# Comparative Studies on *Cephalosporium gramineum* Nisikado et Ikata, which Causes the Stripe Disease of Wheat, and *C. acremonium* Corda.

By

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## I. Introduction.

The stripe disease of wheat plant was first reported in 1933 by the senior writer and his collaborators, and a new name, *Cephalosporium gramineum* Nisikado et Ikata was given to the causal fungus. After then Prof. Joh. Westerdijk, the director of the Centraal Bureau voor Schimmelkultures, Baarn, Holland, wrote to the senior writer, that the causal fungus of the wheat stripe was very similar to *C. acremonium* Corda in respect to the morphological characteristics, and advised him to compare both of these fungi. And she was so kind to send him three strains of *C. acremonium* Corda, almost all the ascertained strains of the fungus in her laboratory. The present writers then set up their studies to compare *C. gramineum* Nisikado et Ikata and *C. acremonium* Corda, not only morphologically but also physiologically and pathologically. The result are here reported, although some of them have not yet been fully ascertained.

The writers wish to express their hearty thanks to Prof. Joh. Westerdijk, who kindly supplied the pure cultures to the writers and induced him to begin the studies. The acknowledgement is also due to Messrs. K. Hirata and K. Kimura, who kindly helped the writers during the course of studies.

## II. Fungus Cultures Studied.

In the present investigation the following cultures of *Cephalosporium gramineum* NISIKADO et IKATA and *C. acremonium* CORDA were studied:

1) *Cephalosporium gramineum* NISIKADO et IKATA, strain No. 530, isolated from a blackened node of the diseased wheat culm on June 10, 1932, collected in Bakuroiti, Kurasiki. The wheat variety was "Tinko-Komugi".

2) *C. gramineum* NISIKADO et IKATA, strain No. 1080, isolated from a diseased culm of wheat in the experimental farm of the Ōhara Institute in Kurasiki on May 30, 1936.

3) *C. gramineum* NISIKADO et IKATA, strain No. 1038, isolated from a diseased culm of *Alopecurus agrestis* L., collected in the experimental farm of the Ōhara Institute on May 6, 1935.

4-6) *Cephalosporium acremonium* CORDA. The following three strains were kindly supplied from Prof. JOH. WESTERDIJK, the director of the Centraal Bureau voor Schimmelkultures, Baarn, Holland on May 9, 1935.

4) *C. acremonium* CORDA, strain No. 1035, isolated by Prof. POLLACCI in Italy.

5) *C. acremonium* CORDA, strain No. 1036, isolated in the Centraal Bureau voor Schimmelkultures, Baarn, Holland.

6) *C. acremonium* CORDA, strain No. 1037, isolated by Dr. KOEHLER in Germany.

## III. Morphological Characteristics.

In pure culture, *C. gramineum* NISIKADO et IKATA and *C. acremonium* CORDA showed no great differences in the morphology of the conidiophore and the mycelium. Therefore the comparative studies were carried out chiefly on the conidia, formed on the potato-dextrose agar.

Among the strains of *C. gramineum*, the strains No. 530 and No. 1080 were very similar in the cultural characteristics as well as in the shape of the conidia. The conidia were hyaline, continuous, ellipsoid to fusoid and often curved to one side. They were rarely septated near the middle part and constricted at the septum. Near the ends they were provided with light-refrecting granules.

The conidia of the strains No. 1035, 1036 and No. 1037 of *C. acremonium*, sent from Holland, were almost similar in shape and somewhat resembling to those of *C. gramineum* N. et I., but the former seemed to be slightly smaller than the latter.

The results of the measurement of the conidia produced on the potato glucose agar after 3 weeks' culture at 20°C. are given in Tables I and II on pages 285 and 286.

The results given in Tables I and II are graphically shown in Fig. 1 and 2 on pages 287 and 288.

Table I.

Variations and Means of the Length of Conidia of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, produced on the Potato-Dextrose Agar Medium after 3 Weeks' Culture at 20°C.

| Fungus strains studied | No. experiment | Classes in length of conidia (μ) | | | | | | | | | | | | | | | | | | Total | Mean (μ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | |
| *C. gramineum* strain No. 530 | I | | | 1 | 1 | 3 | 6 | 17 | 8 | 21 | 6 | 15 | 8 | 7 | 3 | 2 | 1 | 1 | | | 100 | 7.24±0.135 |
| | II | | | | | 5 | 4 | 14 | 8 | 16 | 11 | 20 | 10 | 7 | 2 | 2 | 0 | 1 | | | 100 | 7.36±0.124 |
| | Sum | | | 1 | 1 | 8 | 10 | 31 | 16 | 37 | 17 | 35 | 18 | 14 | 5 | 4 | 1 | 2 | | | 200 | 7.30±0.092 |
| *C. gramineum* strain No. 1080 | I | | | | 2 | 7 | 5 | 14 | 6 | 13 | 8 | 18 | 7 | 7 | 4 | 5 | 2 | 2 | | | 100 | 7.43±0.155 |
| | II | | | 1 | 1 | 14 | 10 | 13 | 10 | 21 | 5 | 5 | 3 | 5 | 3 | 5 | 1 | 1 | 1 | 1 | 100 | 6.95±0.167 |
| | Sum | | | 1 | 3 | 21 | 15 | 27 | 16 | 34 | 13 | 23 | 10 | 12 | 7 | 10 | 3 | 3 | 1 | 1 | 200 | 7.19±0.115 |
| *C. gramineum* | Sum | | | 2 | 4 | 29 | 25 | 58 | 32 | 71 | 30 | 58 | 28 | 26 | 12 | 14 | 4 | 5 | 1 | 1 | 400 | 7.25±0.072 |
| *C. acremonium* strain No. 1035 | I | 1 | 4 | 6 | 6 | 13 | 9 | 16 | 14 | 15 | 4 | 4 | 4 | 3 | 1 | | | | | | 100 | 6.07±0.139 |
| | II | | 2 | 12 | 12 | 10 | 11 | 19 | 12 | 9 | 3 | 8 | 0 | 1 | 0 | 1 | | | | | 100 | 5.80±0.131 |
| | Sum | 1 | 6 | 18 | 18 | 23 | 20 | 35 | 26 | 24 | 7 | 12 | 4 | 4 | 1 | 1 | | | | | 200 | 5.94±0.096 |
| *C. acremonium* strain No. 1036 | I | 10 | 10 | 17 | 10 | 7 | 8 | 13 | 7 | 8 | 2 | 5 | 0 | 1 | 1 | 1 | | | | | 100 | 5.20±0.161 |
| | II | 5 | 10 | 19 | 10 | 11 | 6 | 12 | 4 | 7 | 4 | 5 | 2 | 1 | 1 | 2 | 0 | 1 | | | 100 | 5.42±0.157 |
| | Sum | 15 | 20 | 36 | 20 | 18 | 14 | 25 | 11 | 15 | 6 | 10 | 2 | 2 | 2 | 3 | 0 | 1 | | | 200 | 5.31±0.116 |
| *C. acremonium* strain No. 1037 | I | 1 | 4 | 6 | 10 | 16 | 17 | 17 | 11 | 9 | 4 | 4 | 1 | | | | | | | | 100 | 5.66±0.116 |
| | II | | 2 | 14 | 8 | 17 | 11 | 13 | 8 | 10 | 4 | 10 | 0 | 1 | 0 | 2 | | | | | 100 | 5.83±0.144 |
| | Sum | 1 | 6 | 20 | 18 | 33 | 28 | 30 | 19 | 19 | 8 | 14 | 1 | 1 | 0 | 2 | | | | | 200 | 5.75±0.094 |
| *C. acremonium* | Sum | 17 | 32 | 74 | 56 | 74 | 62 | 90 | 56 | 58 | 21 | 36 | 7 | 7 | 3 | 6 | 0 | 1 | | | 600 | 5.67±0.079 |

