STUDIES ON THE PHYSIOLOGIC SPECIALIZATION IN *FUSARIUM LINI* BOLLEY

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I. Introduction

The discovery of the phenomenon of physiologic specialization in fungi is undoubtedly one of the most important and interesting problems in Plant Pathology. It was first suggested by Schroeter (40), in 1879, but Eriksson (17), in 1894, first divided practically the species *Puccinia graminis* Pers. into six specific forms on the basis of the pathogenicity, and since then it has been found that the physiologic specialization is of very wide occurrence among plant pathogens such as rust fungi (41, 42, 43,) powdery mildews (26), smut fungi (33, 36, 37, 49), and other kinds of fungi (2, 3, 12, 13, 25, 52).

In the early stage of the investigation of this line, physiologic forms were distinguished according only to the differences in pathogenicities, but now they became to be distinguished on the basis of cultural characters (12, 13), physico-chemical reactions (16, 18, 19, 20), and in addition, their biometrical differences in morphology (23, 24, 50), excepting pure parasites such as the rust fungi or the powdery mildews which are unable to be cultured on artificial media.

Thus the following four methods are generally adopted in recognizing physiologic specialization within a species:

1. Discrimination depended upon the difference in cultural characters;
2. Discrimination depended upon the difference in physico-chemical reactions;
3. Discrimination depended upon the biometrical difference of morphlogy;
4. Discrimination depended upon the pathogenicity.

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To study the differences of cultural and physico-chemical characters occurring within a species of a fungus is very important, since they may frequently correspond to the pathogenic differences. In fact, CHRISTENSEN and STAKMAN (13) discriminated fifteen physiologic forms of *Ustilago Zeae* (BECKM.) UNG. on the basis of the difference of cultural characters,and possibly eight, at least seven, of them were differentiated also in their pathogenicity to ten selfed lines of corn plant. Similar results have been obtained by several investigators in other cases (3, 4, 38).

As shown by various authors in cultural studies, the physiologic forms usually differ from each other in their physico-chemical reactions such as temperature-requirements, color-production, and relations to H-ion concentration, to C-N ratio, and to the concentration of culture media, etc.

LEVINE (23) reported that there might be pronounced and significant differences in the size and shape of the uredospores of some of the physiologic forms of *Puccinia gramins tritici* when grown under identical conditions.

According to TOCHINAI and SHIMAMURA (50), nine physiologic forms of *Piricularia Oryzae* classified due to cultural and physico-chemical characters can be distinguished into two types, namely longer and shorter types from the point of view of the biometrical differences in morphology of conidia, and moreover the apex of conidia is attenuate in the longer type and round in the shorter one. From the results of previous studies, it is clearly understood that the differences on cultural, physico-chemical, and biometrical characters may be the first indications of the different physiologic forms and often correspond to the pathogenic differences.

The flax-wilt disease caused by *Fusarium Lini* BOLLEY is occurring commonly in anywhere flax cultivating districts and it has been incessantly an important problem, nevertheless there has been established no satisfactory control measure other than the cultivation of resistant varieties of flax and the crop-rotation of very long intervals.

The breeding and distinction of resistant varieties of flax will contribute materially to the solution of the flax wilt problem, and several hopeful varieties have already been found locally. However, the resistant varieties hitherto found are not alway valid everywhere, and they may be resistant in one locality but not in others.

This difference in the revelation of resistance may be due partly to the influences of environmental conditions as TISDALE (45, 46, 47) said, and BARKER (1) has shown that wilt resistance is only relative and may be modified by environmental conditions. But, it is an important question that the difference in reaction of resistant varieties to the disease in different localities may be due whether to physiologic specialization of the pathogen or not. Then it is of prime importance to determine the occurrence of physiologic specialization in the causal fungus, *Fusarium Lini*.

As far as the authors know, a few work upon the problem of physiologic specialization in the wilt fungus was reported by BROADFOOT (4), BROADFOOT and STAKMAN (5) and LETCHER and WILLAMAN (22).

BROADFOOT (4) and BROADFOOT and STAKMAN (5) distinguished various strains of *Fusarium Lini* into nine physiologic forms according to their pathogenicity to four varieties of flax, and these nine physiologic forms were distinguished due to cultural differences presented on three different media, i.e. potato glucose agar, prune agar, and cornmeal agar, but with some difficulties. BROADFOOT (4) said, however, that no significant differences were found in the amount of radial growth of these physiologic forms on the same medium at various temperatures, and also that there were inherent differences in the spore dimensions of the different physiologic forms when cultured on the same medium under identical conditions.

LETCHER and WILLAMAN (22) found that the physiologic forms of *Fusarium Lini*, which were different in their cultural characters and pathogenicity, differed also in their ability to produce alcohol from sugar.

The present studies have been undertaken to ascertain the occurrence of physiologic specialization in various strains of *Fusarium Lini* collected from various localities in Hokkaido. Experiments were carried out concerning the cultural characters, physico-chemical reactions, pathogenicities, and, in addition, possible difference in morphology of the strains under test.

The saltant produced in the course of cultural experiments were treated as individual strains and the relations between the saltants and the mother strains were examined in detail.

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the Imperial Flax Manufacturing Company who kindly supplied the valuable materials and flax-seeds at the authors' request.

II. Materials used and methods of isolation

The specimens of the diseased flax plants were collected from various localities in Hokkaido in 1933.

Although the wilt fungus attacks flax plants at any stage of growth, the greatest damage is done on the seedlings. The young seedlings are readily killed showing typical wilt symptoms, and a close observation reveals severe rot occurred on the root system by an attack of the fungus. In grown plants, in which lignified tissues developed, such a rapid death is not practical. On the culms of heavily affected plants pinkish conidial masses of the causal fungus are produced.

Isolations were made with such affected flax plants. The wilted seedlings or lower parts of affected mature stems were cut in moderate size and sterilized superficially by means of soaking in 0.1 per cent aqueous solution of mercuric chloride for two minutes, and after thorough washing with sterilized distilled water they were put on apricot decoction agar plates in Petri-dishes, and incubated in a thermostat at 28°C. After one or two days, white cottony mycelia bearing conidia abundantly develop on the cut stems. A possibly small amount of spores were transferred by a sterilized needle to the melted apricot decoction agar medium in test tubes, and poured into Petri-dishes with thorough shaking, and they were incubated in a thermostat at 28°C. After a few days colonies of white mycelium develop on the agar surface. The conidia produced on these colonies showed typical morphological characters of *Fusarium Lini*. They were transferred to the same culture medium slanted in test tubes, in order to obtain abundant spores for the following single spore isolation.

Single spore isolations were made after modified SHERBAKOFF's (39) dilution method.

Spores produced on the apricot decoction agar medium were suspended in the sterilized distilled water in a test tube. Then dilution transfers were made from tube to tube until mostly a single conidium is contained in each small droplet taken by a platinum loop. A droplet of such diluted spore suspension was trans-

ferred on a sterilized cover glass, and it was placed upside down on a Van-Tieghem cell to secure hunging drop. Under a microscope the droplets containing single spore were selected in a germ free glass chamber, and they were singly put into sterilized Petri-dishes, into which about 7 c.c. of melted and properly cooled apricot decoction agar medium was poured. Then it was incubated in a thermostat at 28°C.

At the first observation of the culture plates containing single spore after two or three days, it was revealed that most spores had already germinated and it could be observed from the bottom side with comparative ease. At the time, the mycelial colony grown from single spore was sufficient to be seen by naked eyes. When it was proved by a careful microscopic examination that the mycelium originated from a single spore, it was transferred with a finely pointed needle to the other agar slant.

The isolated 40 strains of *Fusarium Lini* started from single spore with reference to their localities, date of isolation, and varieties of the affected flax plants from which the fungus was isolated are shown in Table I.

TABLE I. The strains of *Fusarium Lini* isolated from the flax plants collected in various localities.

Strain	Variety of flax	Locality	Date of isolation
No. 1	Riga	Sapporo, Prov. Ishikari	June 8, 1933
No. 2	Pernau No. 1	" , "	" , "
No. 3		" , "	June 17, "
No. 4		" , "	July, 3, "
No. 5		" , "	" , "
No. 6	Kotoni No. 1	Kotoni, "	June 9, "
No. 7	Washington No. 14	" , "	June 17, "
No. 8		" , "	July 3, "
No. 9	Pernau No. 1	" , "	" , "
No. 10		Horomui, "	July 16, "
No. 11		" , "	" , "
No. 12		" , "	" , "
No. 13		Asahikawa, "	July 11, "
No. 14		" , "	" , "
No. 15		" , "	" , "

TABLE I (*Continued*)

Strain	Variety of flax	Locality	Date of isolation
No. 16		Biei, Prov. Ishikari	July 3, 1933
No. 17	Saginau	Kakuta, "	June 26, "
No. 18		Tsukigata, "	June 21, "
No. 19		" , "	June 26, "
No. 20		" , "	June 26, "
No. 21		Furano, "	June 21, "
No. 22		Nayoro, Prov. Teshio	June 17, "
No. 23		" , "	July 3, "
No. 24		" , "	" , "
No. 25		Rusutsu, Prov. Iburi	June 17, "
No. 26		Sobetsu, "	" , "
No. 27		" , "	July 3, "
No. 28		Date, "	June 19, "
No. 29		" , "	July 2, "
No. 30		Toyoura, "	June 26, "
No. 31		" , "	" , "
No. 32		" , "	" , "
No. 33		" , "	July 2, "
No. 34		Shimoyubetsu, Prov. Kitami	June 17, "
No. 35		" , "	July 3, "
No. 36		" , "	" , "
No. 37		Toshibetsu, Prov. Shiribeshi	July 11, "
No. 38	Pernau No. 1	Obihiro, Prov. Tokachi	Aug. 5, "
No. 39	"	" , "	" , "
No. 40	"	" , "	" , "

III. Specialization in the growth type on different culture media

(1) Cultures on apricot-juice agar medium.

The apricot-juice agar medium is generally known as one of the most suitable culture medium for the development of most pathogenic fungi. According to TOCHINAI (49) this medium also facilitates the development of aerial mycelium of *Fusarium Lini* BOLLEY.

The apricot-juice agar medium used in this experiment was prepared in the following ways.

Two hundreds grams of dried seedless apricots were soaked in 500 c.c. of distilled water for 24 hours, and filtered through absorbent cotton, and then its original volume was restored by an addition of distilled water. On the other hand, 30 grams of agar were melted in 500 c.c. of distilled water in Koch's steam sterilizer. Having mixed these two solutions the agar medium was obtained, and 15 c.c. of it was distributed in each test tube. After the sterilization for twenty minutes twice on successive two days, they were poured into the sterilized Petri-dishes of uniform size, 85 mm. in diameter.

Inoculations were made at the center of these agar plates with a bit of mycelium and conidia from the stock cultures on the apricot decoction agar medium, and incubated in a thermostat at 28°C.

The observation was made after 8 days regarding three cultures for each strain to examine the cultural behaviours.

In the process of culture, it has been observed that the strains Nos. 1, 2, 4, 5, 9, 10, 32, and 33 produced white aerial mycelium and other did pink one.

The cultural characters of the strain under test were compared in the following respects: diameter, topography, and margin of colonies, proparties of the aerial mycelium, coloration of the immersed mycelium, and the occurrence of zonation and sectoring.

The strains under examination were classified into following five types of growth in regard to their cultural characters above mentioned.

Type 1a: Represented by strain No. 1.

Colony is raised in topography, and the radial growth is good. The aerial mycelium develops densely all over the surface of colony, and is floccose and white in color. Immersed mycelium is colorless. Sectoring is absent and zonation is faint or entirely absent.

Type 1b: Represented by strain No. 36.

Floccose aerial mycelium covers all over the colony being raised in topography and light pinkish lilac to argyle purple in color. Color of the immersed mycelium varies from pale purplish vinaceous to livid brown, and in some of the cultures

the color is light perilla purple at the center. Radial growth is moderate. Zonation is often observed.

Type 1c: Represented by strain No. 17.

This type is resembled to the type 1b, but distinguished from it by more rapid growth of colony and pigmentation of the immersed mycelium being jasper red to madder brown at the inner part and chatenay pink at the margin. Zonation and sectoring are absent.

Type 1d: Represented by strain No. 7.

Rough velvety to velvety aerial mycelium covers densely all over the colony, having concentric alternation of argyle purple and light perilla purple zones. Color of the immersed mycelium is livid brown at the central part and pale purplish vinaceous at the ray part. Radial growth is more or less slow. Zonation is present.

Type 1e: Represented by strain No. 21.

Central portion of the colony is slimy to waxy in appearance and white floccose aerial mycelium grows scantily on it as dots. At the marginal zone of the colony, aerial mycelium develops densely, being floccose to wooly in appearance and purplish lilac in color. Color of the immersed mycelium is livid brown to dark livid brown. Conspicous zonation takes place. Radial growth is more or less slow.

In regard to the cultural characteristics presented on the apricot-juice agar medium, all the strains tested were classified as follows:

Type 1a: Nos. 1, 2, 4, 5, 9, 10, 32, 33.
Type 1b: Nos. 11, 12, 13, 15, 16, 18, 19, 20, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 36, 37, 39, 40, 41.
Type 1c: Nos. 6, 14, 17, 22, 38.
Type 1d: No. 7.
Type 1e: Nos. 3, 21, 27.

(2) Cultures on potato decoction agar medium.

The potato decoction agar medium used in the present experiment was prepared in the following ways.

Two hundreds grams of peeled potatoes were decocted in 1000 c.c. of distilled water for one hour in Koch's steam sterilizer. The decoction was strained through absorbent cotton and the volume

was restored to 1000 c.c. by an addition of proper amount of distilled water. Having added two per cent glucose and two per cent agar, it was steamed until the agar was sufficiently melted, and was strained again through absorbent cotton. The agar medium thus prepared was taken in test tubes 15 c.c. each and sterilized in Koch's steam sterilizer for one hour every day in successive four days. The method of preparation of the culture plates and technique of the experiment were similar to the forgoing experiment.

TISDALE (45) used this medium for the culture of *Fusarium Lini* in temperature experiment. According to TOCHINAI (49), this medium is suitable for the growth of mycelium and also for conidia formation of this fungus.

The tripricate series of cultures of each strain were kept in a thermostat at 25°C. and observations of the growth were made after seven days incubation.

The most remarkable expressions which display the cultural idiosyncrasy of each strain were the color and the appearance of the aerial mycelium and the pigmentation presented by the immersed mycelium.

According to the cultural characters, the strains under examination were classified into following four types.

Type 2a: Represented by strain No. 33.

The topography of the developed colony is raised type. White aerial mycelium grows densely over the colony, being floccose to cottony in appearance. The immersed mycelium presents no special pigmentation. Sectors appear frequently but zonation is absent. Radial growth is rapid.

Type 2b: Represented by strain No. 35.

The colony is raised or more or less umbonate in topography. White floccose aerial mycelium develops densely all over the colony. Color of the immersed mycelium is light purplish vinaceous to purplish vinaceous or light perilla purple at the central part and the remainder is white. Margin of the colony is entire, sometimes undulate or lobate. The radial growth is moderate.

Type 2c: Represented by strain No. 22.

The aerial mycelium develops loosely all over the colony, being floccose in appearance and white in color. Color of

the immersed mycelium is terra cotta to vinaceous-russet in central part of the colony and pinkish buff at margin. Radial growth is very rapid. Sectoring and zonation are absent.

Type 2d: Represented by strain No. 21.

The aerial mycelium develops moderately over the colony, appearing wooly in the inner part and floccose to rough velvety at outside of the colony, and being umbilicate in topography. Color of the aerial mycelium is venetian pink at the center of the colony, pale persian lilac in the ray part, and white to capcine buff at the margin and sometimes concentric alternations of pale persian lilac and venetian pink zones are observed. Color of the immersed mycelium is purplish vinaceous to livid brown in the central part and pale yellowish orange to white at the margin. Colony has entire or lobate margin. Zonation takes place distinctly.
The radial growth is moderate.

