

**Temperature Relations to the Vegetative
and Reproductive Growth and the Pathogenicity
of *Neocosmospora vasinfecta* Smith.**

By

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I. Introduction.

As the morphological characteristics and the occurrence of *Neocosmospora vasinfecta* SMITH in Japan were already reported in the writers' foregoing paper, the results of their experiments on the temperature relations to the vegetative and reproductive growth and the pathogenicity of the said fungus are given in the present paper.

II. Method of Isolation and Strains Studied.

The pure culture of *Neocosmospora vasinfecta* SMITH is easily secured by the common dilution method taking the ascospores from the perithecia formed on the host plant in nature. The fungus is also isolated by transferring cut pieces of the diseased tissue of the host after surface sterilization to agar plates. The following strains, thus isolated, were used in this experiment.

Strain No. 954. This strain was isolated from the diseased tissue of silk-tree, *Albizia julibrissin* DURRAZ., sent by the Kanazawa Local Forestry Office on Sept. 8, 1934. Perithecia were produced pretty easily.

Strain No. 957. The strain was isolated from the diseased tissue of the same specimen, but from a different part. Perithecium production was also observed.

Strain No. 1027. The strain was isolated from ascospores produced on the diseased part of the same specimen sent from Kanazawa. Perithecia were produced profusely.

III. Temperature Relations to the Growth.

(1) Germination of Ascospores.

The ascospores of the strain No. 1027 of *Neocosmospora vasinfecta* SMITH, produced in pure culture, were sown on 3% malt-extract agar aseptically. The germination percentage and the length of the germ-tubes after 24 hours incubation at various temperatures are given in Table 1.

Table 1.
Temperature Relations to the Germination of Ascospores
of *Neocosmospora vasinfecta* Smith.

Strain studied: No. 1027.

Results after 24 hours on malt-extract agar.

Temperature	Germination percentage	Length of germ-tubes	
		Range	Mean
5°C.	0 %	— μ	— μ
10°	0	—	—
15°	13.78	5-60	18.00
20°	13.98	5-60	18.75
24°	39.04	5-125	27.75
27°	42.66	5-200	48.50
30°	67.16	10-370	176.00
32°	69.88	10-325	190.25
35°	63.50	10-200	77.50
38°	60.62	5-125	70.25

According to Table 1, the ascospores began to germinate at about 15°C., at which the germination percentage was 13.78%. The percentage increased with the rise of temperature, the highest percentage, 69.88%, being shown at 32°C. Even at 38°C. the germination was 60%. The length of the germ-tubes after 24 hours incubation at 15°C. was 18 μ in average, at 32°C. 190 μ and at 38°C. 70 μ .

(2) Growth of Mycelium.

The growth of mycelium of two different strains, No. 954 and No. 1027, of *Neocosmospora vasinfecta* SMITH was measured by taking mean diameter of colonies grown on the malt-extract agar at various temperatures, on three different dates. The results of the experiments are shown in Table 2.

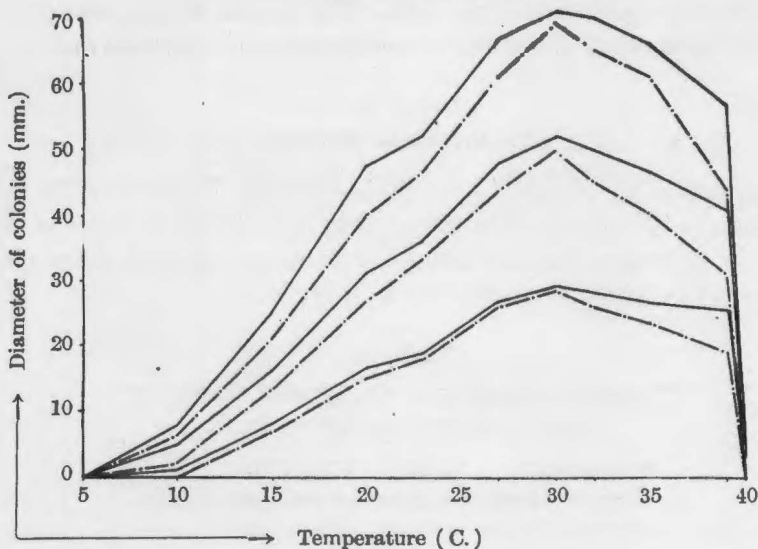
Table 2.
Temperature Relations to the Mycelial Growth of
Neocosmospora vasinfecta Smith.