Table II.

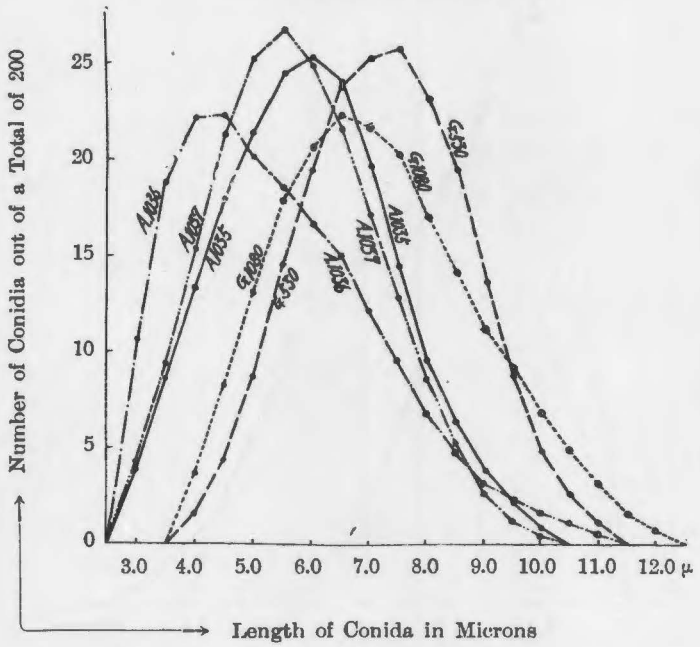
Variations and Means of the Width of Conidia of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, produced on the Potato-Dextrose Agar Medium after 3 Weeks' Culture at 20°C.

| Fungus strains studied | No. experiment | Classes in width of conidia (μ) | | | | | | | | | | | | Total | Means (μ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.0 | 1.25 | 1.5 | 1.75 | 2.0 | 2.25 | 2.5 | 2.75 | 3.0 | 3.25 | 3.5 | 3.75 | | |
| *C. gramineum* strain No. 530 | I | | 4 | 12 | 20 | 23 | 11 | 5 | 12 | 12 | 1 | | | 100 | 2.14±0.052 |
| | II | | 2 | 12 | 19 | 20 | 10 | 9 | 17 | 10 | 1 | | | 100 | 2.19±0.051 |
| | Sum | | 6 | 24 | 39 | 43 | 21 | 14 | 29 | 22 | 2 | | | 200 | 2.16±0.037 |
| *C. gramineum* strain No. 1080 | I | 1 | 10 | 17 | 17 | 22 | 13 | 2 | 8 | 7 | 3 | | | 100 | 2.17±0.056 |
| | II | | 8 | 19 | 19 | 22 | 6 | 7 | 6 | 8 | 4 | 0 | 1 | 100 | 1.87±0.053 |
| | Sum | 1 | 18 | 36 | 36 | 44 | 19 | 9 | 14 | 15 | 7 | 0 | 1 | 200 | 2.04±0.037 |
| *C. gramineum* | Sum | 1 | 24 | 60 | 75 | 87 | 40 | 23 | 43 | 37 | 9 | 0 | 1 | 400 | 2.10±0.018 |
| *C. acremanium* strain No. 1035 | I | 2 | 34 | 32 | 13 | 16 | 2 | 0 | 1 | | | | | 100 | 1.54±0.032 |
| | II | | 20 | 50 | 17 | 8 | 2 | 3 | | | | | | 100 | 1.58±0.028 |
| | Sum | 2 | 54 | 82 | 30 | 24 | 4 | 3 | 1 | | | | | 200 | 1.56±0.022 |
| *C. acremonium* strain No. 1036 | I | | 12 | 19 | 31 | 23 | 7 | 2 | 1 | 5 | | | | 100 | 1.82±0.041 |
| | II | | 26 | 24 | 15 | 16 | 4 | 6 | 6 | 3 | | | | 100 | 1.76±0.054 |
| | Sum | | 38 | 43 | 46 | 39 | 11 | 8 | 7 | 8 | | | | 200 | 1.79±0.033 |
| *C. acremonium* strain No. 1037 | I | | 36 | 37 | 16 | 11 | | | | | | | | 100 | 1.50±0.024 |
| | II | | 34 | 27 | 16 | 19 | 2 | 1 | 0 | 1 | | | | 100 | 1.59±0.024 |
| | Sum | | 70 | 64 | 32 | 30 | 2 | 1 | 0 | 1 | | | | 200 | 1.55±0.021 |
| *C. acremonium* | Sum | 2 | 162 | 189 | 108 | 93 | 17 | 12 | 8 | 9 | — | — | — | 600 | 1.63±0.010 |

The average length of the conidia in the strains No. 530 and No. 1080 of *C. gramineum* N. et I. was $7.30\pm0.09\,\mu$ and $7.19\pm0.12\,\mu$, while in the strains No. 1035, No. 1036 and No. 1037 of *C. acremonium* C., it was $5.94\pm0.10\,\mu$, $5.31\pm0.12\,\mu$ and $5.75\pm0.09\,\mu$, respectively. The average width of the conidia, as shown in Table II and Fig. 1, was $2.16\pm0.037\,\mu$ and $2.04\pm0.037\,\mu$ for the strain Nos. 530

Fig. 1.