The strains belonging to every type are as follows:

Type 2a: Nos. 1, 2, 4, 5, 9, 10, 32, 33.
Type 2b: Nos. 7, 11, 12, 13, 15, 16, 18, 19, 20, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 36, 37, 39, 40, 41.
Type 2c: Nos. 6, 14, 17, 22, 38.
Type 2d: Nos. 3, 21, 27.

(3) Cultures on onion decoction agar medium.

Two hundreds grams of onion scales were boiled in 1000 c.c. of distilled water for one hour in Koch's steam sterilizer, and two per cent of agar was added to the decoction. The method of preparation of culture plates and techniques of the experiment were similar to those of the preceding experiments.

In the present experiment, the comparative observation of the cultural behabiors of each strain was made after eight days' culture in a thermostat at 25°C.

All of the strains under examination were classified into following three types of growth according to their cultural characteristics, especially to the pigmentation of the immersed mycelium and, in addition, to the radial growth of colonies.

Type 3a: Represented by strain No. 2.

White aerial mycelium develops moderately in the central part of the colony, being raised in topography and floccose to rough velvety in appearance. The marginal zone of the colony is waxy to slimy in appearance. The development of aerial mycelium is frequently almost absent in some culture plates. The immersed mycelium presents no special pigmentation. Sectoring and zonation are absent. The radial growth is more or less slow.

Type 3b: Represented by strain No. 3.

The colony is flat in topography and waxy to slimy in appearance. The radial growth is moderate, and the margin is entire or undulate. Aerial mycelium develops in the central part of the colony, and they are white, light vinaceous-fawn, or light pinkish lilac in color and floccose to velvety in appearance. Color of the immersed mycelium is pale purplish vinaceous either at the center or in the ray part. Zonation is present.

Type 3c: Represented by strain No. 6.

Colony is waxy to slimy in appearance and flat in topography. The radial growth is rapid. More or less purplish vinaceous aerial mycelium develops scantily at the inoculated point with velvety appearance. Color of the immersed mycelium is livid brown in most part of the colony and pale vinaceous-fawn or light purplish vinaceous at the margin. Zonation is faint or entirely absent.

All the strains examined in the present experiment on onion decoction agar medium are classified according to their growth types as follows:

Type 3a: No. 1, 2, 4, 5, 9, 10, 32, 33.

Type 3b: Nos. 3, 7, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 38, 39, 40, 41.

Type 3c: Nos. 6, 17, 23.

(4) Cultures on synthetic agar medium A.

The formula of this synthetic agar medium is as follows.

Ammonium nitrate	10.0 gr.
Potassium biphosphate	5.0 gr.
Magnesium sulphate	2.5 gr.

Sucrose ..... 50.0 gr.
Agar ..... 20.0 gr.
Distilled water ..... 1000.0 c.c

Method of preparation of the culture plates and techniques of the experiment were similar to the forgoing experiments.

This medium was once used by TOCHINAI (40) for the cultures of *Fusarium Lini* as liquid medium without agar. According to his experiment, it was suitable for the mycelial growth of the present fungus.

In the present experiment observation was made after eight days' incubation in a thermostat at 25°C.

All the strains under examination were arranged into the following three types according to their cultural characteristics.

Type 4a: Represented by strain No. 2.

White aerial mycelium develops densely all over the colony, being raised in topography and velvety to cottony in appearance. Margin of the colony is entire. The pigmentation of the immersed mycelium does not take place at all. Sectoring and zonation are absent.

Type 4b: Represented by strain No. 29.

Aerial mycelium develops densely all over the colony, being velvety to cottony in appearance, white to faint seashell pink in color, and raised, convex, or slightly umbonate in topography. Margin of the colony is entire. The color of the immersed mycelium is congo pink at the center and pale congo pink in the remainder. Sectoring and zonation are usually absent but rarely present.

Type 4c: Represented by strain No. 17.

White to faint seashell pink colored aerial mycelium develops densely all over the colony, being floccose to cottony in appearance, and raised, umbonate, or slightly convex in topography. Margin of the colony is entire. Pale purplish vinaceous pigmentation is very faintly observed in the medium. Sectoring and zonation are usually absent but rarely present. The radial growth is very rapid.

The strains belonging to each growth type are as follows:

Type 4a: Nos. 1, 2, 4, 5, 9, 10, 32, 33.

Type 4b: Nos. 7, 11, 12, 13, 15, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 39, 40, 41.
Type 4c: Nos. 3, 6, 14, 17, 21, 22, 38.

(5) Cultures on the synthetic agar medium B.

The prescription of the synthetic agar medium used in the present experiment is as follows. Peptone was used as the nitrogen source in place of ammonium nitrate in the synthetic nutrient medium used in the preceding experiment.

Peptone (Teruuchi's)	10.00 gr.
Potassitum biphosphate	0.50 gr.
Magnesium sulphate	0.25 gr.
Glucose	15.00 gr.
Agar	20.00 gr.
Distilled water	1000.00 gr.

The method of preparation of the culture plates and technique of the experiment were similar to the forgoing experiments.

In the present experiment, observation was made after seven days' incubation in a thermostat at 25°C. The cultural aspects presented by these strains under examination on the present agar medium were classified into following three types according to the remarkable differences in their cultural characters.

Type 5a: Represented by strain No. 10.

The colony is raised, slightly convex, or sometimes slightly umbonate in topography. White aerial mycelium develops moderately all over the colony, appearing velvety to cottony. The colors presented by the immersed mycelium are white to faint pinkish buff. The radial growth is moderate and sectoring is sometimes appeared.

Type 5b: Represented by strain No. 36.

White aerial mycelium develops densely all over the colony which is raised to convex in topography and velvety to cottony in appearance. Pigmentation presented by the immersed mycelium is pale purplish vinaceous to livid brown. Sectoring and zonation are faint or entirely absent. The radial growth is moderate.

Type 5c: Represented by strain No. 38.

This type is conspicuously distinguished by the superior radial growth, and a particular pigmentation presented by

the immersed mycelia. It is characterised by the prominent concentric zonation of vinaceous-lilac, pale vinaceous-drab, and light rosset-vinaceous in color developed from center to margin.

All the strains are classified according to their growth-types on this medium as follows:

Type 5a: Nos. 1, 2, 4, 5, 9, 10, 32, 33.
Type 5b: Nos. 3, 6, 7, 11, 12, 13, 15, 16, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 39, 40, 41.
Type 5c: Nos. 14, 17, 22, 38.

(6) Cultures on steamed rice medium.

The medium used in the present experiment was made after WOLLENWEBER'S prescription (53). Two grams of hulled rice grains and 6 c.c. of distilled water were put into each sterilized test tube, and steamed for one hour every day in successive four days.

According to REINKING and WOLLENWEBER (34), cultures on the steamed rice medium are generally very helpful in grouping *Fusaria* into their respective sections according to the color reaction.

In the present experiment, a bit of mycelium and conidia were inoculated to the top of the medium, and incubated in a thermostat at 25°C. during about fourty days until the fungus develops fully and presents the cultural characters thoroughly.

The strains under examination are classified into following five types according to the differences in their cultural characters presented on the medium.

Type 6a: Represented by strain No. 2.

This type is conspicuously distinguished from others by its white to massicot yellow color of aerial mycelium developing densely from the top to the bottom of the culture.

Type 6b: Represented by strain No. 24.

The aerial mycelium being chatenay pink to jasper red in color and floccose to cottony in appearance develops moderately from the top to the bottom of the culture. Wart-like compact mycelial masses are produced in places, which are white to jasper red in color and ranging from 1 to 1.5 mm. in diameter.

Type 6c: Represented by strain No. 27.

The aerial mycelium develops on the upper half of the

culture moderately, being white to verbena violet and in places dark yvette-violet in color and floccose to cottony in appearance. Under the layer of the aerial mycelium, a leathery mycelial sheet develops, and it is purplish vinaceous and flesh pink in color. Rice grains in the upper part of the culture become pea green in color and in the lower part of the substratum where no mycelium develops the rice grains change color from original white to massicot color.

Type 6d: Represented by strain No. 7.

The aerial mycelium develops moderately from the top to the bottom of the culture, being light purplish vinaceous and in some places livid brown in color and rough velvety to cottony in appearance. The rice grains of the lower part of the culture change color from original white to vinaceous-fawn.

Type 6e: Represented by strain No. 6.

The aerial mycelium, being light congo pink in color and velvety to cottony in appearance, develops loosely on the upper surface of the leathery mycelial sheet, which develops on the upper part of the substratum and consists of two or three layers differing in colors of puritan gray, light congo pink, and purplish vinaceous. No marked pigmentation takes place in the substratum on the lower half of the culture.

All the strains under examination are classified as follows according to their growth-types presented on this medium.

Type 6a: Nos. 1, 2, 4, 5, 9, 10, 32, 33.
Type 6b: Nos. 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 36, 37, 38, 39, 40.
Type 6c: Nos. 3, 21, 27, 41.
Type 6d: No. 7.
Type 6e: No. 6.

(7) Summary concerning the cultural experiments.

It is clear from the results obtained in the present cultural experiments that there are remarkable differences in the cultural characters of the various strains of *Fusarium Lini* BOLLEY. Certain distinctions of the cultural behaviours presented by different strains have usually been observed in cultures on each of the six kinds

of culture media used, and the strains could be classified into several groups owing to their cultural appearances presented on each medium.

In the cases of cultures on apricot-juice agar and on steamed rice medium, the growth features of these 41 strains were classified into five types, on potato decoction agar medium into four types, and on onion decoction agar and the synthetic agar media A and B into three types respectively.

Even if some of the strains show the same growth-type on one medium they are not always so on the others, and among these various growth-types presented by the strains some connections may be possible.

In the collating consideration of the various growth types observed on every medium, the authors would like to distinguish the strains under examination into ten groups, as shown in the following table II.

TABLE II. Grouping of the strains according to their growth types.

Group	Combination of growth-types	Strain
I	1a 2a 3a 4a 5a 6a	Nos. 1, 2, 4, 5, 9, 10, 32, 33.
II	1b 2b 3b 4b 5b 6b	Nos. 11, 12, 13, 15, 16, 18, 19, 20, 24, 25, 26,
III		28, 29, 30, 31, 34, 35, 36, 37, 39, 40.
	1b 2b 3b 4b 5b 6c	No. 41.
IV	1b 2b 3c 4b 5b 6b	No. 23.
V	1c 2c 3b 4c 5c 6b	Nos. 14, 22, 38.
VI	1c 2c 3c 4c 6b 6e	No. 6.
VII	1c 2c 3c 4c 5c 6b	No. 17.
VIII	1d 2b 3b 4b 5b 6d	No. 7.
IX	1c 2d 3b 4b 5b 6c	No. 27.
X	1e 2d 3b 4c 5b 6c	Nos. 3, 21.

IV. Inoculation experiment

The causal fungus of the flax wilt disease, *Fusarium Lini*, can live saprophytically in the soil without host plant for several years, and as it can persist also within the seed tissues, it is almost impossible to control the disease without the crop-rotation of adequate

interval and seed disinfection. Under these circumstances attempts to breed wilt resistant varieties of flax have been carried out by several investigators.

According to TISDALE (46, 47) and BARKER (1), the wilt resistance of flax is profoundly influenced by environmental factors, especially temperature. At high temperatures most resistant varieties are readily attacked, while at low temperatures even the susceptible varieties may escape the infection.

The wilt resistance of flax-varieties, however, seems to be influenced not only by the environmental conditions, but also alterable according to the physiologic specialization of the pathogene.

BROADFOOT (4) distinguished nine physiologic forms in *Fusarium Lini* based upon the difference of pathogenicity, and also upon the cultural difference on three kinds of culture media, but with some difficulty.

In short, the wilt resistance of flax should be considered in close connection with the physiologic specialzation of *Fusarium Lini.*

The physiologic forms classified on the basis of the cultural and physico-chemical characters are sometimes correspondent to the discrepancies in their pathogenicity to the host varieties.

The different groups were distinguished in the forgoing cultural experiments of the various strains of *Fusarium Lini.* It is, of course, important and very interesting to know whether and how the strains distinguished into these different groups differ or coincide in their pathogenicities to some flax-varieties. In order to ascertain the question of the pathogenical differences of the strains the present inoculation experiments have been carried out.

Three varieties of flax (*Linum usitatissimum* L.) were used as differential hosts, namely Pernau No. 1, Saginau No. 1, and resistant Taikin-ama, which were supplied from the Hokkaido Agricultural Experiment station.

Apparently healthy seeds of each variety were disinfected by steeping for two minutes in one per cent aqueous solution of mercuric chloride and then washed thoroughly with sterilized distilled water.

The spore suspension containing conidia and fragments of mycelia was obtained by shaking with an addition of distilled water directly to a two- or three-week culture of the fungus on apricot decoction agar medium.

Thirty c.c. of the suspension of each strain of the fungus was poured to sterilized soil in an unglazed pot, being 12 cm. in diameter, previously sterilized in Koch's steam sterilizer for one hour twice on every other day. Fourty seeds of every variety of flax were sown in each pot, and then covered with sterilized soil. For the control, 5 pots were prepared in the same way without inoculation with the spore suspension, and instead, sterilized water poured to uninoculated medium was mixed with soil. For each strain fourty plants in one pot were used.

The present experiments were carried out in a greenhouse. Details of the results obtained in each inoculation experment will be shown in the following descriptions.

Experiment 1.

The present experiment was carried out using the flax variety, Pernau No. 1, and the temperature in the green house was recorded ranging from 6°C. to 36°C. in the first trial carried out from Oct. 5 to Nov. 8, and from 14°C. to 38°C. in the second from Nov. 30 to Dec. 20, 1933.

In the first trial, when the plants grew about 7 to 9 cm. high, first sign of the disease appeared, and most of the plants infected by the fungus gradually lost their green color, and the yellowing of the leaves occurred. Such infected plants eventually died in general, though some of them stunted but survived. In the second trial, however, the typical symptoms of wilt or damping-off were observed in most infected plants. The plants growing in the control pots remained perfectly healthy. The difference of the symptoms observed in these two trials would have been caused by the difference in temperature. In general, the optimal temperature for the growth of flax seedlings is fairly low, while that for the growth of the wilt fungus is markedly high. TISDALE (45, 47) and BARKER (1) reported that the optimal temperature range for the growth of the fungus is higher than that of the flax seedlings. Consequently the wilt disease of flax seedlings prevails more at high temperature than at low one within a limit.

The results obtained in the present experiments are shown summarizingly in the following table.