Strains studied: No. 954 and No. 1027.
Diameter of colonies grown on malt-extract agar.
Average of three repeated experiments.

Temperature	Strain No. 954			Strain No. 1027		
	After 3 days	5 days	7 days	After 3 days	5 days	7 days
5°C.	— cm.	— cm.	— cm.	— cm.	— cm.	— cm.
10°	1.3	4.7	8.3	—	2.0	5.5
15°	8.0	16.5	24.6	6.8	13.3	20.6
20°	16.8	31.1	46.0	15.0	27.2	39.6
23°	19.1	35.9	52.4	17.9	32.5	46.5
27°	27.4	47.9	66.8	27.0	43.7	60.7
30°	29.4	52.0	71.1	29.0	49.5	68.5
32°	28.6	50.1	70.2	25.7	45.1	63.9
35°	27.0	46.7	66.5	23.8	40.2	60.7
38°	15.7	41.3	57.9	18.7	31.1	44.3
40°	3.3	4.5	4.6	—	—	—

From the figures given in Table 2, the mean diameter of the colonies of the both strains after 3, 5 and 7 days respectively are shown in Graph I, in which the abscissa shows the temperature in degree centigrade and the ordinate the average diameter of colonies.

Graph I.
Showing Temperature Relations to the Mycelial Growth of
Neocosmospora vasinfecta Smith.



Remarks: Average diameter of colonies of Strain No. 954 (—) and No. 1027 (---) after 3, 5 and 7 days culture respectively.

According to the results given in Table 2 and Graph I, the both strains of *Neocosmospora vasinfecta* SMITH, Strain No. 954 and No. 1027, showed common reactions to temperature. At 5°C. the both strains did not grow at all within the time studied, but at 10°C. they began to grow. Then the growth became better and better with the rise of temperature and reached to the optimum at 30°C. Between 30° to 38°C., the growth declined with the rise of temperature, and at 40°C. it was hardly observed. Therefore it may be concluded that the minimum temperature for the mycelial growth of *Neocosmospora vasinfecta* SMITH lies between 5°—10°C., the maximum near 40°C. and the optimum at about 30°C.

(3) Formation of Fruit-bodies.

i) Effect of culture media on the formation.

As a preliminary test to the experiment on the thermal relation to the fruit-body formation, reactions of this fungus on some culture media were studied. Strain No. 954, No. 957 and No. 1027 of *Neocosmospora vasinfecta* SMITH were grown at 27°C., a temperature near the optimum for the mycelial growth, and the fruit-body formation was examined after one and four weeks culture respectively. The culture media studied and the constituents were as follows: (1) RICHARD'S solution agar, (2) CURRIE'S solution agar, (3) agar medium with asparagin (water 1000, potassium acid phosphate 5, asparagin 2.5, Mg-sulphate 0.2, cane sugar 10 and agar 20), (4) apricot agar (water 1000, dried apricot 20, agar 20),

- (5) bouillon agar, (6) onion soy agar (water 850, concentrated onion decoction prepared of the same weight of onion and water 100, Japanese soy 50, cane sugar 50 and agar 20), (7) malt-extract agar (water 1000, malt-extract 30 and agar 20), (8) potato glucose agar (water 1000, potato 200, glucose 20 and agar 20).

The results of these experiments are shown in Table 3 and Table 4.

Table 3.

Effects of Culture Media on the Formation of Fruit-bodies of *Neocosmospora vasinfecta* Smith. (I)

Strains studied: No. 954, No. 957 and No. 1027.
Results after a weeks' culture at 27°C.

