliorum*.

Gäuman<sup>8</sup> has separated *Peronospora trifoliorum* into 11 distinct species based on their physiological and morphological differences. He measured 500 conidia of each species, but fails to state the number of oospores and conidiophores measured. Gäuman was unable to transfer the *Peronospora* occurring on alfalfa to any other legume, not even *Medicago lupulina*, a closely related species. Likewise, he was unable to transfer the downy mildew on *M. lupulina* to *M. sativa*. That such marked specificity should exist

<sup>1</sup> Bary, A. de. du development des champignons parasites. Ann. Sci. Nat. Bot. 4 Ser. 20: 117-119. 1863.

<sup>2</sup> Fischer, A. Phycomyces Rabenhorst's Kryptogamenflora. Bd. I. Abt. 4: 457-458. 1892.

<sup>3</sup> Smith, W. G. Diseases of field and garden crops. 263-265. London, 1884.

<sup>4</sup> Butler, E. J. Fungi and diseases in plants. Thacker Spink & Co., Calcutta, 1918.

<sup>5</sup> Nauomoff, N. *Peronospora trifoliorum* DB. var. *Mansburica* N.N. Bul. Soc. Myc. de France 30: 73-74. 1914.

<sup>6</sup> Wolf, F. A. and S. G. Lehman. A new downy mildew of soybeans. Jour. Elisha Mitchell Soc. 39: 164-169. 1924.

<sup>7</sup> In a letter to the senior author, Dr. F. A. Wolf states that *Peronospora sojae* is identical with the variety *manshurica* of *P. trifoliorum* described by Nauomoff from eastern Russia.

<sup>8</sup> Gäuman, E. Beiträge zur Kryptogamenflora der Schweiz. 5 Band. Heft 4: 174-222. Zurich. 1923.

STUDY OF *PERONOSPORA TRIFOLIORUM*

117

is questionable, especially in the light of the cross inoculation trials recorded in this paper.

The size of the conidia, conidiophores and the oospores of *Peronospora trifoliorum* as reported by different investigators are tabulated in the following table.

Table II — Measurements of the Fruiting Bodies of *Peronospora trifoliorum* deBy.

INVESTIGATOR	HOST	MEASUREMENTS		
		CONIDIA	OOSPORES	CONIDIOPHORES
Fischer		20-22x16-19	24-30	360-460
Butler	<i>Medicago sativa</i>	18-24x15-18	24-31	300-450
Gäumann	<i>Glycine hispida</i>	16-37x26-32	20-30	200-500
	<i>Medicago sativa</i>	9-27x20-24		
Melhus & Patel	<i>Medicago sativa</i>	14.5-25.5x	18-37	110-680
		15.5-30.5		
Nauomoff	<i>Glycine hispida</i>	21x18, 24x21	36-48	240-900
		27x24		
Wolf & Lehman	<i>Glycine sojae</i>	24-20.5	18-23	300-500

It should be noted that there prevails a rather wide range in the measurements recorded. Such being the case, it is doubtful whether or not slight differences in size can be considered significant.

## CROSS INOCULATION TRIALS

An attempt was made to learn whether the *Peronospora trifoliorum* occurring on alfalfa would infect the hosts listed in the preceding chapter.

This species of *Peronospora* was easily cultured in the greenhouse on alfalfa seedlings. Stock cultures were maintained in thickly seeded eight-inch pots. The incubation pe-



Fig. 2. *Peronospora trifoliorum* on alfalfa showing systemic infection developed in terminal shoots and profuse conidial development. This type of infection frequently leads to dwarfing. An uninfected branch is shown at the left.

riod varied from 6 to 12 days depending upon the age of the plants and the moisture and temperature conditions in the house.

The organism sporulated more quickly on young than on old plants. A high humidity combined with a temperature ranging from 15° to 22°C. seemed most favorable for the development of the parasite.

It was necessary to infect young alfalfa plants every 15 to 20 days to keep a stock culture in the greenhouse. This was done because the young infected plants during the winter months were killed quickly when conidial production was stopped. Oospores were formed in the dead or dying leaves. This was also true of the infected leaves in the field. The most striking symptom of this disease in the field is its tendency to become systemic in the terminal branches of the plant. (See figure 2.) In order to facilitate sporulation the stock cultures were placed in a closed moist chamber.