TABLE III. The results of inoculation experiments on Pernau No. 1 with various strains of *Fusarium Lini.*

Fungus strain	Total number of seedlings examined		Number of wilted plants		Percentage of the wilted		Averaged Percentage of the wilted in two trials
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	
No. 1	39	30	8	26	20.5	86.6	53.5
No. 2	38	35	11	31	28.9	88.5	58.7
No. 3	37	31	9	22	24.3	70.9	47.6
No. 4	38	36	21	33	55.2	91.6	73.4
No. 5	38	36	7	25	18.4	69.4	43.9
No. 6	39	37	0	0	0	0	0
No. 7	40	35	35	34	87.5	97.1	92.3
No. 9	37	34	13	30	32.4	88.2	60.3
No. 10	38	35	17	30	44.7	85.7	65.2
No. 11	38	32	25	32	65.7	100	82.8
No. 12	37	35	25	34	67.5	97.1	82.3
No. 13	38	28	26	27	68.4	96.4	82.4
No. 14	39	39	0	0	0	0	0
No. 15	40	29	18	11	45	37.9	41.4
No. 16	39	35	12	31	44.4	88.5	61.4
No. 17	37	37	0	0	0	0	0
No. 18	37	40	29	39	78.3	97.5	87.9
No. 19	37	31	12	24	32.4	77.4	54.9
No. 20	39	35	30	34	76.9	97.1	87
No. 21	38	36	9	20	23.6	55.5	39.5
No. 22	40	32	0	0	0	0	0
No. 23	40	34	36	34	90	100	95
No. 24	33	38	15	30	45.4	78.9	62.1
No. 25	40	35	35	31	87.5	88.5	88
No. 26	39	36	5	32	12.8	88.8	50.8
No. 27	39	32	16	28	41	87.8	64.4
No. 28	37	31	28	31	75.6	100	87.8
No. 29	40	36	25	36	62.5	100	81.2
No. 30	34	34	24	33	70.5	97	83.2
No. 31	36	33	24	33	66.6	100	83.3
No. 32	40	31	13	21	32.5	67.7	50.1
No. 33	40	33	11	29	27.7	87.8	57.6

TABLE III (*Continued*)

Fungus strain	Total number of seedlings examined		Number of wilted plants		Percentage of the wilted		Averaged Percentage of the wilted in two trials
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	
No. 34	40	29	26	29	65	100	82.5
No. 35	40	27	36	25	90	92.5	91.2
No. 36	40	31	28	30	70	96.7	83.3
No. 37	38	29	35	28	92.1	96.2	94.1
No. 38	38	35	0	0	0	0	0
No. 39	36	39	23	38	63.8	97.2	80.5
No. 40	38	30	28	28	73.6	93.3	83.4
No. 41	40	33	21	31	52.5	93.9	73.2
Control.	40	32	0	0	0	0	0
	40	34	0	0	0	0	0
	39	33	0	0	0	0	0
	39	37	0	0	0	0	0
	38	40	0	0	0	0	0

The data presented in the table clearly suggest some significant differences occurring in the pathogenicity of various strains examined in the present inoculation experiment, and they may be arranged in order according to their apparent virulence to Pernau No. 1 as follows:

Percentage of wilted plants	Strains
90–100	Nos. 7, 23, 35, 37, 40.
80–89	Nos. 11, 12, 13, 18, 20, 25, 28, 29, 30, 31, 34, 36, 39.
70–79	Nos. 4, 41.
60–69	Nos. 9, 10, 16, 24, 27.
50–59	Nos. 1, 2, 19, 26, 32, 33.
40–49	Nos. 3, 5, 15 ,21.
0	Nos. 6, 14, 17, 22, 38.

The results obtained in the present inoculation experiment indicated that there are some significant connections between the groups classified according to the cultural characters and those classified on the basis of the pathogenicity.

The strains belonging to groups II with exceptions of some

strains, IV, and VII caused the loss more than 80 per cent, those belonging to group III, and the strain No. 4 of group I caused the loss 70 to 79 per cent, those belonging to groups I with exceptions of two strains No. 4 and No. 5, IX, and the strains Nos. 16, 19, 24, 26 belonging to group II caused that of 50 to 69 per cent, and those belonging to group X, the strains No. 5 of group I, and the strain No. 15 of group II caused that of 40 to 49 per cent of total plants in each pot. However the strains belonging to the groups V, VI, and VII showed almost no pathogenicity at least under the present experimental conditions.

Experiment 2.

The present experiment was carried out using the flax variety, Taikin-ama, and the temperature in the green house varied from 15°C. to 37°C during the experimental period, from Nov. 19 to Dec. 19, 1933.

In the present experiment most of the flax plants infected by the present fungus showed the symptoms of wilt and damping-off.

The results obtained in the present experiment are shown summarizingly in the following table. The percentage of wilted plants was calculated on the basis of total numbers of seedlings in each pot.

TABLE IV. The results of inoculation experiments on Taikin-ama with various strains of *Fusarium Lini*.

Fungus strain	Total number of seedlings examined	Number of wilted plants	Percentage of the wilted
No. 1	36	5	13.8
No. 2	34	14	41.1
No. 3	35	8	22.8
No. 4	36	10	27.7
No. 5	35	4	11.4
No. 6	37	0	0
No. 7	36	23	63.8
No. 9	35	14	40
No. 10	38	12	31.5
No. 11	34	20	58.8
No. 12	32	22	68.7

TABLE IV (*Continued*)

Fungus strain	Total number of seedlings examined	Number of wilted plants	Percentage of the wilted
No. 13	37	28	75 6
No. 14	34	0	0
No. 15	37	2	5.4
No. 16	32	8	25
No. 17	34	0	0
No. 18	39	30	76.9
No. 19	33	6	18.1
No. 20	36	30	83.3
No. 21	36	9	25.5
No. 22	36	0	0
No. 23	35	6	17.1
No. 24	40	5	12.5
No. 25	32	21	65.6
No. 26	34	4	11.7
No. 27	40	30	75
No. 28	36	25	69.4
No. 29	39	28	71.7
No. 30	30	17	56.6
No. 31	40	37	92.5
No. 32	38	18	47.3
No. 33	31	10	32.2
No. 34	34	25	73.5
No. 35	36	22	61.6
No. 36	32	29	90.6
No. 37	40	35	87.5
No. 38	35	0	0
No. 39	34	20	58.8
No. 40	36	25	69.4
No. 41	34	7	20.5
Control.	30	0	0
	40	0	0
	35	0	0
	36	0	0
	40	0	0

In the present experiment, the differences in the pathogenicity of various strains was also significant.

The strains may be arranged in order according to their apparent virulence to Taikin-ama as follows:

Percentage of wilted plants	Strains
90–100	Nos. 31, 36.
80–89	Nos. 20, 37.
70–79	Nos. 13, 18, 27, 29, 34.
60–69	Nos. 7, 12, 25, 28, 35, 40.
50–59	Nos. 11, 30, 39.
40–49	Nos. 2, 9, 32.
30–39	Nos. 10, 33.
20–29	Nos. 3, 4, 16, 21, 41.
10–19	Nos. 1, 5, 19, 23, 24, 26.
1–9	No. 15.
0	Nos. 6, 14, 17, 22, 38.

As it was clearly recognized in the present experiment, the strains belonging to groups II with exceptions of five strains, VII, and IX caused the loss of the plants more than 50 per cent, and they were apparently more virulent in the pathogenicity than those belonging to groups I, II, IV, V, VI, VII, and X, and the five strains belonging to group II.

Among 21 members of group II, four strains caused the loss more than 80 per cent, nine strains caused that more than 60 per cent, and three strains caused that more than 50 per cent, but the loss caused by the strains Nos. 15, 16, 19, 24, and 26 was only less than 30 per cent.

The strains belonging to groups VIII and IX caused the loss 60 to 79 per cent.

Among the mumbers of group I, five strains caused the loss 30 to 49 per cent, and three strains caused that 10 to 29 per cent. The strains belonging to groups III, IV, and X caused the loss 10 to 29 per cent. The strains belonging to groups V, VI, and VII, however, showed almost no pathogenicity.

It was noteworthy that the strains belonging to groups III, and IV, which showed moderate and strong pathogenicities on Pernau No. 1 respectively, caused only small damages on the present so-called resistant variety, Taikin-ama.

Experiment 3.

The present experiment was carried out using the variety Saginau.

The temperature in the green house varied from 15°C. to 37°C. during the experimental period from Nov. 30 to Dec. 20, 1933.

In the present experiment the wilt and damping-off were usually observed in most plants infected. The results are shown summarizingly in the following table. The percentage of the wilted plants was calculated on the basis of total members of seedlings in each pot.

TABLE V. The results of inoculation experiment on the flax variety Saginau with various strains of *Fusarium Lini*.

Fungus Strain	Total number of seedlings examined	Numbers of wilted plants	Percentage of the wilted
No. 1	38	27	71
No. 2	30	19	63.3
No. 3	31	12	38.7
No. 4	30	25	83.3
No. 5	28	12	42.8
No. 6	29	0	0
No. 7	32	24	75
No. 9	32	20	62.5
No. 10	31	18	58
No. 11	28	22	78.5
No. 12	32	29	90.6
No. 13	26	17	65.3
No. 14	32	0	0
No. 15	32	11	34.3
No. 16	26	14	53.8
No. 17	33	0	0
No. 18	28	26	92.7
No. 19	29	13	44.8
No. 20	33	30	90.9
No. 21	34	11	32.3
No. 22	32	0	0
No. 23	27	20	79.2

TABLE V. (*Continued*)

Fungus strain	Total number of seedlings examined	Numbers of wilted plants	Percentage of the wilted
No. 24	29	17	58.6
No. 25	32	23	71.8
No. 26	24	12	50
No. 27	33	25	75.7
No. 28	36	28	77.7
No. 29	26	17	65.3
No. 30	32	24	75
No. 31	28	25	89.2
No. 32	31	17	54.8
No. 33	32	13	40
No. 34	30	20	66.6
No. 35	31	7	22.5
No. 36	31	28	90.3
No. 37	27	23	85.1
No. 38	31	0	0
No. 39	34	3	8.8
No. 40	27	17	62.9
No. 41	37	28	75.4
Control.	35	0	0
	34	0	0
	30	0	0
	28	0	0
	24	0	0

As shown in above table, the differences in the pathogenicity were significant among the strains of the fungus. The strains might be classified into following groups according to their differences in the pathogenicity to Saginau variety.

Percentage of wilted plants	Strains
90–100	Nos. 12, 18, 20, 36.
80–89	Nos. 4, 31, 37.
70–79	Nos. 1, 7, 11, 23, 27, 28, 30, 41.
60–69	Nos. 2, 9, 13, 25, 29, 34, 40.

50–59	Nos. 10, 16, 24, 26, 32.
40–49	Nos. 5, 19, 33.
30–39	Nos. 3, 15, 21.
20–29	No. 35.
10–19	No. 39.
0	Nos. 6, 14, 17, 22, 38.

The results obtained in the present experiment were semewhat resemble to those of the previous ones.

Among the members of group II, six strains caused the loss more than 80 per cent, three strains caused that 70 to 79 per cent, five strains caused that 60 to 69 per cent, and strains Nos. 16, 24, and 26 caused that 50 to 59 per cent, but strains 15 and 19 caused the loss of the plants only less than 50 per cent.

The strains belonging to groups III, IV, VIII, and IX caused the loss 70 to 79 per cent, those belonging to group I with exceptions of Nos. 1, and 4 caused that 40 to 69 per cent, and those belonging to group X caused that 30 to 39 per cent of the total plants. As exceptions, strains No. 1 and No. 4 belonging to group I caused the loss more than 70 per cent, and strains Nos. 35, and 39 belonging to group II caused that 22.5% and 8.8% respectively. The strains belonging to groups V, VI, and VII showed no pathogenicity.

Considering the results obtained in the present inoculation experiments on 3 varieties of flax, it is clearly understood that these three varieties are susceptible to most strains of *Fusarium Lini*, with exceptions of those belonging to the cultural groups V, VI, and VII which did not cause the wilt of the seedlings at all at least under the present experimental conditions.

The strains can be distinguished into several groups according to their pathogenicities to these three varieties of flax, and somewhat close connections would be surmised between the pathogenicity to these flax varieties and cultural characters presented on the culture media used.

Comparing the pathogenicities to Pernau No. 1, the strains belonging to group II with exceptions of five strains Nos. 15, 16, 19, 24 and 26, and every single strain belonging to the groups IV. and VIII are the most virulent, the single strain belonging to group III is moderately so, the strains belonging to the group I with an exception of No. 4, and the single strain of the group IX are rather

weak, and the strains belonging to group X are also weak in the pathogenicity. To Tainkin-ama, the strains belonging to the groups II with exceptions of five strains, VIII, and IX are generally more virulent than those belonging to the other groups. The strains belonging to the groups I, III, IV and X are generally weak in the pathogenicity to the present variety of flax. To Saginau, the strains belonging to groups II with exceptions of seven strains, (Nos. 15, 16, 19, 24, 26, 35 and 39), III, IV, VIII, IX, and strains 1 and 4 of group I are generally strong in the pathogenicity to the present variety. Other strains of group I, 7 strains of group II, and 2 strains belonging to group X are generally weak in the pathogenicity.

Every single strain belonging to the groups III, IV, and IX was respectively different in the pathogenicity to those 3 varieties of flax examined. Strain No. 41 of Group III was moderatly virulent to Pernau No. 1 and Saginau, but weak to Taikin-ama. Strain No. 23 of Group IV was markedly virulent to Pernau No. 1, moderate to Saginau, and weak to Taikin-ama. Strain No. 27 of Group IX was moderately virulent to every of these three kinds of flax-varieties.

It was concluded from the results obtained in the present inoculation experiments that most strains of group II, and every single strain belonging to group VIII and IX are strongly or fairly virulent, and those belonging to the group X and the strains 15, 16, 19, 24, and 26 belonging to the group II are weak in pathogenicity to these three varieties of flax. The strains 35 and 39 belonging to group II are weak to Saginau, moderate to Taikin-ama and virulent to Pernau-No. 1 in the pathogenicity. The strains belonging to the groups V, VI, and VII showed almost no pathogenicity to any of these flax-varieties at least under the present experimental conditions. They presented no wilt symptom at all on the flax seedlings raised on the soil inoculated with them and only caused the yellowing of two three leaves. The occurrence of non-pathogenic strains of *Fusarium Lini*, which were isolated from wilted flax plants and are no other than the very species in their morphological and cultural characters, would be understood according only to the idea of biologic specialization in the pathogenicity.

The wilt disease of flax generally prevails at comparatively high temperature. It is frequently experienced that some resistant

varieties are affected badly when temperature is high, while at low temperature even susceptible flaxes occasionally escape from the infection. In observing the results of the present inoculation experiments the temperature relation was taken in consideration, and some interesting instances in this connection were noticed in the experiment 1.

The temperature in the green house was comparatively low, i. e. 6°–36°C., in the first trial, and it was generally high, i. e. 14°–38°C., in the second one. The occurrences of wilted plants per pot, excepting those inoculated with apparently non-pathogenic strains, i. e. Nos. 6, 14, 17, 22, and 28, were 12.8–92.1% in the first trial and 37.9–100% in the second one. Annihilation of whole plants in a pot never took place in the first trial, while it was realized so many as 6 cases by strains 11, 23, 28, 29, 31 and 34 in the second trial.

The apparent pathogenicity of the strains seemed to be rather homogeneally toned up in the second trial owing to high temperature. Consequently the details of varietal differences in the virulence of the strains were revealed better in the cases of the first trial.

V. Influence of temperature on the development of every strain of the fungus

The temperature relation of a fungus often becomes complicated concerning the physiologic specialization.

The temperature requirements of *Fusarium Lini* have been reported by several investigators. TISDALE (46), who studied the relation of temperature to the growth and infecting power of *Fusarium Lini*, reported that the minimum temperature for the growth of the fungus lay between 10°C. and 11°C., the optimum at about 26°C. to 28°C., and the maximum between 35°C. and 36°C., on the potato agar medium, and the critical temperature for the infection of this fungus is 14°C. to 16°C.

According to TOCHINAI (49) the minimum, optimum, and maximum temperatures for the growth of the fungus seems to be at 10°C. to 12°C., 30°C., and 36°C.to 37°C. respectively. BROADFOOT (4), who studied the physiologic specialization in regard to temperature relation of the present fungus, reported that no significant dif-

ference was found in the amount of radial growth among the physiologic forms of the fungus at various temperatures.

The present experiment was undertaken to ascertain whether our strains of *Fusarium Lini* differ in their temperature-relation or there is no significant disparity just as reported by BROADFOOT (4).

The culture plates of the synthetic agar medium A were prepared by pouring 15 c.c. each in Petri-dishes of uniform size being 85 mm. in diameter.

A small piece of the mycelia with conidia taken from the stock culture was inoculated at the center of each agar plate. The plate-cultures were incubated in thermostats at 20°C., 25°C., 28°C., and 32°C. in triplicate for each temperature.