Culture medium	Strain No. 954			Strain No. 957			Strain No. 1027		
	Peri- thecia	Conidia		Peri- thecia	Conidia		Peri- thecia	Conidia	
		Non- sept.	Sep- tate		Non- sept.	Sep- tate		Non- sept.	Sep- tate
1. RICHARD'S agar	+	+	-	+	++	-	++	++	+
2. CURRIE'S agar	+	+	-	+	+	-	+++	+++	+
3. Agar medium with asparagin	+	+	+	+	++	+	+++	+++	+
4. Apricot agar	+	+	-	+	++	+	+	++	+
5. Bouillon agar	+	+	-	+	+	-	++	+	+
6. Onion agar	-	+	+	-	+	-	+	+++	+
7. Malt-extract agar	+	+	-	+	+	-	+	++	+
8. Potato agar	-	++	+	+	++	+	+++	++	+

Remarks: The + signs show the formation of fruit-bodies and the - signs, no formation.

Table 4.

Effects of Culture Media on the Formation of Fruit-bodies of *Neocosmospora vasinfecta* Smith. (II)

Strains studied: No. 954, No. 957 and No. 1027.
Results after four weeks' culture at 27°C.

Culture medium	Strain No. 954			Strain No. 957			Strain No. 1027		
	Peri- thecia	Conidia		Peri- thecia	Conidia		Peri- thecia	Conidia	
		Non- sept.	Sep- tate		Non- sept.	Sep- tate		Non- sept.	Sep- tate
1. RICHARD'S agar	++	++	+	++	++	+	++	++	+
2. CURRIE'S agar	+	++	+	+++	++	+	+++	+++	+
3. Agar medium with asparagin	+++	+	+	+++	++	+	+++	+++	+
4. Apricot agar	++	++	+	+	++	+	++	++	+
5. Bouillon agar	+	+	+	+	+	+	++	++	+
6. Onion agar	+	+	+	+	+	+	+	+++	+
7. Malt-extract agar	++	++	+	++	+	+	++	+++	+
8. Potato agar	+++	++	+	+++	++	+	+++	++	+

Remarks: The + signs show the formation of fruit-bodies and the - signs, no formation.

According to the foregoing tables the strain No. 1027 was much better than the other strains tested, in the formation of perithecia and conidia. And the agar medium with asparagin, CURRIE'S solution agar were very good in the perithecium formation, the potato glucose agar being the next. As these media contain comparatively much nitrogenous substance, the above results seem to agree to those of KRAUSE (1930), who stated that the perithecium formation depended greatly on the nitrogen and carbon contents of the media, the former being more important than the latter. Although the perithecium formation of strain No. 957 was somewhat poor than strain No. 1027, the reaction of the both strains to culture media was almost the same. In the strain No. 954, the formation of the fruit-body was much poor and the reaction to culture media was not always the same with the other.

As to the conidium formation, the strain No. 1027 was the best among the strains tested, and followed by No. 957 and No. 954 in the order. The conidia were formed profusely on CURRIE'S agar, the agar medium with asparagin and the onion soy agar and the malt-extract agar than on the other media.

ii) *Formation of conidium.*

Temperature relations to the conidium formation was studied on the 3% malt-extract agar. The agar plates were inoculated in the center with a bit of culture of the fungus. After a week incubation at various temperatures the conidium formation was examined, the results being given in Table 5.

Table 5.
Temperature Relations to the Formation of Conidia of
Neocosmospora vasinfecta Smith.

Strains studied: No. 954 and No. 1027.

Results after a week culture on malt-extract agar.

Temperature	Strain No. 954		Strain No. 1027	
	Non-septate conidia	Septate conidia	Non-septate conidia	Septate conidia
10°C.	+	-	+	-
15°	+	-	+	-
20°	+	-	+	-
24°	+	-	++	-
27°	+	+	++	+
30°	++	+	++	+
33°	++	+	++	+
35°	++	+	++	+
38°	++	+	++	+
40°	+	-	+	-

Remarks: The + signs show the conidium formation and the - signs, no formation.