**Graph Showing the Variations of the Conidial Length of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, produced on the Potato-Dextrose Agar Medium after 3 Weeks' Culture at 20°C.**




Remarks: G. 530 and G. 1080 in this Figure show the Strain No. 530 and No. 1080 of *Cephalosporium gramineum*, respectively. In the same way, A. 1035, A. 1036 and A. 1037 show the Strain No. 1035, No. 1036 and No. 1037 of *C. acremonium*.

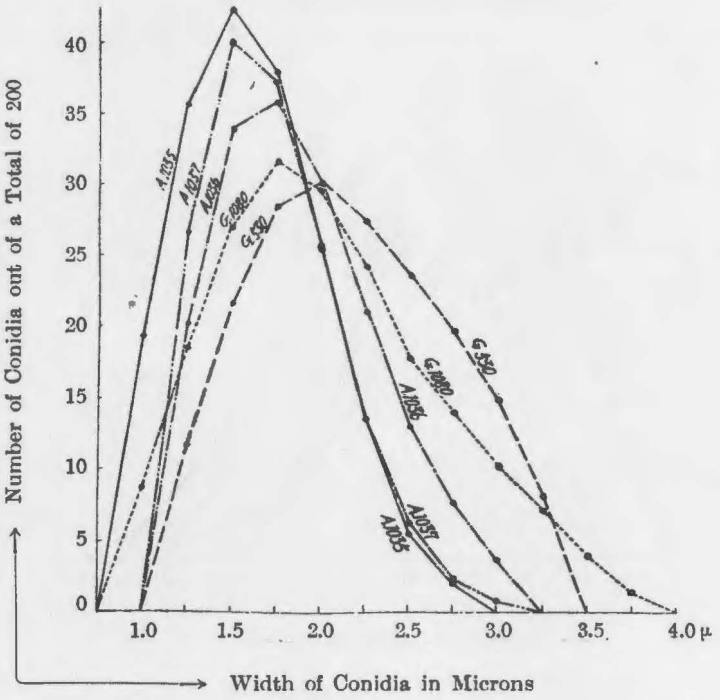
and 1080 of *C. gramineum* N. et I., and $1.56\pm0.022\,\mu$, $1.79\pm0.033\,\mu$ and $1.55\pm0.021\,\mu$ for the strains No. 1035, No. 1036 and No. 1037, respectively. In short the conidia of the fungus *C. gramineum* N. et I. were somewhat larger than those of *C. acremonium* C.

The results of the biometrical comparisons in the conidial size of the both fungi are shown in Table III on page 289.

The figures given in Table III show that, the differences in the average values in the conidial length of the strains among each of the fungi studied, were less than three times of the errors, even in the largest errors. Meanwhile in the comparisons of mean values of the conidial length between *C. gramineum* and *C. acremonium*, the differences attained were 8–13 times of the errors. In regard to the conidial width, the differences in the mean values were four times of the

Fig. 2.

Graph Showing the Variations of the Conidial Width of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, produced on the Potato-Dextrose Agar Medium after 3 Weeks' Culture at 20°C.




Remarks: G. 530 and G. 1080 in this Figure show the Strain No. 530 and No. 1080 of *Cephalosporium gramineum*, respectively. In the same way, A. 1035, A. 1036 and A. 1037 show the Strain No. 1085, No. 1036 and No. 1037 of *C. acremonium*.

errors among the strains of *C. gramineum* and six times among the strain of *C. acremonium*. The differences in the mean values between *C. gramineum* and *C. acremonium* were 5–14 times of the errors. In short, the above stated results seem to show that both the fungi *C. gramineum* and *C. acremonium* are morphologically quite different from each other. Therefore the both fungi may safely be said to be different species, although the conidial size varies greatly according to the cultural conditions.

Table III.

**Comparisons in the Conidium Size of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, grown on the Potato-Dextrose Agar Medium after 3 Weeks' Culture at 20°C.**

| Fungi studied | No. of strains studied | Length of conidia | | Width of conidia | |
|---|---|---|---|---|---|
| | | Difference in means | Ratio $\frac{\text{Diff. in M.}}{\text{Diff. in E.}}$ | Difference in means | Ratio $\frac{\text{Diff. in M.}}{\text{Diff. in E.}}$ |
| *C. gramineum* | 530 I ~ II | 0.12±0.184 | 0.65 | 0.05±0.073 | 0.69 |
| | 530 S ~ I | 0.06±0.163 | 0.37 | 0.02±0.036 | 0.56 |
| | 530 S ~ II | 0.06±0.154 | 0.39 | 0.03±0.036 | 0.84 |
| | 1080 I ~ II | 0.48±0.127 | 2.12 | 0.03±0.077 | 3.90 |
| | 530 S~1080 S | 0.11±0.014 | 0.78 | 0.12±0.052 | 2.24 |
| *C. acremonium* | 1035 I ~ II | 0.27±0.191 | 1.41 | 0.04±0.043 | 0.92 |
| | 1035 S ~ I | 0.13±0.170 | 0.86 | 0.02±0.036 | 0.96 |
| | 1035 S ~ II | 0.14±0.163 | 0.86 | 0.02±0.033 | 0.61 |
| | 1036 I ~ II | 0.22±0.244 | 0.99 | 0.06±0.067 | 0.90 |
| | 1037 I ~ II | 0.17±0.184 | 0.92 | 0.09±0.047 | 1.90 |
| | 1035 S~1036 S | 0.23±0.151 | 1.58 | 0.23±0.036 | 6.22 |
| | 1035 S~1037 S | 0.19±0.014 | 1.41 | 0.01±0.027 | 0.38 |
| | 1036 S~1037 S | 0.44±0.150 | 2.95 | 0.24±0.039 | 6.23 |
| *C. gramineum* and *C. acremonium* | 530 S~1035 S | 1.36±0.013 | 10.46 | 0.60±0.042 | 14.29 |
| | 530 S~1036 S | 1.99±0.015 | 13.27 | 0.37±0.049 | 7.55 |
| | 530 S~1037 S | 1.55±0.013 | 11.92 | 0.61±0.043 | 14.19 |
| | 1080 S~1035 S | 1.25±0.151 | 8.28 | 0.48±0.042 | 11.43 |
| | 1080 S~1036 S | 1.88±0.166 | 11.33 | 0.25±0.050 | 5.00 |
| | 1080 S~1037 S | 1.44±0.147 | 9.80 | 0.49±0.043 | 11.40 |