Table III shows that only *Medicago sativa* and *M. lupulina*

Table III—Infection Trials with *Peronospora trifoliorum* on Twenty-eight Leguminous Hosts Occurring on Alfalfa

HOSTS	NO. OF TRIALS	APPROXIMATE NUMBER PLANTS INOCULATED	APPROXIMATE NUMBER PLANTS INFECTED	REMARKS
<i>Medicago sativa</i>	47	740	740	Only a scanty infection was secured.
<i>Medicago lupulina</i>	5	110	8	
<i>Medicago hispida</i>	6	60	0	
<i>Astragalus canadensis</i>	4	80	0	
<i>Melilotus alba</i>	7	80	0	
<i>Melilotus officinalis</i>	7	90	0	
<i>Melilotus indica</i>	2	40	0	
<i>Glycine hispida</i>	2	30	0	
<i>Trifolium pratense</i>	31	310	0	
<i>Trifolium repens</i>	3	80	0	
<i>Trifolium fragiferum</i>	4	40	0	
<i>Trifolium reflexum</i>	3	30	0	
<i>Trifolium alexandrinum</i>	4	50	0	
<i>Trifolium incarnatum</i>	5	60	0	
<i>Trifolium hybridum</i>	5	60	0	
<i>Trifolium subterraneum</i>	3	100	0	
<i>Trifolium arvense</i>	3	40	0	
<i>Trifolium dubium</i>	3	30	0	
<i>Lotus villosa</i>	5	60	0	
<i>Lotus corniculatus</i>	1	20	0	
<i>Lespedeza striata</i>	1	20	0	
<i>Trigonella coernella</i>	1	20	0	
<i>Cytissus prolifer</i>	1	30	0	
<i>Mimosa prolifer</i>	1	70	0	
<i>Lathyrus sp.</i>	2	30	0	
<i>Phaseolus acutifolius</i>	4	40	0	
<i>Glottidium floridium</i>	4	50	0	
<i>Cicer aceratimum</i>	4	50	0	
<i>Vicia sativa</i>	4	50	0	
<i>Pisum sativum</i>	8	80	0	

became infected with the *Peronospora* taken from alfalfa in the vicinity of Ames, Iowa. The latter species is seemingly much more resistant than the former. Only eight plants out of 110 became infected. Another allied species, *M. hispida* Gaertn., known as bur clover, which has round and hairy leaves, has given no infection in six trials comprising 60 plants. Closely related genera such as *Lespedeza*, *Lotus*, *Glycine*, *Melilotus*, *Trigonella*, *Trifolium*, *Astragalus*, *Vicia*, *Pisum*, *Cicer*, *Glottidium*, *Phaseolus*, *Mimosa*, and *Cytissus*, did not become infected. It would seem that the *Peronospora* occurring on alfalfa in the vicinity of Ames, Iowa, used in these studies is quite definitely restricted in its host range, which suggests that there may be one or more biologic forms.

#### SUMMARY

Temperatures ranging from 4°C. to 28°C. permitted germination of the conidia of *Peronospora trifoliorum*; the optimum temperature for germination was about 18°C. The minimum time for the germination of conidia at the optimum temperature was three hours. The conidia always germinated by a germ tube.

The conidia of *P. trifoliorum* retained their viability when kept frozen for seven days. Continuous freezing of the conidia for periods longer than seven days (176 hours) resulted in no germination.

Twelve varieties of alfalfa were successfully inoculated. *Medicago lupulina* was successfully infected in only two trials. No infection was secured on the following legumes: *Astragalus canadensis*, *Cicer aceratinum*, *Cytissus prolifer*, *Glottidium floridium*, *Glycine hispida*, *Lathyrus* sp., *Lespedeza striata*, *Lotus villosa*, *L. corniculatus*, *Medicago hispida*, *Melilotus alba*, *M. indica*, *M. officinalis*, *Mimosa pudica*, *Phaseolus acutifolius*, *Pisum sativum*, *Trigonella coerulea*, *Trifolium alexandrinum*, *T. arvense*, *T. dubium*, *T. fragiferum*, *T. hybridum*, *T. incarnatum*, *T. pratense*, *T. reflexum*, *T. repens*, *T. subterranean*, *Vicia villosa*, *V. sativa*.

The host range of *Peronospora trifoliorum* occurring on alfalfa in Iowa is very restricted. This fact coupled with the large number of hosts reported suggests that there may be biological forms in this species.

IOWA STATE COLLEGE,  
AMES, IOWA.