As shown in Table 5, the both strains, No. 954 and No. 1027 produced non-septate conidia at all the temperatures between 10°–38°C. The conidium formation of the strain No. 1027 was very good at the temperatures between 24°–38°C. and that of the strain No. 954 at between 30°–38°C. Moreover, between 27°–28°C. comparatively large, 1-3-septated conidia were produced. In short the minimum temperature for the conidium formation was about 10°C., the maximum 38°C. and the optimum about 32°C.

iii) *Formation of perithecium.*

Further, the temperature relations to the perithecium formation were studied in the same way as stated above. The fungus strains were grown on the 3% malt-extract agar, and the perithecium formation was examined after 1, 2, 3, 4, 5 and 6 weeks culture respectively. The results are given in Table 6.

Table 6.
Temperature Relations to the Formation of Perithecia of
Neocosmospora vasinfecta Smith.

Strains studied: No. 954 and No. 1027.
Results on 3% malt-extract agar.

Temperature	Strain No. 954						Strain No. 1027					
	After 1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	After 1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
5°C.	-	-	-	-	-	-	-	-	-	-	-	-
10°	-	-	-	-	-	-	-	-	-	-	-	-
15°	-	-	-	-	+	+	-	-	-	-	+	+
20°	-	-	+	+	+	++	-	-	++	++	++	++
24°	-	-	+	+	++	++	-	±	++	++	+++	+++
27°	-	-	++	++	++	++	-	+	++	++	+++	+++
30°	-	-	+	+	++	++	-	-	++	++	++	++
32°	-	-	-	-	+	+	-	-	-	+	+	+
35°	-	-	-	-	-	-	-	-	-	+	+	+
38°	-	-	-	-	-	-	-	-	-	-	-	-
41°	-	-	-	-	-	-	-	-	-	-	-	-
45°	-	-	-	-	-	-	-	-	-	-	-	-

Remarks: The + signs show the perithecium formation and the - signs, no formation.

An examination of the results given in Table 6 shows that the both strains tested, were almost the same in the perithecium formation, although they varied slightly. The formation began at 15°–20°C. and best at 27°C. Above 32°–35°C. no perithecia were formed. The results coincide with those of KRAUSE (1930).

IV. Temperature Relations to the Pathogenicity.

(1) Pathogenicity to Silk-tree.

i) Germination of seeds of silk-tree, sown into inoculated soil.

Experiment I.

As to the pathogenicity of *N. vasinfecta* SMITH to silk-tree, experiments (a) on the germination of the seeds sown in inoculated soil and (b) on the infection of silk-tree seedlings transplanted to inoculated soil, were undertaken. In the first experiment, loamy soil was put into glass-bottle of 10 cm. high and 8 cm. in diameter, autoclaved and then inoculated with pure culture of *N. vasinfecta*. After the hyphae of the inoculated fungus were grown for some days, silk-tree seeds were sown, which were previously soaked in water and then surface-disinfected. They were then kept at various temperatures from 10° to 35°C. The germination of the seeds after 7, 13 and 17 days was examined. The results are given in Table 7.

Table 7.

Temperature Relations to the Germination of Seeds
of *Albizia Julibrissin* Durraz., which were Sown into the Soil inoculated
with *Neocosmospora vasinfecta* Smith.

Date of inoculation: March 11, 1935.

Date of sowing: March 14, 1935.

Date of examination of results: March 31, 1935.

Temperature	Germination percentage after		
	7 days	13 days	17 days
10°C. { Inoculated soil	0 %	0 %	0 %
{ Control	0	0	0
15° { Inoculated soil	0	0	0
{ Control	0	0	40
20° { Inoculated soil	0	0	0
{ Control	80	80	100
25° { Inoculated soil	0	0	0
{ Control	60	60	80
30° { Inoculated soil	10	10	10
{ Control	60	60	60
35° { Inoculated soil	0	0	0
{ Control	0	0	0

According to Table 7, the germination percentages of the seeds in the control after 7 days at 20°, 25° and 30°C. was 80, 60 and 80% respectively, the percentage

at 20°C. being the best. The seeds sown in the inoculated soil did not germinated at all the temperatures tested, with one exception of 30°C., at which only 10% of the seeds germinated.

Experiment II.

In this experiment, water-soaked seeds were surface-disinfected, then inoculated with the culture of *N. vasinfecta* and sown in autoclaved soil in galvanized-iron pots. The pots, 20 cm. high and 16 cm. in diameter, and containing 3.5 kgr. of loamy soil in each, were sterilized under a pressure of 20 pounds for one hours. They were placed on the constant temperature tanks, set at various temperatures.