The diameter of colony developed on the agar plate was measured every other day continuously during eight days. The measurement was made of the longest and the shortest diameters of each colony passing through the inoculated point, or of several diametral axes when the colony had irregular margin, and then the average was taken. The results are shown in Table VI.

TABLE VI. The radial growth of colonies of different strains at various temperature.

Strain	Temperature (centigrade)	Increases of the diameter of colony in every two days (mm.)				Average diameter of colony after 8 days
		2nd day	4th day	6th day	8th day	
No. 1	32	18	19	16	19	72
	28	16.6	18.4	19	20	74
	25	14.3	16.7	18	14	63
	20	10	15	19	15	56
No. 2	32	17	20	18	21.3	76.3
	28	17.3	19	20.7	20	77
	25	14.3	17	20	20	70.3
	20	9.6	15.4	15	17	57
No. 3	32	16	17	16	16	63
	28	16	18	19.6	20	74.6
	25	12	18	18.3	22	70.3
	20	8	16	14	17	55
No. 4	32	16	17	16	16	65

TABLE VI. (*Continued*)

Strain	Temperature (centigrade)	Increases of the diameter of colony in every two days (mm.)				Average diameter of colony after 8 days
		2nd day	4th day	6th day	8th day	
	28	19.3	19	21.7	21.6	81.5
	25	16	18	23	16.3	73.3
	20	10	17	16	16	59
No. 5	32	15	17	16	16	64
	28	17	19.5	21.5	23	81
	25	15	19	19	21	74
	20	9	17	15	14	55
No. 6	32	20	18	17	21	76
	28	20	21	21	18	80
	25	18	19	20	19	76
	20	14	16	17	15.6	62.6
No. 7	32	15.3	15.7	12	14	57
	28	17	15	17.6	14.4	64
	25	14	16	16	14	60
	20	7.6	14.4	12	11	45
No. 9	32	17	19	17	20	73
	28	18.5	20.5	22	20	81
	25	16.3	19	19	18	72.3
	20	13.3	15.7	15.3	15.7	60
No. 10	32	17	18.3	16.7	15	67
	28	17.5	21.5	21	22	82
	25	15.3	19.7	19	21	75
	20	9	17.6	15	13.4	55
No. 11	32	14	15.3	17.7	13	60
	28	17	14	17	13	61
	25	16.3	16	16.7	13.3	62.3
	20	10	15	13	12	50
No. 12	32	15	17	14	16	62
	28	17	14	14	11	56
	25	14	17	14.3	12.7	58
	20	11.6	13.4	12	12	49

TABLE VI. (*Continued*)

Strain	Temperature (centigrade)	Increases of the diameter of colony in every two days (mm.)				Average diameter of colony after 8 days
		2nd day	4th day	6th day	8th day	
No. 13	32	15	16	19	16	66
	28	17	18	17	14	67
	25	14.3	16.7	18.3	15.7	65
	20	8	15	12	13	48
No. 14	32	20	20.3	19	18.7	78
	28	19	20.6	22	20.4	82
	25	16.6	21.4	21	22	81
	20	12.6	17.4	17	16.3	63.3
No. 15	32	12.3	12.7	11	11	47
	28	15	14.5	16	13	58
	25	12	16	15.3	13	56.3
	20	8	15	11	11	45
No. 16	32	17.3	16.7	16	16	66
	28	18.3	19.7	17	16	71
	25	16.3	18.7	14	14.4	63.3
	20	10	15	15	15	55
No. 17	32	17	21	19	20	77
	28	19.3	20.3	22	20	82
	25	17.3	17.7	14	15.6	64.6
	20	7	20	17.3	16.7	61
No. 18	32	18	15	13	14	60
	28	18	16	14	12	60
	25	15	17	12	12	56
	20	10	16.6	11.4	11	49
No. 19	32	16	17	16	16	65
	28	19.3	18.7	17	15	71
	25	15	18	18	19	70
	20	9	16	13	12	50
No. 20	32	14	14	17	15	60
	28	18	18	13	19	68
	25	14	16	15.6	13.4	59
	20	10.6	15.4	11	11	48

TABLE VI. (*Continued*)

Strain	Temperature (centigrade)	Increases of the diameter of colony in every two days (mm.)				Average diameter of colony after 8 days
		2nd day	4th day	6th day	8th day	
No. 21	32	16	15	16	19	66
	28	17	19	19	17	70
	25	10	17	15	16	58
	20	8.3	15.7	12.3	10.7	47
No. 22	32	20	18	19	16	73
	28	20	21	19	18.3	78.3
	25	17	18.3	18.7	19	73
	20	13	18	17	16	64
No. 23	32	15	15	13.3	14.7	58
	28	16	15.6	14.4	16	62
	25	15.3	17.7	18	13	64
	20	12.6	16.4	9	9	47
No. 24	32	18.3	15.7	14.3	12.7	61
	28	15	16	18.3	17.7	67
	25	14.6	17.7	17.7	14	64
	20	9	16	12.3	11.7	49
No. 25	32	18	15	12	17	62
	28	17	18	17	18	70
	25	16.6	17.4	15.6	17.4	67
	20	13.3	13	11	8.7	46
No. 26	32	15	17	15	17	64
	28	18	17	17	16	68
	25	14	17	17	15	63
	20	10	14.3	14.7	12	51
No. 27	32	18	15	17	16	66
	28	18.3	15	17.7	9	60
	25	15	15	16	14	60
	20	8	15.6	12.4	12	48
No. 28	32	16	17	16	15	62
	28	17.3	18.7	18.3	16.7	71
	25	14	18	18	13	63
	20	9.3	12.7	12.6	12.4	50

TABLE VI. (*Continued*)

Strain	Temperature (centigrade)	Increases of the diameter of colony in every two days (mm.)				Average diameter of colony after 8 days
		2nd day	4th day	6th day	8th day	
No. 29	32	16	14	16	15	61
	28	15.3	15.7	16	16	63
	25	15	15	16	13	59
	20	9	16	12	12	49
No. 30	32	17	14	14	13	58
	28	17	16	18	16	67
	25	16	16.6	13.4	15	64
	20	9	16	12	12	49
No. 31	32	16.3	15.7	15	14.3	61.3
	28	16	20	16	16	68
	25	14	18	18	18	68
	20	10	15.3	12.7	13	51
No. 32	32	19	18	20	20	77
	28	18	19.5	23.5	21	82
	25	14	18	19.3	18.7	70
	20	10	18	13	13	54
No. 33	32	16	19	19	18.3	72.3
	28	16.5	19	22.5	25	83
	25	14	18.3	18.7	19	70
	20	10.3	16.7	15	15	57
No. 34	32	18	19	20	18	75
	28	17	18.6	22.4	19.3	77.3
	25	17	20	18	18	73
	20	10	15	14	14.6	53.6
No. 35	32	16	18.3	18	15.7	68
	28	16	16.6	19	17.4	69
	25	15	17	15	15	62
	20	9	15	13.4	12	50
No. 36	32	15.3	15.7	15.3	15	61.3
	28	15.6	15.4	18	16.3	65.3
	25	13	17	17	13	60
	20	8	16.3	13	12	49.3

TABLE VI. (*Continued*)

Strain	Temperature (centigrade)	Increases of the diameter of colony in every two days (mm.)				Average diameter of colony after 8 days
		2nd day	4th day	6th day	8th day	
No. 37	32	16	15	15	16	62
	28	16.3	15.7	16	15	63
	25	12	17	16.3	13.7	59
	20	8	16	13	12	49
No. 38	32	20	17	19	16	72
	28	18.6	17.4	18.6	23.4	78
	25	16	19.3	16	17.7	69
	20	12.6	16.7	16.7	16	62
No. 39	32	20	17	19	16.3	72.3
	28	19	21	22	19.6	81.6
	25	14	19	20	17.3	70.3
	20	10	16	14	13	53
No. 40	32	15.3	16	15.7	15.3	62.3
	28	15.6	15.4	19	17	68
	25	16	16	17	15	64
	20	10	14.6	12.4	12	49
No. 41	32	16.3	17	15.7	19	68
	28	18	20	19.6	17.4	75
	25	14	18	19	18	69
	20	9.3	15.7	14	16	55

The strains under examintion generally showed the most vigorous growth at 28°C. with exceptions of Nos. 11 and 12 which showed the maximal growth at 25°C. and 32°C. respectively. The growth at 20°C. was the worst with no exception.

In comparison of the growth at 32°C. and at 25°C., some strains showed better growth at 25°C. than at 32°C., while the others at 25°C. than at 32°C. A few strains showed equal growth at both temperatures.

The strains showed better growth at 32°C. than at 25°C. were Nos. 1, 2, 9, 12, 13, 16, 17, 18, 20, 21, 27, 29, 32, 33, 34, 35, 36, 37, 38, 39, and those develped better at 25°C. than at 32°C were, Nos. 3, 4, 5, 7, 10, 11, 14, 15, 19, 23, 24, 25, 26, 28, 30, 31, 40, 41. Strains Nos. 6 and

22 showed the equal growth at 32°C. and 25°C.

Most strains showed more or less the staling tendency in their radial growth of the colony, but any detailed racial connection could not be recognized.

It was notable that the strains belonging to cultural groups V, VI, VII, always showed better growth than the other strains at any grade of temperature examined.

In general, it has been concluded that the difference of the temperature induced a great effect upon the radial growth of the colony of each strain examined in the present experiment, and among some strains fairly significant differences in their growing reactions to varying temperature have been observed.

The strains belonging to group I have produced no special pigmentation in the medium throughout every temperature examined in the present experiment, while the other strains produced various pigmentations and sometimes conspicuous zonations.

In the cases of the cultures at 32°C., strains belonging to groups II, III, IV, VIII, IX, and X produced pale purplish vinaceous to purplish vinaceous colors in the medium and conspicuous zonations, while the strains belonging to groups V, VI, and VII presented only faint shell pink pigmentation in the medium but no zonation at all.

At 28°C., strains belonging to groups II, III, IV, VIII, and IX produced pale to light congo pink color, those of group X produced pale purplish vinaceous color, and those of groups V, VI, and VII did only faint shell pink color. Zonation was absent throughout every strain.

At 25°C., strains belonging to groups II, III, IV, VIII, IX, and X produced pale to light congo pink color, and those of V, VI, and VII did faint shell pink color. Zonation did not take place at all.

At 20°C., the strains belonging to groups II, III, IV, VIII, and IX produced pale to light congo pink color in the medium and conspicuous zonations, while in the cases of those belonging to groups V, VI, VII, and X the same coloration was observed but faintly and zonation was quite absent.

These results are shown summarizingly in the following Table VII. In the table, p.p.v. means pale purplish vinaceous, l.p.v. light purplish vinaceous, p.v. purplish vinaceous, p.c.p. pale congo pink, l.c.p. light congo pink, c.p. congo pink, and s.p. shell pink.

TABLE VII. Racial differences in the color production and zonation at various cultural temperature.

Strain	Color presented in the medium				Zonation			
	32°C.	28°C.	25°C.	20°C.	32°C.	28°C.	25°C.	20°C.
No. 1	none	none	none	none	+	−	−	−
No. 2	"	"	"	"	±	−	−	−
No. 3	l.p.v.	p.p.v.	l.c.p.	p.c.p.	+++	−	−	±
No. 4	none	none	none	none	±	−	−	−
No. 5	"	"	"	"	−	−	−	−
No. 6	s.p.	s.p.	s.p.	p.c.p.	±	−	−	−
No. 7	l.p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 9	none	none	none	none	±	−	−	−
No. 10	"	"	"	"	±	−	−	−
No. 11	p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 12	p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	++
No. 13	p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	++
No. 14	s.p.	s.p.	s.p.	p.c.p.	−	−	−	±
No. 15	p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	++
No. 16	p.p.v.	p.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 17	s.p.	s.p.	s.p.	p.c.p.	−	−	−	−
No. 18	p.p.v.	p.c.p.	p.c.p.	l.c.p.	+++	−	−	+++
No. 19	l.p.v.	p.c.p.	l.c.p.	l.c.p.	++	−	−	+++
No. 20	p.v.	l.c.p.	l.c.p.	l.c.p.	++	−	−	+++
No. 21	p.p.v.	p.p.v.	p.c.p.	p.c.p.	++	−	−	±
No. 22	s.p.	s.p.	s.p.	p.c.p.	−	−	−	−
No. 23	p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	++
No. 24	p.p.v.	l.c.p.	l.c.p.	p.c.p.	+++	−	−	+++
No. 25	l.p.v.	l.c.p.	l.c.p.	l.c.p.	+++	±	−	+++
No. 26	p.v.	p.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 27	l.p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 28	p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 29	p.v.	l.c.p.	p.c.p.	p.c.p.	+++	−	−	+++
No. 30	l.p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 31	l.p.v.	p.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 32	none	none	none	none	±	−	−	−
No. 33	"	"	"	"	±	−	−	−
No. 34	p.v.	l.c.p.	l.c.p.	l.c.p.	++	−	−	++

Strain	Color presented in the medium				Zonation			
	32°C.	28°C.	25°C.	20°C.	32°C.	28°C.	25°C.	20°C.
No. 35	l.p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 36	p.p.v.	l.c.p.	l.c.p.	p.c.p.	+++	−	−	+++
No. 37	p.v.	p.c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 38	s.p.	s.p.	s.p.	p.c.p.	−	−	−	−
No. 39	p.v.	l.c.p.	l.c.p.	l.c.p.	++	−	−	++
No. 40	p.v.	c.p.	l.c.p.	l.c.p.	+++	−	−	+++
No. 41	p.v.	l.c.p.	l.c.p.	l.c.p.	+++	−	−	++

Grouping the strains according to their pigmentation in the medium and zonation at different temperature, it is as follows.

Group 1. (Temp.) No marked pigmentation took place in the medium throughout varying temperature examined. Zonation was generally absent, but occurred faintly at 32°C. Strains 1, 2, 4, 5, 9, 10, 32, 33 belong to this group.

Group 2. (Temp.) The pigmentation was pale purplish vinaceous to purplish vinaceous at 32°C., and pale congo pink to congo pink at 28°C., 25°C. and 20°C. Conspicuous zonation took place at 32°C. and 20°C., but not at 28°C. and 25°C. Strains 7, 11, 12, 13, 15, 16, 18, 19, 20, 23, 24, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 39, 40, and 41 belong to this group.

Group 3. (Temp.) This was distinguished from the group 2 (Temp.) by absense of zonation at 20°C. and also by pale purplish vinaceous pigmentation at 28°C. Strains 3 and 21 belong to this group.

Group 4, (Temp.) The pigmentation was shell pink at 32°C., 28°C., and 25°C., and pale congo pink at 20°C. Zonation was absent. Strains 6, 14, 17, 22, and 38 belong to this group.

The strains belonging to cultural group I belong to the group 1 (Temp.), those belonging to cultural groups II, III, IV, VIII, and IX belong to group 2 (Temp.), those belonging to cultural group X belong to group 3 (Temp.), and those belonging to cultural groups V, VI, and VII belong to group 4 (Temp.).

It was deeply interesting that the strains 6, 14, 17, 22, and 38

which are non-pathogenic to flax seedlings coincided perfectly with each other in their pigmental reactions at various temperature examined. These produced uniformly shell pink color at 32°, 28°, and 25°., and pale congo pink color at 20°C., and moreover they almost commonly lacked the zonation. These non-pathogenic strains were quite plainly distinguished from the other pathogenic strains according to their coloring characters exhibited in the present experiment. This fact undoubtedly suggested a possible correlation between the cultural character and the pathogenicity of the fungus.

It has been concluded from the results obtained in the present experiment that the difference of the cultural temperature induced a great effect upon the radial growth of colony, color production in the medium and the occurrence of zonation, and among some strains fairly significant racial differences were observed in their growing reactions to varying temperature. In regard to several non-pathogenic strains, an interesting correlation between the coloring reaction and the pathogenicity was recognized, and 5 non-pathogenic strains examined were plainly distinguished from the other strains in their common characters of special color-production in the medium.