## IV. Characteristics on Culture Media.

For the comparisons of the cultural characteristics, one strain (No. 530) of *Cephalosporium gramineum* Nisikado et Ikata and three strains (No. 1035, 1036 and 1037) of *C. acremonium* Corda were grown on malt extract agar (tap water 1000 cc., malt extract 30 g. and agar 20 g.), potato-dextrose agar (tap water 1000 cc., potato 200 g., extracted, and agar 20 g.), onion-soja agar (concentrated onion decoction 100 cc., soja 50 cc., saccharose 50 g., tap water 850 cc. and agar 20 g.), apricot decoction agar (dried apricot 30 g., decocted, diluted to 1000 cc. and agar 20 g.), rice straw decoction agar (tap water 1000 cc., rice straw 100 g., decocted, and agar 20 g.), meat extract agar (tap water 1000 cc., meat extract 20 g. and agar 20 g.), asparagin agar (distilled water 1000 cc., potassium acid phosphate, second., 5 g., asparagin 2.5 g., magnesium sulphate 2 g., saccharose 10 g. and agar 20 g.),

CURRIE's agar (distilled water 1000 cc., ammonium nitrate 2.5 g., potassium acid phosphate, prim., 1 g., magnesium sulphate 0.25 g., saccharose 150 g. and agar 20 g.) and HOPKINS' agar (distilled water 1000 cc., potassium nitrate 2 g., potassium acid phosphate, prim., 0.1 g., magnesium sulphate 0.5 g., glucose 10 g. and agar 20 g.). They were grown in an incubator at 24°C. and the diameter and the compactness of the colonies and the formation of aerial mycelium and conidium, were studied after one and three weeks' culture, respectively. The data secured in this experiment are given in Table IV in tabular form.

Table IV.

**Comparisons in the Cultural Characteristics of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, grown on various Culture Media at 24°C.**

| Fungus | Strain | Diameter of colonies (mm.) | | Aerial mycerium | | Compactness of colonies | | Conidium formation |
|---|---|---|---|---|---|---|---|---|
| Duration of culture (weeks) | | 1 week | 3 weeks | 1 week | 3 weeks | 1 week | 3 weeks | 1 week |
| **Malt Extract Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 17.0 | 63.0 | — | — | T | T | + |
| *C. acremonium* | No. 1035 | 27.0 | 70.0 | — | — | T | TT | + |
| „ | No. 1036 | 26.0 | 80.0 | — | — | T | TT | ++ |
| „ | No. 1037 | 30.0 | 85.0 | — | — | T | T | + |
| **Potato-Dextrose Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 19.0 | 56.0 | ++ | ++ | C | C | +++ |
| *C. acremonium* | No. 1035 | 40.0 | 80.0 | ++ | ++ | C | C | +++ |
| „ | No. 1036 | 45.0 | 100.0 | ++ | +++ | C | C | +++ |
| „ | No. 1037 | 34.0 | 85.0 | — | ++ | C | C | + |
| **Onion-Soja Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 15.0 | | | | C | C | +++ |
| *C. acremonium* | No. 1035 | 35.0 | 56.0 | — | + | C | C | +++ |
| „ | No. 1036 | 23.0 | 60.0 | + | ++ | C | C | +++ |
| „ | No. 1037 | 34.0 | 90.0 | + | ++ | C | C | +++ |
| **Apricot Extract Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 18.0 | 46.0 | + | + | CT | CT | + |
| *C. acremonium* | No. 1035 | 30.0 | 90.0 | + | + | CT | CT | +++ |
| „ | No. 1036 | 38.0 | 85.0 | ++ | ++ | C | CT | +++ |
| „ | No. 1037 | 34.0 | 94.0 | + | + | C | C | ++ |

Table IV. (Continued.)

| Fungus | Strain | Diameter of colonies (mm.) | | Aerial mycelium | | Compactness of colonies | | Conidium formation |
|---|---|---|---|---|---|---|---|---|
| Duration of culture (weeks) | | 1 week | 3 weeks | 1 week | 3 weeks | 1 week | 3 weeks | 1 week |
| **Rice Straw Decoction Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 16.0 | 45.0 | ++ | + | CT | CT | +++ |
| *C. acremonium* | No. 1035 | 33.0 | 80.0 | + | − | CT | CT | +++ |
| „ | No. 1036 | 30.0 | 85.0 | + | + | CT | CT | ++ |
| „ | No. 1037 | 31.0 | 90.0 | + | + | C | CT | +++ |
| **Meat Extract Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 13.0 | 17.0 | − | − | CT | CT | +++ |
| *C. acremonium* | No. 1035 | 30.0 | 55.0 | + | + | CT | CT | + |
| „ | No. 1036 | 28.0 | 50.0 | − | + | CT | CT | ++ |
| „ | No. 1037 | 27.0 | 40.0 | − | − | C | CT | ++ |
| **Asparagin Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 17.0 | 37.0 | − | + | T | CT | +++ |
| *C. acremonium* | No. 1035 | 32.0 | 75.0 | − | + | C | CT | + |
| „ | No. 1036 | 29.0 | 65.0 | − | + | C | CT | +++ |
| „ | No. 1037 | 34.0 | 70.0 | − | − | C | CT | + |
| **Currie's solution Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 16.0 | 23.0 | − | − | T | C | +++ |
| *C. acremonium* | No. 1035 | 14.0 | 25.0 | − | − | CC | CC | + |
| „ | No. 1036 | 14.0 | 25.0 | − | + | C | CC | +++ |
| „ | No. 1037 | 28.0 | 45.0 | − | + | C | C | +++ |
| **Hopkins' solution Agar.** | | | | | | | | |
| *C. gramineum* | No. 530 | 17.0 | 30.0 | + | + | T | CT | +++ |
| *C. acremonium* | No. 1035 | 28.0 | 60.0 | + | + | CT | C | + |
| „ | No. 1036 | 27.0 | 90.0 | + | + | C | C | +++ |
| „ | No. 1037 | 33.0 | 94.0 | + | + | C | C | + |