In the same time, the autoclaved soil was inoculated with culture of *N. vasinfecta* as in the Experiment I. In this soil the surface-disinfected seeds were sown. During the experiments best cares were paid to raise the seedlings in good conditions.

After 25 days at temperatures of 10°, 15°, 20°, 25°, 30° and 35°C. the germination percentage of silk-tree seeds, number of seedlings affected after germination and the percentage of healthy seedlings were determined. The results are given in Table 8.

Table 8.
Temperature Relations to the Germination of Seeds
of *Albizia Julibrissin* Durraz., inoculated with *N. vasinfecta* Smith
and to the Seedling Blight.

Date of inoculation: March 30, 1935.
Date of examination of results: April 24, 1935.
Number of seeds sown: 20 seeds per set.

Temperature		10° C.	15°	20°	24°	27°	30°	33°	Average
Inoculated to seeds	Germination percentage . . .	0	0	0	0	0	0	0	0
	No. of infected seedlings . . .	0	0	0	0	0	0	0	0
	Percentage of healthy seedlings	0	0	0	0	0	0	0	0
Inoculated to soil	Germination percentage . . .	0	0	50	60	30	40	20	28.5
	No. of infected seedlings . . .	0	0	0	4	2	4	4	0
	Percentage of healthy seedlings	0	0	50	40	20	5	0	16.4
Control . .	Germination percentage . . .	0	70	75	90	85	85	85	70.0
	No. of infected seedlings . . .	0	0	0	0	0	0	0	0
	Percentage of healthy seedlings	0	70	75	90	85	85	85	70.0

As shown in Table 8, in the control more than 70% of the seeds sown, germinated at all the temperatures tested above 15°C., although they did not germinate at 10°C. On the contrary the seeds directly inoculated with the fungus did not germinate at all. The seeds sown in the inoculated soil did not germinated below 15°C., but above 20°C. 20—50% of them germinated. At high tempera-

tures the seedlings were affected after germination. Thus the percentage of healthy seedlings grown from inoculated soil was best at 20°C. and was 50%. At 33°C. all the seedlings wilted.

Experiment III.

The same inoculation experiment was repeated in the constant temperature tanks. Pure cultures of *N. vasinfecta* were inoculated to seeds and to soil as in the case of Experiment II. On April 25, 1935, the silk-tree seeds were sown into the pots, which were kept at various constant temperatures. The results, examined on May 16, 1935, are given in Table 9.

Table 9.
Temperature Relations to the Germination of Seeds
of *Albizia Julibrissin* Durraz., inoculated with *N. vasinfecta* Smith
and to the Seedling Blight.

Date of inoculation: April 26, 1935.

Date of examination of results: May 16, 1935.

Number of seeds sown: 20 seeds per set.

Temperature		15° C.	20°	24°	27°	30°	33°	36°	Average
Inoculated to seeds	Germination percentage . . .	0%	0%	0%	0%	0%	0%	0%	0%
	No. of infected seedlings . . .	0	0	0	0	0	0	0	0
	Percentage of healthy seedlings	0	0	0	0	0	0	0	0
Inoculated to soil	Germination percentage . . .	70	30	80	80	70	70	70	68.5
	No. of infected seedlings . . .	2	3	5	5	4	3	6	—
	Percentage of healthy seedlings	50	0	30	30	30	40	10	27.1
Control . .	Germination percentage . . .	80	60	60	60	60	60	80	65.7
	No. of infected seedlings . . .	0	0	0	0	0	0	0	0
	Percentage of healthy seedlings	80	60	60	60	60	60	80	65.7

According to Table 9, the average germination percentage in the control was 65.7%, while no germination was observed in the inoculated seeds. In regards to the germination of the healthy seeds, sown to inoculated soil, the percentage was pretty good and was 68.5% in average at all the temperatures tested. After the germination, however, many of the seedlings were infected by the wilt disease. The mean percentage of the healthy seedlings after 20 days was 27% in the case of soil inoculation and 0% in seed inoculation, while it was 65.7% in the control.

ii) Infection of seedlings, transplanted to inoculated soil.