VI. Effect of varying C-N ratio in the medium upon the development of the fungus

The ratio of the carbon and nitrogen contained in medium influences greatly the mycelial growth as well as the spore-formation of fungi.

According to BROWN (6, 7, 8), a high C-N ratio in a medium generally causes non-staling type growth, and the maximal rate of growth is maintained almost constantly throughout a certain cultural period, and a low C-N ratio causes the staling type, and the rate of growth decreases after the maximum was reached. The septation of the spores of *Fusaria* increases in the former case and diminishes in the latter one. He (8) also said that when the C-N ratio is increased by raising C-content of the medium, the influence of high C-N ratio is counteracted and reduced to some extent by the effects of increased total concentration and in the consequence the results brought about on the fungus is not so striking, and in some cases it is obscure at all. However, an increased C-N ratio being

given by diminishing N-content of the medium, the favoring effects of high C-N ratio and dilution of the medium reinforce each other, and very pronounced results are obtained.

The present experiment was made to study how respond the various strains to different C-N ratio.

In the present experiment, a synthetic solution of the following formula was used as the standard solution.

Mono-potassium phosphate ..... 0.50 gr.
Magnesium sulphate ..... 0.25 gr.
Sucrose ..... 20.00 gr.
Distilled water ..... 1000.00 c.c.

The cultural agar media were prepared by additions of 2 per cent agar and different amount of ammonium nitrate, varying 0.02, 0.1, 0.5 per cent, to the standard nutrient solution.

The method of preparation of culture plates and techniques of the experiment were similar to the preceding experiments, and the culture was carried out at 25°C. in a thermostat.

The rate of radial growth of each strain was compared by measuring the diameter of colony, and colors produced in the media and zonation of the colony were observed in making comparison of each strain and each medium differing in the C-N ratio. The measurements, of develped colonies are shown in the Table VIII and the pigmentation and zonation in Table IX. The media containing 0.02, 0.1, and 0.5 per cent of ammonium nitrate are represented by the letters A, B, and C respectively.

The indications of colors in Table IX are as follows: p.p.v. pale purplish vinaceous, l.p.v. light purplish vinaceous, p.v. purplish vinaceous, deep v. deep vinaceous, dark v. dark vinaceous, ar.p. argyle purple, l.j.r. light jasper red, and p.c.p. pale congo pink.

TABLE VIII. The radial growth of colonies of various strain on three kinds of media differing in the C-N ratio.

Strain	Medium	Diametral growth rate in every two days					Averaged diameter of colony
		2nd day	4th day	6th day	8th day	10th day	
No. 1	A	15	15	12.6	13.7	13.3	69.6
	B	14	13	12.3	11.3	11.3	61.3
	C	15.6	18	23	21.4	—	(78)

TABLE VIII. (*Continued*)

Strain	Medium	Diametral growth rate in every two days					Averaged diameter of colony
		2nd day	4th day	6th day	8th day	10th day	
No. 2	A	16.3	18.7	14.3	14.7	10	74
	B	13	14	11.6	12.4	10	61
	C	10.6	23.4	22.3	15.3	12.4	84
No. 3	A	16.6	18.7	12	10.7	11	69
	B	13.6	14	11	12.7	9.7	61
	C	13	20.6	25.4	21.6	—	(80.6)
No. 4	A	15	12.3	14	15.7	15	72
	B	13	13	11.6	11.7	10.7	60
	C	18	20.3	20.7	14.6	10	83.6
No. 5	A	15.3	16.7	12	15.3	14.7	74
	B	12.3	13	12	13.3	11	61.6
	C	13	22	19.6	20.7	7.7	83
No. 6	A	17	14.6	8.3	10	10	60
	B	16.6	10.7	9.7	9.6	8.7	55.3
	C	20.3	10.3	11.4	17	—	(77)
No. 7	A	15.6	17.4	13	15	10.6	71.6
	B	13	12	10.6	11	12.4	58
	C	1.53	17	16.7	12.3	14.3	75.6
No. 9	A	15	16	14	14	14	73
	B	14.3	13.3	12.4	12	11	63
	C	16.6	20	22	17	8	83.6
No. 10	A	16	17	15.6	16.4	10.6	75.6
	B	13.3	13.7	13	12	11.3	63.3
	C	13	23.3	21.7	17	7	82
No. 11	A	15.6	17.4	13.3	15.7	11	73
	B	14	13.6	12.4	12.3	11.7	62
	C	15.3	18.7	18	13	13.3	78.3
No. 12	A	14.3	16.7	12	12.3	11.7	67
	B	14.3	11.7	9	10.3	9.7	55
	C	14.6	17	16.4	13	12	73

TABLE VIII. (*Continued*)

Strain	Medium	Diametral growth rate in every two days					Averaged diameter of colony
		2nd day	4th day	6th day	8th day	10th day	
No. 13	A	18	18	11	13	10	70
	B	13	15	9.6	10.7	8.3	56.6
	C	16.3	19	15.7	14	11	76
No. 14	A	15.6	15.4	12	15	12	70
	B	16	12.3	9.7	17.6	10.4	66
	C	19.3	21.3	22.7	15.3	—	(78.6)
No. 15	A	14	15	13	12	11.3	65.3
	B	14	12	12	9	6.6	51.6
	C	15	16.6	15	12	13.4	72
No. 16	A	14.6	16.4	12.6	11.4	11.3	66.3
	B	13.6	15.7	10.7	11	11	62
	C	14	21	20	12	13	80
No. 17	A	18	11	10	15.6	11.4	76
	B	16.6	12.4	9	11	9.6	58.6
	C	18	11	12.3	15.3	—	(76.6)
No. 18	A	15.6	15.4	12	12	10	65
	B	14	13	9.6	11	10	57.6
	C	15	17	14.3	12.7	11.3	70.3
No. 19	A	15	18.3	13.7	13.3	10.7	71
	B	15.3	13.7	10.6	10.7	9.3	59.6
	C	13.6	16	17.4	13	12	72
No. 20	A	15.3	14.7	13	12	13	68
	B	13.6	12.4	10.3	10.7	10	57
	C	15.6	15.4	14.6	14.4	12	72
No. 21	A	16.6	16.4	13.3	12.7	10	69
	B	16	11	12	10.3	10	59.3
	C	13	21.7	19	15.4	13.3	82.4
No. 22	A	17	19.3	14.7	17	12.6	80.6
	B	18	12.3	10.3	11.4	11	63
	C	17	20	21	18	—	(76)

TABLE VIII. (*Continued*)

Strain	Medium	Diametral growth rate in every two days					Averaged diameter of colony
		2nd day	4th day	6th day	8th day	10th day	
No. 23	A	15.6	15.7	11.3	9.4	11	63
	B	15.6	14	12	11.4	8.6	61.6
	C	15	17.6	18	14.4	14	79
No. 24	A	15.3	18	12.7	12	10	68
	B	15	12	19.6	13	10.4	60
	C	16	17	13.6	13.4	15	78
No. 25	A	14.6	15.4	12	13	11.6	66.6
	B	15	12	10	12	11	60
	C	16.6	18.4	13	14.3	14	76.3
No. 26	A	14.6	16.4	12	14	10	67
	B	14	14	10.3	8.7	8	55
	C	14.3	19	22	19	10.7	85
No. 27	A	16.3	18.7	13	12	8	68
	B	14	12	11	14	10	61
	C	12.3	19.7	16.3	15.3	11.4	75
No. 28	A	15	17	15	15	11	73
	B	15.6	14	10.4	9.3	13	62.3
	C	15	18	16	13.3	14.7	77
No. 29	A	16	18	14	12	12	72
	B	15.6	12.4	10	11	11	60
	C	17.6	19.7	15.7	14	13.3	77.3
No. 30	A	16	17	13.3	15.7	18	70
	B	16.6	12.4	10.3	10.3	10.7	60.3
	C	17.3	16.7	1.56	11.4	15.3	76.3
No. 31	A	16.6	17.4	13	15	12	74
	B	15	13	12.3	11.7	13	65
	C	17	19	14	13.3	12.7	76
No. 32	A	14	17.3	15	18	12.7	77
	B	15	14	12.3	14	11.7	67
	C	12.3	21.7	22.6	20.7	—	(77.3)

TABLE VIII. (*Continued*)

Strain	Medium	Diametral growth rate in every two days					Averaged diameter of colony
		2nd day	4th day	6th day	8th day	10th day	
No. 33	A	14.3	14.7	14	15.6	13.4	72
	B	14	16.3	13.7	12	12	68
	C	12	23	21.6	15.7	12.7	85
No. 34	A	16	17	14	15	10.3	72.3
	B	16.6	13	9.4	10.3	10.7	60
	C	16.6	17.4	16.3	12.7	12	75
No. 35	A	16	18	14	15	12	75
	B	16.6	13.7	9.6	12	10.7	62.3
	C	16	16.3	16	14.7	14	77
No. 36	A	16	18	15	15	11	75
	B	16	11	10	13	12	62
	C	15	17.6	13.4	15	11.6	75.6
No. 37	A	15.6	17.4	13	14	10	70
	B	16	13	10.6	10.4	11.6	61.6
	C	14.3	19	15.6	14	11.6	74.6
No. 38	A	18	21.3	15.7	14	11	80
	B	16	10.3	7.7	10	8	52
	C	19.3	21.7	20.6	17.7	—	(79.3)
No. 39	A	16	18	12	11	10	67
	B	15	13	11	9.3	11.7	60
	C	17	20	19	13.3	10.7	80
No. 40	A	15	18	12.6	10	12.4	68
	B	14.6	13.4	12	11	10	61
	C	16	16.6	16	14.4	11.6	74.6
No. 41	A	16.6	16.4	11.6	13.4	17.3	65.3
	B	15.3	12.7	11	12	12.3	63.3
	C	16	20.3	21.3	20.4	—	(78)

All the strains showed the best radial growth on medium C, containing 0.5% NH_4NO_3, the second on medium A containing 0.02% NH_4NO_3, and the worst on medium B containing 0.1% NH_4NO_3, with

no exception. No remarkable racial difference was observed in the effect of varying C–N ratio of the medium upon the radial growth of various strains.

As it is recognizable in the data given in table VIII, several strains showed the growth of staling type, and they were more or less differing from each other in the degree of staling and in the time required untill the staling taking place. However, any decided racial correlation in these connections could not be recognized.

In most strains development of aerial mycelium decreased remarkably on the medium A, and in general, it was produced scantily in the central part of a colony, or sometimes it was entirely absent.

The strains belonging to group I presented no special pigmentation in these three kinds of media, and the zonation was faint or entirely absent. The sectoring, however, sometimes occurred on media A and B.

On medium A, most strains produced deep vinaceous to dark vinaceous colors in the medium, while Nos. 6, 14, 17, 22, and 38 produced light jasper red color.

On media B and C, most strains produced pale purplish vinaceous to purplish vinaceous color. Strains 6, 14, 17, 22, and 38 produced light jasper red color in medium A, pale congo pink color in medium B, and pale purplish vinaceous color in medium C, while No. 23 (group IV) produced purplish vinaceous color in medium B, and argyle purple color in medium C.

Zonation was conspicuous in the cultures of most strains on medium A and B, and faint on medium C, except Nos. 6, 14, 17, 22, and 38 which showed no marked zonation on every of these three kinds of media, and No. 23 which showed conspicuous zonation on medium C.

TABLE IX. Pigmentation and zonation of various strains in three kinds of media differing in the C–N ratio.

Strain	Colors presented in			Zonation on		
	Medium A	Medium B	Medium C	Medium A	Medium B	Medium C
No. 1	none	none	none	±	−	−
No. 2	"	"	"	−	−	−
No. 3	dark. v.	p.v.	p.p.v.	++	+++	±
No. 4	none	none	none	−	−	−
No. 5	"	"	"	−	−	−

TABLE X1 (*Continued*)

Strain	Colors presented in			Zonation on		
	Medium A	Medium B	Medinm C	Medium A	Medium B	Medium C
No. 6	l.j.r.	p.c.p.	p.p.v.	−	−	−
No. 7	dark. v.	p.v.	l.p.v.	+++	+++	±
No. 9	none	none	none	−	±	−
No. 10	"	"	"	−	±	−
No. 11	dark. v.	p.v.	p.p.v.	++	+++	+
No. 12	"	"	p.v.	++	+++	±
No. 13	"	"	p.p.v.	+	+++	−
No. 14	l.j.r.	p.c.p.	"	−	−	−
No. 15	deep v.	p.v.	l.p.v.	++	+++	±
No. 16	"	"	p.p.v.	++	+++	−
No. 17	l.j.r.	p.c.p.	p.p.v.	+	−	−
No. 18	dark. v.	p.v.	p.v.	+++	+++	+
No. 19	"	"	"	+++	+++	+
No. 20	deep v.	"	l.p.v.	++	+++	±
No. 21	"	"	"	++	+++	±
No. 22	l.j.r.	p.c.p.	p.p.v.	±	−	±
No. 23	dark v.	p.v.	ar.p.	++	+++	+++
No. 24	"	"	p.p.v.	++	+++	±
No. 25	"	"	p.v.	++	+++	+
No. 26	"	"	p.p.v.	+++	+++	−
No. 27	"	"	l.p.v.	++	+++	+
No. 28	deep v.	"	"	++	+++	−
No. 29	dark v.	"	p.p.v.	++	+++	−
No. 30	"	"	"	+++	+++	±
No. 31	"	"	"	++	+++	−
No. 32	none	none	none	−	−	−
No. 33	"	"	"	±	−	−
No. 34	dark v.	p.v.	l.p.v.	++	+++	+
No. 35	"	"	p.p.v.	++	+++	±
No. 36	deep v.	"	l.p.v.	+++	+++	+
No. 37	"	"	"	++	+++	−
No. 38	l.j.r.	p.c.p.	p.p.v.	±	−	±
No. 39	deep v.	p.v.	"	++	+++	±
No. 40	dark v.	"	p.v.	++	+++	+
No. 41	deep v.	p.v.	p.p.v.	++	+++	±

From the results obtained in the present experiment, it was concluded that the varying C–N ratio in the medium caused different radial growth of the colonies, but any decided racial difference in this connection was hardly observed among the strains examined, although its influences upon the pigmentation of immersed mycelium and the zonation of colony differed markedly due to strains, and some of them were plainly distinguished from each other according to these cultural reactions.

The strains belonging to group I were distinguished from the others by their no marked pigmentation of the immersed mycelium and by perfect absence of zonation on three kinds of media differing in the C–N ratio. Strain No. 23 of group IV was characterized by an algyle purple pigmentation and by the occurrence of conspicuous zonation on medium C, while most of the other strains produced pale purplish vinaceous to purplish vinaceous colors and faint or no zonation on the same medium. The strains belonging to groups V, VI, VII were distinguished by the pigmentations of light jasper red color in medium A and pale congo pink color in medium B, and by faint or no zonation on every of these three kinds of media.

The strains examined were classified into the following 4 groups according to the pigmentation of immersed mycelium and the zonation of colony concerning the different C–N ratio of the media used in the present experiment.

Group A (C–N ratio). No pigmentation took place in the media A, B, and C. The zonation was faint or entirely absent. Strains Nos. 1, 2, 4, 5, 9, 10, 32, and 33 belong to this group.

Group B (C–N ratio). Pigmentations were deep or dark vinaceous in medium A, purplish vinaceous in medium B, and pale purplish vinaceous to purplish vinaceous in medium C. The zonation was conspicuous on media A and B, but faint or absent on medium C. Strains Nos. 3, 7, 11, 12, 13, 15, 16, 18, 19, 20, 21, 25, 26, 27, 28, 29, 30, 31, 34, 36, 37, 39, 40, and 41 belong to this group.

Group C (C–N ratio). This was distinguished from the group B (C–N ratio) by an occurrence of conspicuous zonation on medium C. Strain No. 23 belongs to this group.