Remarks: In the columns of the formation of the aerial mycelium and the conidium, the plus sign means the formation and the minus sign no formation. The more the plus signs the better the formation.
In the columns of the compactness of the colonies C shows that the colonies are compact T, thin, CT the intermediate between C and T, and CC or TT, much more compact or thinner.

According to the results given in Table IV, *C. gramineum* showed much better growth than *C. acremonium* on all the media studied, and the diameter of the colonies of the former species was much larger than the latter. As to the temperature relation to the mycelial growth and the effect of light to the coloring of the colonies are reported in the following lines.

## V. Effect of Light on the Coloring of Colonies.

Effect of light on the coloring of the colonies *C. gramineum* and *C. acremonium* grown on the potato-dextrose agar were studied. Strain No. 1035 of *C. acremonium*, grown on the above stated medium at 20°C., in an incubator kept quite dark, formed colorless colonies. When the cultures were placed near the window and exposed to the diffused day light, the colonies began to turn to orange yellow even within a day. At the end of a week's exposure to the diffused day light, the colonies turned to bitter sweet pink or light salmon orange (after the color name given by RIDGWAY), although the central part of the colonies was somewhat lighter color. But the cultures kept dark in a tin box and placed on the same place as stated above, showed no coloring at all.

In the same way, the colonies of the strains No. 1036 and No. 1037 belonging to *C. acremonium*, turned to bitter sweet pink, when they were exposed to the diffused day light.

On the contrary the strains No. 530, No. 1038 and No. 1080 of *C. gramineum* showed no bright coloring as in the strains No. 1035, No. 1036 and No. 1037 of *C. acremonium*, even when they were exposed to the diffused day light.

In regard to the coloring of the colonies, *C. gramineum* seems to be quite different from *C. acremonium*.

REDDY and HOLBERT (1924) reported that *C. acremonium* grew rapidly on the media containing such carbohydrates as glucose, maltose, lactose, sucrose, etc. and formed pink colored colonies. In this point of view the senior writer had decided in 1933 his fungus to be different from *C. acremonium*. The above stated result of the present investigation on the coloring of the colonies coincides to the report of REDDY and HOLBERT (1924).

## VI. Temperature Relations on the Fungus Growth.

The temperature relations to the growth of the both fungi *C. gramineum* and *C. acremonium* were tested. Small, circular bits of agar culture, 3 mm. in diameter, were transferred to the center of the slants of the 3% malt extract agar and the potato-dextrose agar.

In the comparative measurement of the diameter of fungus colonies, the plate culture in Petri dishes seems to be more convenient than the slant culture in test-tubes. However, the temperature in an incubator is very variable according

## Table V.

**Effect of Temperature on the Mycelial Growth of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, on the Malt Extract Agar. (I)**

Growth on the Malt Extract Agar after 4, 7 and 10 days' culture.

| Fungus strain studied | After | Temperature C. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 5° | 10° | 15° | 20° | 25° | 27° | 30° |
| *C. gramineum* No. 530 | 4 days | — | — | 4.0 | 8.0 | 4.5 | 4.5 | — |
| | 7 „ | — | — | 12.0 | 16.0 | 6.0 | 6.0 | 4.5 |
| | 10 „ | ± | 7.0 | 18.7 | 26.3 | 9.3 | 9.3 | 7.3 |
| „ No. 1080 | 4 „ | — | — | 6.7 | 8.7 | 6.7 | 6.3 | — |
| | 7 „ | — | 6.0 | 13.7 | 16.0 | 9.0 | 8.3 | 6.0 |
| | 10 „ | ± | 8.3 | 22.3 | 27.3 | 13.7 | 11.3 | 8.0 |
| „ No. 1038 | 4 „ | — | — | 6.7 | 8.7 | 6.7 | 6.3 | — |
| | 7 „ | — | 6.0 | 13.7 | 16.0 | 9.0 | 8.3 | 6.0 |
| | 10 „ | ± | 8.3 | 22.3 | 27.3 | 13.7 | 11.3 | 8.0 |
| „ No. 1035 | 4 „ | — | — | 4.0 | 8.0 | 13.0 | 13.7 | 13.0 |
| | 7 „ | — | — | 8.7 | 16.0 | 23.3 | 26.0 | 25.3 |
| | 10 „ | — | — | 14.0 | 26.0 | 34.7 | 38.3 | 37.3 |
| „ No. 1036 | 4 „ | — | — | 5.0 | 9.0 | 13.3 | 15.0 | 16.0 |
| | 7 „ | — | — | 11.3 | 18.0 | 26.0 | 26.3 | 27.7 |
| | 10 „ | — | — | 17.7 | 29.3 | 38.0 | 39.3 | 40.7 |
| „ No. 1037 | 4 „ | — | — | + | 8.0 | 17.0 | 20.3 | 21.3 |
| | 7 „ | — | — | 10.0 | 18.0 | 32.3 | 34.3 | 35.0 |
| | 10 „ | — | — | 16.7 | 29.7 | 50.0 | 50.7 | 51.7 |

## Table VI.

**Effect of Temperature on the Mycelial Growth of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, on the Malt Extract Agar. (II)**

Growth on the Malt Extract Agar after 4, 7 and 10 days' culture.