Experiment IV.

To the autoclaved soil in glass bottles, 10 cm. high and 8 cm. in diameter, pure culture of *N. vasinfecta* on boiled rice was inoculated. They were kept at

24°C. for four days. Young seedlings of silk-tree, grown in sterilized soil, were transplanted to the pots, which were then kept at 10°, 15°, 20°, 25°, 30° and 35°C. The results are given in Table 10.

Table 10.

Temperature Relations to the Infection of Seedlings of *Albizia Julibrissin*, transplanted to Soil inoculated with *N. vasinfecta* Smith.

Date of inoculation: March 11, 1935.

Date of transplanting: March 14, 1935.

Date of examination of results: March 31, 1935.

Temperature	Soil, to which transplanted	Percentage of seedling blight				
		After 4 days	7 days	10 days	13 days	17 days
10°C.	Inoculated soil . .	0 %	0 %	0 %	0 %	25 %
	Control	0	0	0	0	0
15°	Inoculated soil . .	0	0	25	50	75
	Control	0	0	0	0	0
20°	Inoculated soil . .	0	50	20	75	100
	Control	0	0	0	0	0
25°	Inoculated soil . .	25	50	75	100	100
	Control	0	0	0	0	0
30°	Inoculated soil . .	0	50	75	100	100
	Control	0	0	0	0	0
35°	Inoculated soil . .	0	50	100	100	100
	Control	0	0	25	25	50

The result of this experiment, as given in Table 10, showed that the seedling infection appeared at first in those kept at 25°C., and 25% of the seedlings were infected after 4 days. After 7 days, however, 50% of the seedlings wilted at all the temperatures of 20°, 25° and 30°C. After 10 days the infection was observed at 15°C. and after 17 days even at 10°C.

Experiment V.

On March 30, 1935, a further experiment was repeated in the same way as stated above in glass bottles. On 5 days after transplanting, one seedling wilted at 20°C., three at 25°C. and four at 30°C., while after 7 days five seedlings wilted at 20°C., three at 25°C. and six at 30°C. Results, examined on April 15, 1935, are shown in Table 11, in which 100% of seedlings were infected at 30°C., 88.9% at 20° and 25°C., 57.1% at 15°C. and 44.4% at 10°C., respectively.

In short, the wilt of silk-tree seedlings outbroke at all the temperatures tested, between 10° and 30°C., although the infection was very severe at above 25°C. and somewhat slight at below 15°C.

Table 11.

Temperature Relations to the Infection of Seedlings of *Albizia Julibrissin*,
transplanted to Soil inoculated with *N. vasinfecta* Smith.

Date of inoculation: March 30, 1935.

Date of transplanting: April 2, 1935.

Date of examination of results: April 15, 1935.

Temperature	Inoculated soil		Control	
	Infection percentage	Formation of perithecia on affected part of host	Infection percentage	Formation of perithecia
10°C.	44.4 %	—	0 %	—
15°	57.1	—	0	—
20°	88.9	+	0	—
25°	88.9	++	0	—
30°	100	###	0	—

(2) Pathogenicity to Cotton Plant.

Experiment VI.

Cotton seeds inoculated with pure culture of *N. vasinfecta* were sown into sterilized soil in pots of galvanized-iron, placed at the constant temperature tanks. The temperatures tested were 10°, 15°, 20°, 25°, 30° and 35°C. The date of sowing was March 30, 1935, and the examination of results, April 24, 1935. The results are given in Table 12.

Table 12.

Temperature Relations to the Germination of Seeds
of *Gossypium Nanking* Meyen., inoculated with *N. vasinfecta* Smith
and to the Seedling Infection. (I)

Date of sowing: March 30, 1935.

Date of examination of results: April 24, 1935.

Number of seeds sown: 20 seeds per set.