Group D (C–N ratio.) Pigmentations were light jasper red in

medium A, pale congo pink in medium B, and pale purplish vinaceous in medium C. The zonation was almost absent on every medium. Strains Nos. 6, 14, 17, 22, and 38 belong to this group.

The strains of cultural group I belong to Group A (C–N ratio), those of cultural groups II, III, VIII, IX, and X belong to Group B (C–N ratio), strain No. 23, the single member of cultural group IV substantiates Group C (C–N ratio), and those of cultural groups V, VI, and VIII belong to Group D (C–N ratio).

VII. The aversion phenomenon

The word "aversion" means the phenomenon of antagonistic action occurring among two or more inocula from different sources inoculated to be opposite to each other on the same culture plate, and this name was first given by CAYLEY (11) in his investigation on *Diaporthe perniciosa* MARCHEL in 1923.

Before him similar phenomena had been observed and reported by DODGE (15) and PORTER (32).

When two or more strains, varieties or species of certain fungi were inoculated antithetically on the same agar plate, in some cases the mycelia developing from the inoculated points mix together without showing any apparent mutual influences, and in other cases they avert each other leaving a narrow clear strip entirely free from the mycelium, or they meet each other forming a boundery line. The latter cases are, namely, known as the phenomena of aversion.

According to CAYLEY (11), PORTER (32) and NAKATA (29) the phenomenon of aversion is presented among different strains but not between differential inocula of the same strain.

PORTER (32), who studied the antagonistic action among various species of fungi, or bacteria, said that the phenomenon of aversion occurs among different species of fungi or bacteria, and according to him the aversion phenomenon is grouped into following five types, namely (A) mutual intermingling; (B) superficial growing over the contending organisms; (C) slight inhibition; (D) growing around the contending organisms; and (E) mutual inhibition at a considerable distance.

NAKATA (29, 30) has undertaken a study of aversion phenomenon

in *Sclerotium Rolfsii* SACC. in order to throw light on the systematic problems of this species. He has endorsed CAYLEY's opinion and classified the phenomena of aversion into two types. The first is the mutual aversion in which the colonies developed side by side on the same plate avert each other and a clear line of demarcation is often found between them. The second is the one-side aversion, in which the averting action is perceivable in one inoculum, while the other remains indifferent, and in this case the demarcation is recognized usually along the line where one of the inocula averted. Further he (29) said that the width of the averted region in the case of mutual aversion or the distinctness of the averted line in one-side aversion appeared to have some significance corresponding to the affinity among the strains. He also said that the aversion phenomenon occurred irrespective of the environmental conditions and growing stages of the fungus.

The present study was intended to ascertain the occurrence of aversion phenomenon between two of the various strains of *Fusarium Lini* belonging to the different cultural groups classified conforming to the cultural characters.

The synthetic agar medium B and the strains Nos. 2, 3, 6, 7, 10, 17, 18, 21, 22, 23, 24, 27, 36, 38, and 41 were used in the experiments.

The belongings of the strains under test to the groups classified due to the cultural type (Cult. Group), the pigmentation and the zonation at different temperature (Temp. Group), the same in three kinds of media differing in C–N ratio (C–N ratio Group,). and the pathogenicity to three kinds of flax varieties are shown in the following table.

TABLE X. Belongings of the strains examined in the aversion experiment.

Strain	Cult. Group	Temp. Group	C–N ratio Group	Pathogenicity
2	I	1	A	medium
3	X	3	B	weak
6	VI	4	D	none
7	VIII	2	B	strong
10	I	1	A	medium
17	VII	4	D	none

TABLE X. (*Continued*)

Strain	Cult. Group	Temp. Group	C-N ratio Group	Pathogenicity
18	II	2	B	violent
21	X	3	B	weak
22	V	4	D	none
23	IV	2	C	strong
24	II	2	B	medium
27	IX	2	B	strong
36	II	2	B	violent
38	V	4	D	none
41	III	2	B	strong

All possible combinations of every two of these fifteen strains were examined by means of inoculating side by side on the same culture plate and incubated. The observation was made after two weeks incubation. Though a distinct aversion should not be expected among such inocula as treated in the present experiment, still some mutual hindrance of the development of counteracting colonies were observed among some strains.

The boundary between two colonies appeared as an indistinct furrow. The phenomena of aversion occurred in the present experiments might appertain to so called "slight inhibition" after PORTER (32).

The combinations of any two strains in which the slight inhibition took place were as follows.

No. 2 and Nos. 3, 17, 24.
No. 3 and Nos. 18, 23.
No. 6 and Nos. 7, 10, 21, 23, 38.
No. 7 and Nos. 10, 21, 24.
No. 10 and Nos. 21, 24.
No. 17 and Nos. 22, 36, 38.
No. 18 and Nos. 24, 38.
No. 21 and Nos. 27, 28, 41.
No. 22 and No. 24.
No. 23 and Nos. 24, 27.
No. 24 and Nos. 27, 38, 41.
No. 27 and No. 41.

The results obtained in the present experiment are shown in

the following Table XI. In the table the mark + shows the occurrence of slight inhibition, and the mark − shows the absence of aversion phenomenon.

As it was shown in Table XI, the occurrence of aversion among the different strains of *Fusarium Lini* was undoubtedly recognized in the present experiment, and the aversion phenomena pertaining to so called slight inhibition took place among different strains belonging either to the same cultural group or to different ones, but not between two inocula taken from the same strain. These facts suggested that the cultural expressions do not always positively denote the affinitive relations among the strains. In consequence, about the same weight should be laid on some requisite cultural characters, the aversion phenomena, and the pathogenicity in distinguishing the differences of strains. On the whole, however, even though some close resembrances were perceived in the cultural characters or the pathogenicity, we can not presume an affinity between the strains averting mutually. From this point of view it should be said that the aversion is a significant factor in ascertaining the affinitive relation among strains.

TABLE XI. Presence or absence of aversion phenomena among the different strains.

	2	3	6	7	10	17	18	21	22	23	24	27	36	38	41
2	−	+	−	−	−	+	−	−	−	−	+	−	−	−	−
3	+	−	−	−	−	−	+	−	−	+	−	−	−	−	−
6	−	−	−	+	+	−	−	+	−	+	−	−	−	+	−
7	−	−	+	−	−	−	−	−	−	−	−	+	−	+	+
10	−	−	+	−	−	−	−	+	−	−	+	−	−	−	−
17	+	−	−	−	−	−	−	−	+	−	−	−	+	+	−
18	−	+	−	−	−	−	−	−	−	−	+	−	−	+	−
21	−	−	+	−	+	−	−	−	−	−	−	+	−	+	+
22	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−
23	−	+	+	−	−	−	−	−	−	−	+	+	−	−	−
24	+	−	−	−	+	−	+	−	+	+	−	+	−	+	+
27	−	−	−	+	−	−	−	+	−	+	+	−	−	−	+
36	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
38	−	−	+	+	−	+	+	+	−	−	+	−	−	−	−
41	−	−	−	+	−	−	−	+	−	−	+	+	−	−	−

VIII. Biometrical experiments

Slight but apparently inherent differences in the spore dimentions of some different physiologic forms have occasionally been found in certain species of fungi. The differences, however, are somewhat significant from the biometrical point of view, but not so conspicuous as for a characteristic of an independent species.

The present experiments were intended to ascertain whether or not some biometrical differences in morphology of conidia produced by different strains would be found.

The biometrical variations of spores produced by different physiologic forms within a species have been reported of some fungi by several investigators (4, 8, 23, 50).

The external factors sometimes undoubtedly influence the morphology of conidia. It has been stated of the genus *Fusarium* by BROWN (8) that the external factors such as temperature, kinds of media, and the presence or absence of growth-retarding substances give considerable effects on the morphology of spores. BROADFOOT (4) said that there were marked and significant differences in spore-sizes of a given form of *Fusarium Lini* when it has grown on several different media, and that therefore it was important to maintain uniform cultural conditions with respect to media and environmental conditions when the comparative study of the morphology of the fungus was made. He also said that there were inherent differences in the spore dimensions of different physiologic forms when cultured on the same medium under identical conditions, although spore-size alone was not a sufficient basis for definite separation of physiologic forms of *Fusarium Lini*.

Seventeen strains of *Fusarium Lini* used in the present experiments were inoculated on potato glucose agar slants and incubated in a thermostat at 28°C. After 10 to 13 days measurement of spore-length was made of 200 spores of each strain.

By pouring distilled water to cultures in test tubes, suspenison of spores of each strain was obtained.

A drop of the suspension was placed on a slide glass, and the spores were stained with 2 per cent aequeous solution of gentian violet so that the septation of conidia could be examined distinctly and readily.

The spores examined were classified into groups due to the

number of their septation, and the spore-lengths measured were arranged distributing to respective groups. The frequencies of occurrence of spores having a certain number of septa were estimated by an average of observational results obtained in several microscopic fields.

The single celled micro-conidia were the most abundantly produced, but septate macro-conidia were relatively small in number and sometimes they were entirely absent in some strains. In regard only to septate macro-conidia, however, 3-septate conidia were produced far more than the others.

The results obtained in the present experiment are shown in the following table.

TABLE XII. Variation in spore-lengths of various strains cultured on potato glucose agar slants.

Strain	Septation	Frequency (%)	Spore-length (μ)	Mode (μ)	Mean (μ)	Standard deviation (μ)
No. 1	0	82.0	5.0—12.5	10.0	9.00±0.19	± 2.23
	1	3.0	17.5—22.5			
	2	1.5	20.0—22.5			
	3	13.6	27.5—42.5	35.0	34.42±0.49	± 3.82
No. 2	0	57.8	7.5—15.0	10.0	9.58±0.18	± 1.84
	1	7.8	12.5—22.5	20.0		
	2	4.2	25.0—35.0	30.0		
	3	37.5	30.0—50.0	37.5	38.89±0.67	± 5.29
	4	4.2	42.5—52.5	47.5		
No. 3	0	99.0	5.0—17.5	10.0	9.08±0.16	± 2.28
	1		7.5—20.0			
	2		25.0			
No. 5	0	98.0	5.0—12.5	10.0	9.53±0.14	± 1.79
	1		17.5—22.5			
	2		25.0			
	3	1.0	32.5—50.0	35.0	37.81±0.67	± 3.36
No. 6	0	53.5	5.0—12.5	7.5	8.36±0.32	± 2.99
	1	1.40	12.5—22.5	17.5		
	2					

TABLE XII (*Continued*)

Strain	Septation	Frequency (%)	Spore-length (μ)	Mode (μ)	Mean (μ)	Standard deviation (μ)
	3	23.5	30.0—50.0	40.0	39.48±0.58	± 4.41
	4	5.0	42.5—55.0	47.5		
	5	4.0	45.0—60.0	50.0		
No. 7	0	67.8	7.5—12.5	10.0	10.33±0.25	± 1.90
	1	13.1	15.0—22.5	20.0		
	2	3.4	20.0—22.5	22.5		
	3	15.7	20.0—32.5	25.0	26.43±0.35	± 2.77
No. 9	0	93.0	5.0—12.5	10.0	8.66±0.19	± 2.13
	1	3.0	12.5—20.0	17.5		
	2		22.5—35.0	22.5		
	3	3.0	32.5—42.5	35.0	37.00±0.43	± 3.00
	4		40.0—50.0			
	5		45.0			
No. 12	0	95.0	5.0—12.5	7.5	8.22±0.15	± 1.75
	1	1.5	15.0—20.0	17.5		
	2		20.0—25.0			
	3	3.0	25.0—40.0	30.0	32.30±0.66	± 4.60
	4		32.0—42.5	35		
	5		42.5			
	6		45.0—50.0			
No. 17	0	99.0	5.0—12.5	7.5	8.29±0.08	± 1.24
	1	1.0	1.50—20.0	17.5		
No. 21	0	99.0	5.0—17.5	10.0	10.47±0.18	± 2.60
	1		15.0—20.0	17.5		
No. 22	0	82.0	5.0—12.5	10.0	9.31±0.18	± 2.11
	1	17.0	1.25—20.0	15.0		
	2		17.5—22.5	17.5		
	3		22.5—27.5	27.5		
No. 23	0	100.0	7.5—10.0	7.5	8.26±0.12	± 1.82
No. 27	0	99.0	5.0—12.5	7.5	8.57±0.15	± 2.09
	1	1.0	12.5—20.0	15.0		

TABLE XII (*Conuinued*)

Strain	Septation	Frequency (%)	Spore-length (μ)	Mode (μ)	Mean (μ)	Standard deviation (μ)
No. 30	0	95.0	5.0—12.5	7.5	8.17±0.19	± 2.19
	1	3.0	17.5—22.5	17.5		
	2		20.0—27.5	25.0		
	3	1.5	25.0—42.5	30.0	32.33±1.07	± 6.38
	4		37.5			
	5		42.5—47.5			
No. 34	0	98.0	5.0—12.5	7.5	8.5±0.15	± 1.75
	1		12.5—20.0	17.5		
	2		20.0—25.0	22.5		
	3	1.0	25.0—37.5	30.0	30.22±0.14	± 3.10
No. 35	0	81.2	5.0—12.5	7.5	8.71±0.25	± 2.89
	1	7.6	12.5—22.5	17.5		
	2	2.2	20.0—22.5	22.5		
	3	9.0	25.0—37.5	27.5	29.26±0.46	± 3.31
No. 41	0	100.0	5.0—12.5	5.0	6.65±0.13	± 1.84

Marked differences in lengths of conidia were recognized among the strains examined.

The three-septate conidia, the typical macro-conidia of *Fusarium Lini* were produced by the strains 1, 2, 5, 6, 7, 9, 12, 22, 30, 34, and 35, but not by Nos. 3, 17, 21, 23, 27, and 41, in the present experiments. Among the strains produced 3-septate conidia, the strains 1, 2, 5, 6, and 9 produced longer ones than the other strains. The mean lengths of 3-septate conidia produced by the strains of the former group were ranging from 34.42±0.49 microns in the strain No. 1 to 39.48±0.58 microns in the strain No. 6, and the modes were ranging from 35 microns in the strain No. 1 to 40 microns in the strain No. 6, while in the latter group including the strains 7, 12, 22, 30, 34, and 35, the mean lengths and the modes of 3-septate conidia were ranging from 26.43±0.35 microns in the strain No. 7 to 32.33±1.07 microns in the strain 0.30 and from 25 microns in the strain No. 7 to 30 microns in the strain No. 30 respectively.

The former group included the strains belonging to the cultural

groups I and VI and the latter one included those of II and VIII.

In regard to the length of single celled micro-conidia the strains examined in the present experiments also differed from each other. The mean lengths of micro-conidia produced by strains 1, 2, 3, 5, 7, 9, 21, and 22 were ranging from 8.66 ± 0.19 microns in the strain No. 9 to 10.47 ± 0.18 in the strain No. 21 and the mode was 10 microns, while in the strains 6, 12, 17, 23, 27, 30, 34, 35, the mean lengths are ranging from 8.17 ± 0.19 microns in the strain No. 30 to 8.71 ± 0.25 microns in the strain No. 35 and the mode was 7.5 microns. The strain No. 41 seemed to be particular in the smaller size of micro-conidia which were measured only 6.65 ± 0.13 microns in the mean length and 5.0 microns in the mode. The strains included in the first group concerning the size of micro-conidia belong to the cultural group I, VIII, and X, the second group consisted of the strains belonging to groups II, IV, VI, VII, and IX, and strain No. 41 which produced particularly small micro-conidia belongs to the cultural group III.

These facts suggested an occurrence of racial differences in the dimention of conidia produced by different strains and also some possible connections between the size of conidia and the growth types on certain media.

IX. The occurrence of saltation

In the course of cultural experiments, wedge- or fan-shaped sectors occurred occasionally in the colonies of some strains of *Fusarium Lini*.