| Fungus strain studied | After | Temperature C. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5° | 10° | 15° | 20° | 24° | 27° | 30° | 32° | 34° | 38° | 40° |
| *C. gramineum* No. 530 | 4 days | — | 4.5 | 5.8 | 10.5 | 10.5 | 5.3 | — | — | — | — | — |
| | 7 „ | — | 10.0 | 14.0 | 21.5 | 20.8 | 10.8 | 5.0 | — | — | — | — |
| | 10 „ | 6.0 | 14.8 | 21.8 | 29.3 | 28.3 | 14.5 | 6.0 | — | — | — | — |
| „ No. 1080 | 4 „ | — | 4.5 | 10.0 | 11.5 | 9.3 | 6.0 | — | — | — | — | — |
| | 7 „ | 5.0 | 10.5 | 17.8 | 21.5 | 19.8 | 12.3 | 5.0 | — | — | — | — |
| | 10 „ | 6.5 | 16.3 | 23.8 | 29.8 | 28.0 | 15.0 | 6.0 | — | — | — | — |
| „ No. 1083 | 4 „ | — | 5.0 | 8.0 | 11.3 | 11.0 | 7.3 | — | — | — | — | — |
| | 7 „ | 5.0 | 10.5 | 16.0 | 21.5 | 20.3 | 14.0 | 5.5 | — | — | — | — |
| | 10 „ | 6.0 | 15.3 | 22.0 | 29.5 | 28.0 | 16.5 | 6.0 | — | — | — | — |
| *C. acremonium* No. 1035 | 4 „ | — | — | 4.0 | 10.3 | 12.5 | 14.5 | 16.3 | 13.5 | 11.8 | 5.0 | — |
| | 7 „ | — | 4.8 | 10.5 | 17.5 | 23.3 | 28.0 | 28.3 | 22.0 | 20.3 | 10.0 | — |
| | 10 „ | — | 8.5 | 14.5 | 26.3 | 33.3 | 39.0 | 39.5 | 32.3 | 27.5 | 14.0 | — |
| „ No. 1036 | 4 „ | — | 4.5 | 9.8 | 13.3 | 15.3 | 17.3 | 17.5 | 16.0 | 15.8 | — | — |
| | 7 „ | — | 9.5 | 15.8 | 22.0 | 28.0 | 30.3 | 31.3 | 27.3 | 25.8 | 5.5 | — |
| | 10 „ | — | 14.3 | 21.3 | 30.3 | 37.0 | 41.8 | 42.3 | 37.0 | 32.5 | 9.0 | — |
| „ No. 1037 | 4 „ | — | 4.5 | 6.0 | 12.5 | 14.8 | 19.3 | 21.3 | 19.0 | 18.5 | 11.3 | — |
| | 7 „ | — | 6.0 | 14.0 | 22.0 | 27.8 | 36.5 | 37.3 | 36.3 | 35.8 | 28.3 | — |
| | 10 „ | — | 11.0 | 17.8 | 31.3 | 39.5 | 49.3 | 54.3 | 47.8 | 44.3 | 33.0 | — |

Table VII.

**Effect of Temperature on the Mycelial Growth of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, on the Potato-Dextrose Agar. (I)**

| Fungus strain studied | After | Temperature C. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 5° | 10° | 15° | 20° | 25° | 27° | 30° |
| *C. gramineum* No. 530 | 4 days | — | — | 5.5 | 7.5 | 7.0 | 5.0 | — |
| | 7 „ | — | — | 12.5 | 15.5 | 8.0 | 7.0 | — |
| | 10 „ | ± | 8.0 | 18.0 | 22.5 | 10.0 | 10.0 | ± |
| „ No. 1080 | 4 „ | — | — | 5.0 | 7.0 | 7.5 | 6.5 | ± |
| | 7 „ | — | ± | 11.0 | 15.0 | 7.5 | 7.5 | 5.5 |
| | 10 „ | — | 7.0 | 16.0 | 21.5 | 9.5 | 8.5 | 6.5 |
| „ No. 1038 | 4 „ | — | — | 5.0 | 8.0 | 5.0 | 5.0 | — |
| | 7 „ | — | 5.0 | 11.0 | 13.5 | 7.0 | 7.0 | 4.0 |
| | 10 „ | + | 7.0 | 15.0 | 19.0 | 8.0 | 8.0 | 4.5 |
| *C. acremonium* No. 1035 | 4 „ | — | — | 4.0 | 7.0 | 14.0 | 16.5 | 17.0 |
| | 7 „ | — | — | 10.0 | 16.5 | 26.0 | 28.5 | 29.0 |
| | 10 „ | — | 4 0 | 16.0 | 28.0 | 38.5 | 42.5 | 42.5 |
| „ No. 1036 | 4 „ | — | — | 6.0 | 9.0 | 13.0 | 15.5 | 14.5 |
| | 7 „ | — | — | 13.0 | 19.5 | 28.0 | 28.5 | 27.5 |
| | 10 „ | — | 4.0 | 23.0 | 32.5 | 46.0 | 47.5 | 42.0 |
| „ No. 1037 | 4 „ | — | — | 4.0 | 8.0 | 19.0 | 19.0 | 19.5 |
| | 7 „ | — | — | 10.0 | 18.0 | 33.0 | 35.0 | 39.0 |
| | 10 „ | — | — | 17.0 | 35.0 | 55.0 | 55.5 | 56.0 |

Table VIII.