Temperature		10° C.	15°	20°	24°	27°	30°	33°	Average
Inoculated to seeds	Germination percentage . . .	0 %	20 %	25 %	35 %	55 %	30 %	35 %	28.5 %
	No. of infected seedlings . . .	0	0	2	0	0	1	0	0
	Percentage of healthy seedlings	0	20	15	35	55	35	35	26.4
Control . . .	Germination percentage . . .	0	90	90	90	100	90	100	80.0
	No. of infected seedlings . . .	0	0	0	0	0	0	0	0
	Percentage of healthy seedlings	0	90	90	90	100	90	100	80.0

At 10°C., even after 25 days both the control and the inoculated seeds did not germinate. At all the temperatures tested above 15°C. more than 90% of the control seeds germinated, while in the inoculated seeds about 30% germination was observed at all the temperatures tested, with two exceptions of 20% at 15°C. and 55% at 27°C.

Experiment VII.

In the similar method as in Experiment VI, germination of cotton seeds inoculated with *N. vasinfecta*, and those sown in the inoculated soil were tested. The results after 20 days are given in Table 13.

Table 13.

Temperature Relations to the Germination of Seeds
of *Gossypium Nanking* Meyen., inoculated with *N. vasinfecta* Smith
and to the Seedling Infection. (II)

Date of sowing: April 26, 1935.

Date of examination of results: May 16, 1935.

Number of seeds sown: 20 seeds per set.

Temperature		15° C.	20°	25°	27°	30°	33°	36°	Average
		%	%	%	%	%	%	%	%
Inoculated to seeds	Germination percentage . . .	60	90	70	80	60	70	60	70.0
	No. of infected seedlings . . .	4	0	1	4	4	5	2	0
	Percentage of healthy seedlings	20	90	60	40	20	20	40	41.3
Inoculated to soil	Germination percentage . . .	20	80	100	100	80	0	80	65.7
	No. of infected seedlings . . .	0	4	2	2	6	0	4	0
	Percentage of healthy seedlings	20	40	80	80	20	0	40	40.0
Control . .	Germination percentage . . .	30	80	100	100	100	70	90	80.0
	No. of infected seedlings . . .	0	0	0	0	0	0	0	0
	Percentage of healthy seedlings	30	80	100	100	100	70	90	80.0

In this experiment the germination of the cotton seeds in the control was comparatively good, except that at 15°C. and the average percentage was 80%. Even in the inoculated seeds, the germination was also pretty good, except that at 33°C. The percentage of healthy seedlings from inoculated seeds, however, was near a half of that of the control and was about 40%. The growth of the seedlings in the seed as well as the soil inoculation was much worse than in the control, especially at temperatures above 30°C.

Experiment VIII.

On March 30, 1935, a similar inoculation experiment was undertaken with cotton seedlings. Healthy, aseptically grown, young seedlings of cotton were transplanted to soil in galvanized-iron pots inoculated with *N. vasinfecta*. On April 4, 1935, 14 days after transplanting, the result was examined. It is shown in Table 14.

Table 14.
Temperature Relations to the Infection of Seedlings
of *Gossypium Nanking* Meyen., transplanted to Soil inoculated with
N. vasinfesta Smith.

Temperature	Inoculated soil		Control	
	Infection percentage	Formation of perithecia	Infection percentage	Formation of perithecia
10°C.	0 %	—	0 %	—
15°	0	—	0	—
20°	100	+	0	—
25°	100	+	0	—
30°	100	+	0	—

In short *Neocosmospora vasinfesta* is able to infect the cotton seeds and the seedlings and cause the wilt disease. The pathogenicity seems to be severer at comparatively higher temperatures.

(3) Pathogenicity to Watermelon.

In the same way as above, inoculation experiment of *N. vasinfesta* to seeds of watermelon was undertaken. On April 26, 1935, the inoculated seeds were sown to sterilized soil in pots, which were then kept at 15°, 20°, 25°, 30°, 33° and 36°C. Germination percentage from the seeds and number of seedlings affected after germination were studied on May 16, 1935. The results are given in Table 15.

Table 15.
Temperature Relations to the Germination of Seeds
of *Citrullus vulgaris* Schrad., inoculated with *N. vasinfesta* Smith
and to the Seedling Infection.

Date of sowing: April 26, 1935.

Date of examination of results: May 16, 1935.

Number of seeds sown: 20 seeds per set.