The phenomenon of saltation in micro-organisms was first discussed thoroughly by STEVENS (44). He distinguished that by the name of saltation from the mutation occurring in higher plants by reason of lacking definite data of cytological and genetical structures in fungi.

The variation in fungi has been often believed merely as the phenotypic change and was interpreted by segregation or hybridization. However, in some fungi, it seems to be nothing but a true mutation.

BRIERLY (3) suggested that one of the underlying causes of sectorial segregations among the colonies of fungi may be traced to the possible phenomenon of "mixochimaera" whereby germ tubes

and hyphae of different strains, varieties, and even species are supposed to fuse and give rise to mycelial threads containing cytoplasm and nuclei of distinct types. However, LEONIAN (21) who attempted to induce mixochimaera in *Fusarium moniliforme* concluded that, in so far as the organism and the conditions of his experiments are concerned, mixochimaera is not a factor in dissociation phenomena, and a mere mixture of protoplasms cannot give rise to new characteristics, unless there be a sexual phenomenon involved, because each nucleus will behave as an unit and will develop its own characteristics.

NAKATA (31) reported, in the study of two mutants of *Sclerotium Rolfsii*, that the new forms are asserted to be true mutants caused by genotypic change, not by segregation or hybridization.

It has been stated by some investigators that the saltants differ from their parent in morphological and physiological characters, and occasionally in pathogenicity, and that they can be induced sometimes artificially by change of environmental factors, such as temperature (14, 27, 38), or properties of culture media (14, 27, 48), irradiating X-ray or ultra violet ray (28), and by treatment with electric current (28).

In the phenomena of variation in fungi, two cases have been generally considered. The first is so-called "saltation", in which the saltants grow on several different kinds of media without failing in their distinctive characters under different conditions for a considerable period of time. STEVENS (44) and BURGER (10) demonstrated that the spores of saltants again developed in the colonies of the same type. The second is the temporary modification, in which the resulted colonies of distinct type revert readily to its parental form by renewed growings. VASDEVA (52) gave a name "false-sector" to such a variant occurring in *Fusarium fructigenum*.

The present experiments were carried out to study the cultural characters, biometry of spores, and the pathogenicity of the sectors occurred in strains 5, 34 and 39 making comparison with their corresponding parents.

The sectors were transfered on the apricot-juice agar slants, and the stableness of their distinct appearances were preliminarily ascertained.

The saltants examined in the present experiments are indicated with an addition of the mark S to the strain-number of the respec-

tive parent as follows:

No. 5. S. It occurred in a culture of strain No. 5 on the synthetic agar medium A at 25°C. in the experiments of varying C-N ratio in the medium. This was distinguished from its parent by scanty aerial growth and floccose appearance of the colony.

No. 34. S. It occurred in a culture on the synthetic agar medium A at 28°C. in the experiments of temperature-relation. This was distinguished from its parent by an absence of aerial growth and lacking pigmentation.

No. 39. S. This resembled closely No. 34. S., and the mother strains of these two saltants bore also a close resemblance to each other.

1. Cultural character of the saltants on various culture media

The cultural characters of the saltants presented on the following three kinds of media, namely apricot-juice agar, potato decoction agar (containing 2% glucose), and synthetic agar medium A, were studied by making comparison with their mother-strains. The cultures were carried out in triplicate for each strain and saltant in a thermostat at 28°C., and observations were made after seven days.

Details of the results obtained in the present cultural experiments are described in the following lines in which the parental strain and its saltant are discriminated with respective affixes P and S to the strain number.

A. Cultures on apricot-juice agar medium

No. 5. P. The colony was raised in topography, 59 mm. in diameter, and white aerial mycelia developed loosely over the surface. No marked pigmentation took place in the medium.

No. 5. S. The colony was flat in topography, waxy to slimy in appearance, and 60.6 mm. in diameter. White aerial mycelia, being floccose in appearance, tufted at the center of the colony. No marked pigmentation took place in the medium.

No. 34. P. The colony was 60.3 mm. in diameter and effuced

in topography. Aerial mycelia being rough velvety to floccose in appearance and light pinksh lilac in color developed loosely in the ray part of the colony. The immersed mycelium produced purplish vinaceous color in the medium. Conspicuous zonation took place.

No. 34. S. Diameter of the colony was 66.3 mm. The characters of the colony closely resembled those of No. 5. S consequently being different from those of the mother strain beyond comparison.

No. 39. P. Diameter of the colony was 59 mm., and the general characters resmbled those of No. 34. P described above.

No. 39. S. Diameter of the colony was 62.6 mm. The characters resembled those of No. 5. S and No. 34. S.

B. Cultures on potato decoction agar medium

No. 5. P. The colony was 63.3 mm. in diameter, flat in topography, and slimy to waxy in appearance. Zonation was absent.

No. 5. S. The diameter of the colony was 70 mm., being obviously larger than the mother strain, but the other characters resembled those of the parent.

No. 34. P. Aerial mycelia, being velvety to cottony in appearance and light purplish vinaceous in color, developed moderately at the center of the colony. The colony was flat in topography, slimy to waxy in appearance, and 68 mm. in diameter. The pigmentation by the immersed mycelium was light purplish vinaceous. Zonation was faint and sectoring was rare.

No. 34. S. The diameter of the colony was larger than the mother strain, being 75.3 mm. The other characters of the colony resembled those of No. 5. S and No. 5. P.

No. 39. P. The colony was 73.3 mm. in diameter and its characters resembled those of No. 34. P.

No. 39. S. The colony was 78 mm. in diameter, and its characters resembled those of No. 5. P, No. 5. S, and No. 34. S.

C. Cultures on synthetic agar medium A

No. 5. P. The colony was raised in topography, velvety to cottony in appearance, 62 mm. in diameter, and white

aerial mycelia developed densely all over the surface. No marked pigmentation took place. Zonation and sectoring were absent.

No. 5. S. The colony was 64 mm. in diameter, and no marked difference from the mother strain was recognized in general cultural characters.

No. 34. P. The colony was 60 mm. in diameter, velvety to cottony in appearance, and raised to slightly convex in topography. Aerial mycelia, being white to faint shell pink in color, developed densely all over the colony. Pigmentation by immersed mycelia was pale purplish vinaceous. Zonation and sectoring were absent.

No. 34. S. The colony was far larger than the mother strain, being 71 mm. in diameter, and the characters resmbled those of No. 5. S.

No. 39. P. The colony was 68 mm. in diameter, and the characters resembled those of No. 34. P.

No. 39. S. The colony was 71.6 mm. in diameter, and the characters resembled those of No. 5. P, No. 5. S, and No. 34. S.

Any of the strains and their saltants examined in the present cultural experiments produced almost no aerial myceium on potato decoction agar, notwithstanding they had proproduced it on the same medium in the forgoing experiments.

The saltants of the strains No. 5, No. 34, and No. 39 resembled the strain No.5 in general characters, with an exception of that the latter produced white aerial mycelia on apricot-juice agar medium.

The saltants, No. 34. S and No. 39. S, were clearly distinguished from their parents by lacking pigmentation of immersed mycelia, wherefore they resembled No. 5 and its saltant.

2. Biometrical experiment

The present experiment was undertaken to examine whether any biometrical difference occurs or not in length of spores between the saltant and its parent, and it was exemplified by comparative measurements of the conidia of saltant No. 34. S and its parental strain No. 34.

The lengths of the conidia obtained from 14-day cultures of saltant No. 34. S and strain No. 34 are contrasted in the following

table, in which P and S indicate the parental strain No. 34 and its saltant No. 34. S respectively.

TABLE XIII. Contrast of spore length between strain No. 34 and its saltant.

Septation	Frequency (%)		Range of spore-length (μ)		Mode (μ)		Mean (μ)		Standard deviation	
	P	S	P	S	P	S	P	S	P	S
0	98.0	87.7	5.0—12.5	5.7—15.0	7.5	10.0	8.5±0.15	9.09±0.19	± 1.75	± 2.02
1		55.5	12.5—20.0	12.5—22.5	17.5	17.5				
2			20.0—25.0	22.5—25.0	22.5	22.5				
3	1.0	6.0	25.0—37.5	27.5—45.0	30.0	35.0	30.22±0.14	35.05±0.51	± 3.10	± 3.63
4				40.0—45.0						
5				45.0—47.0						

The length of conidia of the saltant No. 34. S was larger than those of the mother strain No. 34. P, namely the former was 35.05 ±0.15 microns and the latter was 30.22±0.14 microns in the mean length of 3-septate conidia.

Comparing the mean length of conidia of saltant No. 34. S obtained in the present measurements with those of various strains given in the Table XI, it is also recognizable that the spore length in saltant No. 34. S bears a close resemblance to those of the strains Nos. 1, 2, 5, and 9 belonging to the cultural group I, which are from 34.42±0.49 to 38.89±0.69 microns.

3. Pathogenicity

The present experiments were undertaken to examine whether the pathogenicity of saltants differs or not from that of their parents. For that purpose the saltants of strains No. 5, No. 34 and No. 39 were inocultated to two varieties of flax, namely Pernau No. 1 and Takin-ama in comparison with their respecitive parent. The method and technique of the inoculation were similar to those used in the preceding same experiments.

Results obtained in the present inoculation experiments are shown in the following tables.

TABLE XIV. The results of the inoculation experiments on Pernau No. 1.

Inocula	Number of seedlings	Wilted plants	
		Numbers	Percentage
No. 5. P	36	6	16.6
No. 5. S	33	5	15.1
No. 34. P	31	28	90.3
No. 34. S	33	5	15.1
No. 39. P	34	16	44.4
No. 39. S	35	8	23.8
Control.	35	0	0

TABLE XV. The results of inoculation experiments on Taikin-ama.

Inocula	Number of seedlings	Wilted plants	
		Numbers	Percentage
No. 5. P	36	1	2.9
No. 5. S	35	0	0
No. 34. P	35	9	25.7
No. 34. S	33	0	0
No. 39. P	33	6	18.1
No. 39. S	34	0	0
Control.	36	0	0

It was greatly interesting that the results of the present inoculation experiments substantially suggested a falling tendency in the pathogenicity of the saltants in comparison with their respective parent. To Pernau No. 1 variety, the saltant of strain No. 5, the No. 5. S, showed almost equal pathogenicity to its parent, but the saltants of strains No. 34 and No. 39 were far less virulent than their respective parent. To Taikin-ama, the resistant variety, the pathogenicity of 3 saltants was uniformly reduced to naught, in spite of the parental strains were altogether more or less virulent and caused 2.4–25.7% wilted plants out of over 30 seedlings examined.

Pondering over the results of the cultural, biometrical, and pathogenical experiments concerning the present three saltants, it was concluded that the peculiarities of these saltants distinguished themselves from their respective parent and were almost enough to deal with them as particular strains. The saltants of the strains No. 34 and No. 39, i. e. No. 34. S and No. 39. S, were quite different from their parental strains, and on the contrary resembled strain No. 5 and its saltant, No. 5. S, not only in cultural and biometrical characters but also in the pathogenicity. When these four members, i. e. No. 5. P, No. 5. S, No. 34. S and No. 39. S, be dealt with as particular strains respectively, they would be included in one and the same group.

X. Summary to the experimental works and conclusion of the present studies

(1). *Forty one strains of Fusarium Lini* BOLLEY *were obtained* by means of single spore isolation from the materials of wilted flax plants collected in various localities in Hokkaido, and the phenomena of physiologic specialization in this species have been studied from morphological, physiological and pathogenical points of view with regard to these strains.

(2). These 41 strains were classified according to the growth-types presented on steamed rice medium and on apricot-juice agar into five groups respectively, on potato decoction agar into four groups, and on onion decoction agar and two kinds of synthetic agar into three groups respectively. In consequence of a correlating scrutiny on the results of the comparative cultures, the strains were arranged in ten different cultural groups as follows:

Cultural Group	Strain
I	Nos. 1, 2, 4, 5, 9, 10, 32, 33.
II	Nos. 11, 12, 13, 15, 16, 18, 19, 20, 24, 25, 26, 28, 29, 30, 31, 34, 35, 36, 37, 39, 40.
III	No. 41.
IV	No. 23.
V	Nos. 14, 22, 38.
VI	No. 6.
VII	No. 17.

VIII	No. 7.
IX	No. 27.
X	Nos. 3, 21.

(3). Inoculation experiments of these 41 strains to three kinds of flax varieties, namely Pernau No. 1, Saginau No. 1, and Taikin-ama, a resistant variety, were carried out by means of inoculating sterilized pot-soil with spore suspension. The results suggested a diverse difference of the pathogenicity occurring among the strains examined as shown in the following table.

TABLE XVI. Racial difference in pathogenicity of the strains.

Occurrence of the wilting (%)	Causal strains		
	on Pernau No. 1	on Saginau No. 1	on Taikin-ama
90 —100	35, 37, 40 (II); 23 (IV); 7 (VIII)	12, 18, 20, 36 (II)	31, 36 (II)
80 — 89	11, 12, 13, 18, 20, 25, 28, 29, 30, 31, 34, 36, 39 (II)	4 (I); 31, 37 (II)	20, 37 (II)
70 — 79	4 (I); 41 (III)	1 (I); 11, 28, 30 (II); 41 (III); 23 (IV); 7 (VIII); 27 (IX)	13, 18, 29, 34 (II); 27 (IX)
60 — 69	9, 10 (I); 16, 24 (II); 27 (IX)	2, 9 (I); 13, 25, 29, 34, 40 (II)	12, 25, 28, 35, 40 (II); 7 (VIII)
50 — 59	1, 2, 32, 33 (I); 19, 26 (II)	10, 32 (I); 16, 24, 26 (II)	11, 30, 39 (II)
40 — 49	5 (I); 15 (II); 3, 21 (X)	5, 33 (I); 19 (II)	2, 9, 32 (I)
30 — 39		15 (II); 3, 21 (X)	10, 33 (I)
20 — 29		35 (II)	4 (I); 16 (II); 41 (III); 3, 21 (X)
10 — 19		39 (II)	1, 5 (I); 19, 24, 26 (II); 23 (IV)
1 — 9			15 (I)
0	14, 22, 38 (V); 6 (VI); 17 (VII)	14, 22, 38 (V); 6 (VI); 17 (VII)	14, 22, 38 (V); 6 (VI); 17 (VII)

Roman figure in parentheses means the cultural group number to which belong the strain or strains mentioned ahead.

The strains are diversely different in the pathogenicity to these three kinds of flax varieties, and somewhat close connections would be surmised between the pathogenicities and the cultural characters. Most strains of cultural group II and every single strain belonging to the groups VIII and IX are strongly or fairly virulent, and two strains (3 and 21) belonging to the group X and the strains 15, 16, 19, 24 and 26 belonging to the group II are weak in pathogenicity to these flax varieties. The strains 35 and 39 of cultural group II are weak to Saginau No. 1, moderate to Taikin-ama and virulent to Pernau No. 1 in the pathogenicity. The strains belonging to the groups V, VI and VII showed almost no pathogenicity to any of these flax-varieties other than caused the yellowing of two three leaves occasionally.

(4). The studies of temperature-relation were carried out by means of comparative plate-cultures in thermostats at 20°, 25°, 28° and 32°C.

In the radial growth of colonies, the influence of varying temperature was remarkable but any significant racial difference could not be assumed among the strains, while in the pigmentation presented by the immersed mycelium in media and the zonation occurred on colonies, the racial difference was fairly conspicuous among some strains concerning the temperature. In this connection the strains were classified into 4 groups. The first group consisted of the strains belonging to cultural group I, which showed almost no marked pigmentation and zonation. The second group consisted of strains 11, 16, 18, 19, 20, 24, 26, 29, 30, 31, 34, 35, 36, 37, 39, 40 (II), No. 41 (III), No. 23 (IV), No. 7 (VIII) and No. 27 (IX), which presented pale purplish vinaceous to purplish vinaceous colors at 32°C. and pale congo pink to congo pink colors at 28°C, 25°, and 20°C., and couspicuous zonation at 32° and 20°C., but not at 28° and 25°C. The third group consisted of strains 3 and 21 (X), which differed from the former group in the absense of zonation at 20°C. and pale purplish vinaceous pigmentation at 28°C. The forth group consisted of strains 14, 22, 38 (V), No. 6 (VI) and No. 17 (VII), which presented shell pink color at 32°, 28° and 25°C. and pale congo pink color at 20°C. and entirely no zonation.