**Effect of Temperature on the Mycelial Growth of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, on the Potato-Dextrose Agar. (II)**

| Fungus strain studied | After | Temperature C. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5° | 10° | 15° | 20° | 24° | 27° | 30° | 32° | 34° | 38° | 40° |
| *C. gramineum* No. 530 | 4 days | — | 5.0 | 8.0 | 10.5 | 10.0 | 7.0 | — | — | — | — | — |
| | 7 „ | + | 11.5 | 14.0 | 21.0 | 19.0 | 11.5 | 6.0 | — | — | — | — |
| | 10 „ | 6.0 | 15.0 | 20.5 | 31.5 | 28.5 | 14.0 | 7.0 | — | — | — | — |
| „ No. 1080 | 4 „ | — | 6.0 | 8.0 | 10.5 | 8.5 | 7.0 | — | — | — | — | — |
| | 7 „ | + | 12.0 | 14.0 | 20.5 | 17.0 | 11.5 | 6.0 | — | — | — | — |
| | 10 „ | 6.0 | 15.5 | 20.5 | 30.0 | 23.5 | 15.0 | 7.0 | — | — | — | — |
| „ No. 1038 | 4 „ | — | — | + | 6.0 | 8.0 | 6.5 | 5.5 | — | — | — | — |
| | 7 „ | — | + | 10.5 | 13.5 | 14.5 | 13.0 | 8.0 | 5.0 | — | — | — |
| | 10 „ | — | 6.5 | 14.0 | 17.5 | 25.5 | 19.0 | 11.0 | 6.0 | — | — | — |
| *C. acremonium* No. 1035 | 4 „ | — | + | 5.0 | 10.0 | 13.0 | 16.5 | 17.5 | 13.0 | 12.0 | 5.5 | — |
| | 7 „ | — | 7.5 | 10.0 | 19.5 | 25.5 | 29.0 | 30.0 | 21.8 | 21.5 | 10.5 | — |
| | 10 „ | — | 10.0 | 15.5 | 28.5 | 35.0 | 40.5 | 41.0 | 33.0 | 27.5 | 14.5 | — |
| „ No. 1036 | 4 „ | — | 4.5 | 9.0 | 14.0 | 16.0 | 18.5 | 19.0 | 16.5 | 15.0 | — | — |
| | 7 „ | — | 9.5 | 16.0 | 23.0 | 28.0 | 32.5 | 33.0 | 28.5 | 26.5 | 6.0 | — |
| | 10 „ | — | 14.0 | 22.5 | 32.0 | 38.5 | 45.0 | 45.5 | 38.5 | 33.0 | 9.5 | — |
| „ No. 1037 | 4 „ | — | 4.5 | 8.0 | 15.0 | 18.0 | 23.5 | 27.0 | 21.5 | 20.5 | + | — |
| | 7 „ | — | 7.5 | 15.0 | 26.5 | 34.5 | 43.0 | 46.0 | 37.0 | 33.5 | 13.0 | — |
| | 10 „ | — | 13.5 | 22.0 | 38.0 | 48.5 | 56.0 | 60.0 | 49.5 | 42.5 | 23.0 | — |

to the positions, where the culture plates are placed, as already reported in 1926, by the senior writer. In many cases, it may be impossible to expect the uniform growth in Petri dish cultures, which are placed in various places in an incubator. For this reason, the agar slants were used for the comparisons of the diameter of the fungus colonies, although the measurements were somewhat inaccurate.

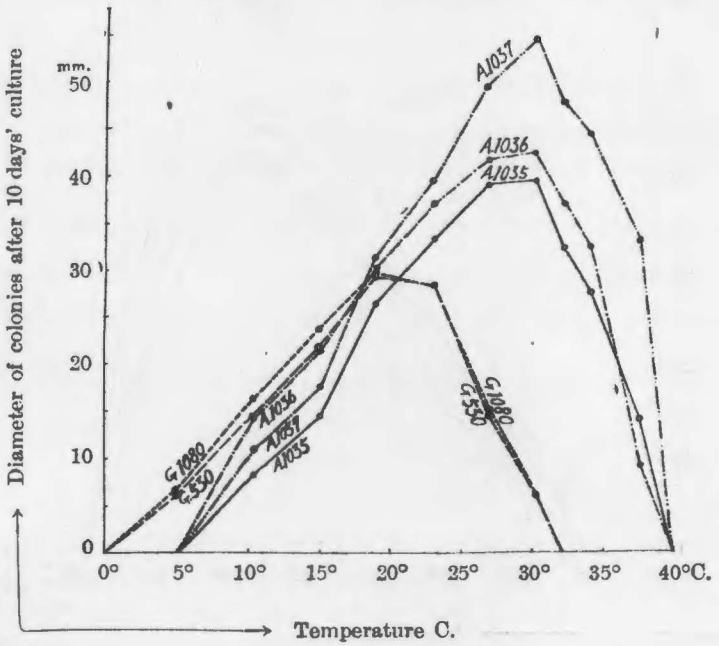
The test-tube cultures were grown in the incubators set at 5°, 10°, 15°, 20°, 24°, 27°, 30°, 32°, 34°, 38° and 40°C., respectively. The diameter of the colonies were measured after 4, 7, 10 and 14 days, respectively. The general appearance of the colonies were recorded after 14 and 21 days. The results are given in Tables V–VIII on pages 293 and 294.

In the first experiment, the growth of the fungi was studied between the temperatures of 5° and 30°C. As shown in Tables V and VII, on both the media used, the malt extract agar and the potato-dextrose agar, *C. gramineum* did not grow at all the temperatures 27°–30°C., while *C. acremonium* showed vigorous growth at these temperatures.

Fig. 3.

Graph Showing the Effect of Temperature on the Mycelial Growth *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda.

Growth on the Malt Extract Agar after 7 days' culture.




Remarks: G. 530, G. 1035 and G. 1080 in this Figure show the Strain No. 530, No. 1035 and No. 1080 of *Cephalosporium gramineum*, respectively. In the same way, A. 1035, A. 1036 and A. 1037 show the Strain No. 1035, No. 1036 and No. 1037 of *C. acremonium*.

In the second experiment, the temperature range studied was from 5° to 40°C. The results are shown in Table VI and VIII. The diameter of the fungus colonies after 10 days' culture are shown graphically in Fig. 3 on the preceding page and Fig. 4 on this page.