Temperature		15° C.	20°	24°	27°	30°	33°	36°	Average
Inoculated to seeds	Germination percentage . . .	50	10	30	30	20	40	60	34.2
	No. of infected seedlings . . .	0	0	1	0	0	0	1	
	Percentage of healthy seedlings	50	10	20	30	20	40	50	31.4
Control . .	Germination percentage . . .	0	30	40	60	60	50	60	42.8
	No. of infected seedlings . . .	0	0	0	0	0	0	0	
	Percentage of healthy seedlings	0	30	40	60	60	50	60	42.8

According to Table 15, the germination of watermelon seeds tested was comparatively bad, not only in the inoculated seeds, but also in those not inoculated.

(4) Discussion.

The above given results of the present writers' experiment showed that *Neocosmospora vasinfecta* SMITH was able to infect the seedlings of silk-tree, cotton and watermelon. It causes sometimes a serious damage to these plants. The seeds inoculated with the fungus showed no germination almost always, and if any germinated, the seedlings were then attacked by the disease and wilted. So far as the present experiments concerned, the higher the temperature was, the severer the pathogenicity of the fungus was.

As to the pathogenicity of this fungus, SMITH (1899) described *N. vasinfecta*, as the cause of the wilt of cotton, watermelon and cowpea. Later, however, BUTLER (1910) ascribed the cause of the cotton wilt to *Fusarium* spp. and considered *N. vasinfecta* to be secondary non-pathogenic saprophyte. It is true that the *Fusarium* spp. are able to cause the cotton wilt and the opinion was admitted by many authors, but it can't be said that *N. vasinfecta* has no relations to the wilt. Recently MITRA (1934) described this fungus as a cause of wilt of *Crotalaria funcea* L., beside *Fusarium* spp. The fact agrees the present writers' result of the experiment on the pathogenicity of *N. vasinfecta* to silk-tree, cotton and watermelon.

As stated above, *N. vasinfecta* SMITH may be a cause of the wilt of silk-tree and other plants, at least in favourable conditions. Therefore, it can not be safe to consider *N. vasinfecta* as a mere harmless saprophyte.

V. Summary.

1) This is the second report on *Neocosmospora vasinfecta* SMITH, a causal fungus of seedling wilt of silk-tree, *Albizia Julibrissin* DURRAZ., and deals with the temperature relations to the vegetative and reproductive growth as well as the pathogenicity of the fungus.

2) The ascospores of this fungus began to germinate at a temperature of 10°—15°C., and the germination was the best at about 30°C. and slightly observed even at 38°C. The minimum temperature for the mycelial growth was at 10°C., the maximum 40°C. and the optimum 30°C. approximately.

3) The temperature relations to the conidium formation were almost the same with those to the mycelial growth, the conidia were formed between temperatures from 10° to 38°C. and the best at 24°—29°C.

4) The minimum temperature to the perithecium formation was 10°—15°C., the maximum, 32°—35°C. and the optimum, about 27°C.

5) According to the writers' experiment *Neocosmospora vasinfecta* SMITH is able to infect the seedlings of silk-tree, cotton, watermelon, etc., although its pathogenicity has been denied since the BUTLER'S report.

6) The pathogenicity of *Neocosmospora vasinfecta* SMITH to seeds and seedlings of these plants was positively demonstrated by the seed as well as the soil inoculations, at all the temperatures between 10° and 35°C. The attack, however, was severer at above 25°C. and somewhat slight at below 15°C.

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Explanation of Plates.

Showing results of inoculation experiments of *Neocosmospora vasinfecta* SMITH (Strain No. 1027) to the seedlings of silk-tree.

Fig. 1. A bird's eye view of silk-tree seedlings on 7 days after transplanting of healthy seedlings to the soil, previously inoculated with *N. vasinfecta* SMITH. A and B show the pots containing inoculated soil and C, not inoculated.

Fig. 2. Three seedlings of silk-tree showing the result of an inoculation experiment of *N. vasinfecta* SMITH. B and C show the inoculated, diseased seedlings and A, the control.

Fig. 3. The same as Fig. 2. In this picture, A and B are the inoculated, diseased seedlings and C, the control.

Fig. 1.



Fig. 2.



Fig. 3.