(5). The reactions of different strains to varying C–N ratio in

culture medium were examined. The culture media A, B and C differing in C–N ratio were prepared by means of changing the quantity of ammonium nitrate to fixed amonunt of sucrose as follows:

Ammonium nitrate	0.02% (A), 0.1% (B), 0.5% (C).
Mono-potassium phosphate	0.50 gr.
Magnesium sulphate	0.25 gr.
Sucrose	20.00 gr.
Agar-agar	2 %
Distilled water	1000.00 c.c.

All the strains showed the best growth on medium C containing 0.5% NH_4NO_3, the second on medium A containing 0.02% NH_4NO_3, and the worst on medium B containing 0.1% NH_4NO_3 with no exception. The development of aerial mycelium decreased markedly on medium A in most strains. Several strains exhibited the staling phenomenon, and they were more or less differing from each other in the degree of staling and in the time required until the staling takes place. However, any decided racial relation in these connections could not be recognized.

For all that, the pigmentation presented by immersed mycelia in media and the zonation of colonies occasionally displayed racial differences due to the varying C–N ratio, and some of the strains were obviously distinguished from each other according to these cultural reactions. In this connection, the strains examined were classified into four groups.

The first group consisted of strains 1, 2, 4, 5, 9, 10, 32 and 33 belonging to cultural group I, whicn presented no pigmentation at all and no or faint zonation on all three kinds of the media.

The second group consisted of strains 11, 12, 13, 15, 16, 18, 19, 20, 24, 25, 26, 28, 29, 30, 31, 34, 35, 36, 37, 39, 40 (II), No. 41 (III), No. 7 (VIII), No. 27 (IX), and Nos. 3 and 21 (X), which presented deep of dark vinaceous pigmentations in medium A, purplish vinaceous one in medium B, and pale purplish vinaceous to purplish vinaceous ones in medium C, and conspicuous zonations on media A and B, and no or faint one on medium C.

The third group consisted of strain 23, the single member of cultural group IV, which was distinguished from the former group by an occurrence of conspicuous zonation on medium C.

The fourth group consisted of strains 14, 22, 38 (V), No.6 (VI), and No. 17 (VII), which presented light jasper red pigmentation in medium A, pale congo pink one in medium B, and pale purplish vinaceous one in medium C, and almost no zonation on every medium.

(6). Aversion phenomena were examined with following fifteen strains belonging to every cultural group, namely Nos. 2 and 10 (I), Nos. 18, 24, 36 (II), No. 41 (III), No. 23 (IV), Nos. 22 and 38 (V), No. 6 (VI), No. 17 (VII), No. 7 (VIII), No. 27 (IX), Nos. 3 and 21 (X). All possible combinations of every two of these fifteen strains were tested by means of inoculating side by side on the same culture plate of the synthetic agar medium in a thermostat at 25°C. After a fortnight the results were observed, and it was revealed that the aversion phenomenon pertaining to so-called "slight inhibition" took place among different strains in the following combinations:

No. 2 and Nos. 3, 17, 24.
No. 3 and Nos. 18, 23.
No. 6 and Nos. 7, 10, 21, 23, 38.
No. 7 and Nos. 10, 21, 41.
No. 10 and Nos. 21, 41.
No. 17 and Nos. 22, 36, 38.
No. 18 and Nos. 24, 38.
No. 21 and Nos. 27, 38, 41.
No. 22 and No. 24.
No. 23 and Nos. 24, 27.
No. 24 and Nos. 27, 38, 41.
No. 27 and No. 41.

(7). Racial differences in the measurements of conidia produced on potato glucose agar medium at 28°C. were studied with the strains 1, 2, 5, 9 (I), 12, 30, 34, 35 (II), 41 (III), 23 (IV), 22 (V), 6 (VI), 17 (VII), 7 (VIII), 27 (IX), 3, 21 (X).

In the present experiment, single celled micro-conidia were produced most abundantly, while septate conidia were relatively small in number and sometimes they were entirely absent in some strains, e.g. Nos. 23 and 41. Triseptate conidia, the typical macro-conidia of the present fungus, were produced by strains 1, 2, 5, 6, 7, 9, 12, 22, 30, 34 and 35, but not by strains 3, 17, 21, 23, 27 and 41.

Marked differences in lengths of either macro-conidia or micro-conidia were recognized among the strains examined.

In regard to the length of 3-septate macro-conidia, the strains which produced those spores were devided into two groups, and in the former group consisted of the strains 1, 2, 5, 9 (I) and 6 (VI) the mode of the spore-length was ranging from 35 μ (strain No. 1) to 40 μ (strain No. 6), and in the latter group consisted of the strains 12, 30, 34, 35 (II), 22 (V) and 7 (VIII) it was raging from 25 μ (strain No. 7) to 30 μ (strain No. 30). Concerning the length of micro-conidia the strains examined were classified into 3 groups. In the first group consisted of the strains 1, 2, 5, 9 (I), 22 (V), 7 (VIII) and 3, 21 (X) the mode was 10 μ, in the second group consisted of the strains 12, 30, 34, 35 (II), 23 (IV), 6 (VI), 17 (VII) and 27 (IX) it was 7.5 μ, and the third group conisisted of strain No. 41 (III) only produced particularly small micro-conidia in which the mode of the length was 5 μ.

These results mentioned above suggested an occurrence of racial difference in the dimentions of conidia produced by different strains and also some possible connections between the size of spores and the growth-types on certain media.

(8). The sectoring occurred occasionally in some strains. The saltant of strain No. 5 (I) was distinguished from the parent only by scanty aerial growth and floccose appearance, while those of strains 34 and 39 (II) were greatly different from their parents in lacking aerial growth and pigmentation. These three saltants should be classed with the cultural group I according to their growth-type.

(9). As the conclusion of the present studies, the 41 strains of *Fusarium Lini* BOLLEY examined were classified into ten different physiologic forms according to collating the differences in cultural and biometrical characters and also in the pathogenicity to 3 kinds of flax-varieties, i. e. Pernau No. 1, Saginau No. 1 and Taikin-ama.

Physiologic form I. No marked pigmentation was presented in most media but a faint massicot yellow color in steamed rice medium and a faint pinkish buff color rarely in synthetic agar medium B were observed. The conidia were generally large, and the mode of length of 3-septate macro-conidia was measured 35–37.5 μ and that of single-celled micro-conidia was 10 μ. The pathogenicity was moderate or weak to those flax-varieties.

The strains 1, 2, 4, 5, 9, 10, 32 and 33, the whole members of

cultural group I, belonged to this physiologic form.

Physiologic form II. Aerial growth was prominent and floccose to cottony in appearance.

The pigmentation was conspicuous, and it was purplish vinaceous to livid brown in apricot-juice agar, pale purplish vinaceous in onion decoction agar, light purplish vinaceous to purplish vinaceous in potato decoction agar, congo pink in synthetic agar medium A, pale purplish vinaceous to livid brown in synthetic agar medium B, and chatenay pink to jasper red on steamed rice medium. The mode of length of 3-septate macro-conidia was comparatively small, i. e. 30 μ, and that of micro-conidia was intermediate, i. e. 7.5 μ. The pathogenicity was violent or strong to Pernau No. 1 and strong or medium to Saginau No. 1 and Taikin-ama.

The strains 11, 12, 13, 18, 20, 25, 28, 29, 30, 31, 34, 36, 37, 39 and 40, a large majority of the members of cultural group II, belonged to this physiologic form.

Physiologic form III. This was distinguished from the former physiologic form by weak pathogenicity to these 3 kinds of flax-varieties.

The strains 15, 16, 19, 24 and 26, a part of the members of cultural group II belonged to this physiologic form.

Physiologic form IV. This was distinguished from physiologic from II by the difference in pathogenicity which was strong to Pernau No. 1, medium to Taikin-ama, and very weak to Saginau No. 1.

The strain 35 and 39, the remaining members of cultural group II belonged to this physiologic form.

Physiologic form V. On steamed rice medium, the color of aerial growth was white to vervena violet and that of leathery mycelial sheet was purplish vininaceous and flesh pink. The mode of length of micro-conidia was as small as 5 μ. The other cultural characters were almost similar to those of phsiologic form II.

The pathogenicity was moderate to Pernau No. 1 and Saginau No. 1 and weak to Taikin-ama.

This physiologic form consisted of strain 41 only, the single member of cultural group III.

Physiologic form VI. This was almost similar to physiologic form II in cultural characters, but was distinguished from it by livid brown pigmentation in onion decoction agar medium and also

by differences in pathogenicity which was moderate to Pernau No. 1, and Saginau No. 1, and weak to Taikin-ama.

Strain No. 23, the single member of cultural group IV, was alone assigned to this physiologic form.

Physiologic form VII. The differences from physiologic form II in cultural characters were slower development of colony and velvety aerial growth on apricot-juice agar medium and a light purplish vinaceous pigmentation tinged in places with livid brown color in steamed rice medium. The length of miro-conidia was as large as 10 μ in mode. The pathogenicity was strong to Pernau No. 1, and moderate to Saginau No. 1 and Taikin-ama.

Strain No. 7, the single member of cultural group VIII, was assigned alone to this physiologic form.

Physiologic form VIII. The cultural characters generally resembled physiologic form II, but discriminated in wooly aerial growth developing on apricot-juice agar and potato decoction agar, and pigmentation in steamed rice medium being similar to that of physiologic form V. The pathogenicity was moderate to 3 flax-varieties examined.

Strain No. 27, the single member of cultural group IX, was alone assigined to this physiologic form.

Physiologic form IX. This was distinguished from the former physiologic form by pale purplish vinaceous pigmentation in synthetic agar medium A, by larger micro-conidia being 10 μ in the mode of length contrasting to 7.5 μ of those of physiologic form VIII, and also by the weak pathogenicity to the flax-varieties.

The present physiologic form consisted of strains 3 and 21, the members of cultural group X.

Physiologic form X. The radial growth of colonies was very rapid on all media. The zonation was obscure or entirely absent on most media excepting synthetic agar medium B on which a conspicuous zonation took place. The pigmentation was jasper red to madder brown in apricot juice agar, terra cotta to vinaceous-russet in potato decoction agar, livid brown or pale purplish vinaceous in onion decoction agar, pale purplish vinaceous in synthetic agar medium A, and vinaceous-lilac, pale vinaceous-drab, and light russet-vinaceous or sometimes livid brown to pale purplish vinaceous from center to margin in synthetic agar medium B.

This was practically none-pathogenic to three kinds of flax-

varieties examined.

Strains 14, 22, 38 (V), No. 6 (VI), and No. 17 (VII) belonged to this physiologic form.

These strains were isolated actually from wilted flax-seedlings and were no other than *Fusarium Lini* BOLLEY in the morphological and physiological characteristics, nevertheless they did not cause the wilting of flax-seedlings in inoculation experiments.

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EXPLANATION OF PLATES

PLATE VIII

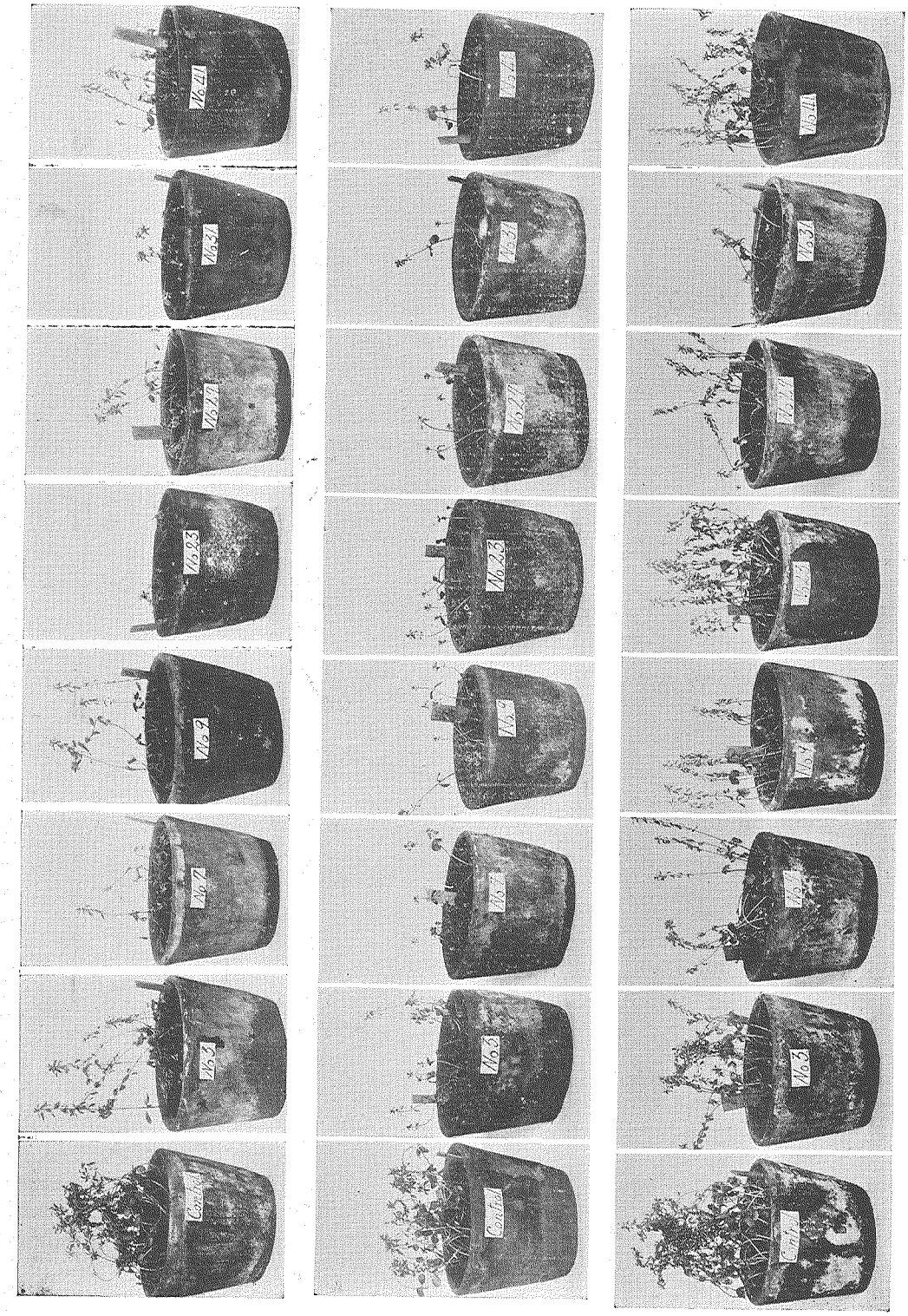
Photographs showing the comparative virulence of different strains of *Fusarium Lini* to flax seedlings.

Upper series: Pernau No. 1 variety.
Middle series: Saginau No. 1 variety.
Lower series: Taikin-Ama, the resistant variety.

PLATE IX

I. Photographs showing a result of soil-inoculation experiment with apparently non-virulent strains of *Fusarium Lini* regarding Pernau No. 1 variety.

II. Photographs showing the comparative virulence of strain No. 34 and its saltant, No. 34. S, to Pernau No. 1 variety (upper) and Taikin-Ama, the resistant variety (lower).


No.44
No.24
No.14
No.23
No.9
No.2
No.3
Control
No.44
No.24
No.14
No.23
No.9
No.2
No.3
Control
No.44
No.24
No.14
No.23
No.9
No.2
No.3
Control

I

II