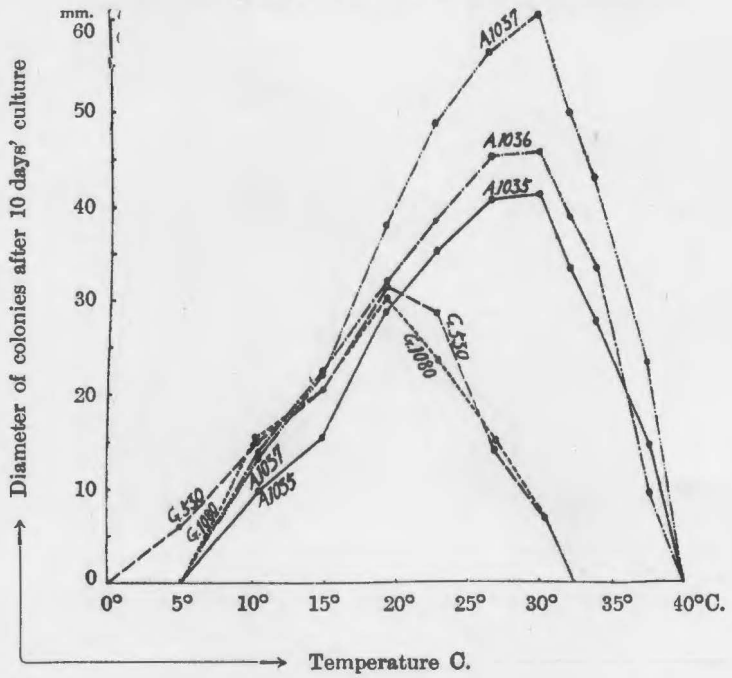
Tables V–VIII and Figures 3 and 4 show that three strains of *C. gramineum* studied, began to grow at 5°C. and formed pretty large colonies at 10°C., the diameter being 10–15 mm. The growth became better with the rise of temperature tested up to 20°C., at which the diameter of the colonies was about 30 mm. Above 20°C. the growth became worse with the rise of temperature and at 30°C. the growth was very scanty and no growth at 32°C.

On the contrary, *C. acremonium*, all the three strains studied, showed no trace of the growth at 5°C. at all, and then formed colonies of 10–15 mm. in diameter at 10°C. Above this temperature the growth rate became larger with the rise

Fig. 4.

**Graph Showing the Effect of Temperature on the Mycelial Growth of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda.**

Growth on the Potato-Dextrose Agar after 7 days' culture.




Remarks: G. 530, G. 1035 and G. 1080 in this Figure show the Strain No. 530, No. 1035 and No. 1080 of *Cephalosporium gramineum*, respectively. In the same way, A. 1035, A. 1036 and A. 1037 show the Strain No. 1035, No. 1036 and No. 1037 of *C. acremonium*.

of temperature. Thus the optimum for the fungus growth was shown at about 20°C. Above 30°C. the growth became worse with the rise of temperature rapidly. Slight growth was shown at 38°C. but no growth at 40°C.

The general appearance of the colonies grown on the media after 2 weeks' culture at various temperature was also recorded and the data are given in Tables IX and X.

Table IX.

**Mycerial Growth of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, grown on the Malt Extract Agar after 2 Weeks' Culture at various Temperatures.**

| Temperature | *C. gramineum* Nisikado et Ikata | | *C. acremonium* Corda | |
|---|---|---|---|---|
| | Strain | Mycelial growth | Strain | Mycelial growth |
| 5°C. | No. 530<br>1080<br>1038 | Poor growth, colonies hyaline, without aerial hyphae. | No. 1035<br>1036<br>1037 | No growth. |
| 10°C. | No. 530<br>1080<br>1038 | Growth was better than that at 5°, colonies hyaline without aerial hyphae. | No. 1035<br>1036<br>1037 | Colonies were thin and colorless, without aerial hyphae. |
| 15°C. | No. 530<br>1080<br>1038 | As above. | No. 1035<br>1036<br>1037 | As above. |
| 20°C. | No. 530<br>1080<br>1038 | As above. | No. 1035<br>1036<br>1037 | As above. |
| 24°C. | No. 530<br>1080<br>1083 | Colonies were colorless, without aerial hyphae, but with wet hyphae crept on the wall of the test-tubes. | No. 1035<br>1036<br>1037 | As above. |
| 27°C. | No. 530<br>1080<br>1038 | Growth was much worse than the above. Colonies were colorless and thin. | No. 1035<br>1036<br>1037 | Growth was very good. Colonies were thick, colorless, without aerial hyphae. |
| 30°C. | No. 530<br>1080<br>1038 | Growth was very bad, forming small colonies. | No. 1035<br>1036<br>1037 | As above. |
| 33°C. | No. 530<br>1080<br>1038 | No growth. | No. 1035<br>1036<br>1037 | As above. |
| 35°C. | No. 530<br>1080<br>1038 | No growth. | No. 1035<br>1036<br>1037 | As above. |
| 38°C. | No. 530<br>1080<br>1038 | No growth. | No. 1035<br>1036<br>1037 | As above. |
| 40°C. | No. 530<br>1080<br>1038 | No growth. | No. 1035<br>1036<br>1037 | No growth. |

Table X.

**Mycerial Growth of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda, grown on the Potato-Dextrose Agar after 2 Weeks' Culture at various Temperatures.**

| Temperature | *C. gramineum* NISIKADO et IKATA | | *C. acremonium* CORDA | |
|---|---|---|---|---|
| | Strain | Mycelial growth | Strain | Mycelial growth |
| 5°C. | No. 530<br>1080<br>1038 | Growth was slight, forming about 2 mm. long bristles in the colonies. | No. 1035<br>1036<br>1037 | No growth. |
| 10°C. | No. 530<br>1080 | As above, but somewhat better growth. | No. 1035<br>1037 | Pretty good growth. Colonies thick, provided with 2–3 mm. long bristles. |
| | No. 1038 | Colonies thick, without bristles. | No. 1038 | Colonies cottony, with slender, short bristles. |
| 15°C. | No. 530<br>1080 | In the central part of the colonies many, short bristles were formed. | No. 1035<br>1037 | Bristles attained to 7 mm. |
| | No. 1038 | Almost as above. | No. 1036 | Aerial hyphae were cottony. |
| 20°C. and 24°C. | No. 530<br>1080 | Colonies were thin, aerial hyphae cottony, with long bristles. | No. 1035 | Good growth, forming thick colonies and bristles richly. |
| | No. 1038 | As above, but without bristles. | | As above, but bristles were poor. |
| 27°C. | No. 530<br>1080<br>1038 | Slight growth, forming hemispherical colonies, and not bristles. | No. 1035<br>1037 | Good growth, forming bristles all over the surface of the colonies. |
| | | | No. 1036 | Cottony hyphae. |
| 30°C. | No. 530<br>1080<br>1038 | Small high colonies were formed. | No. 1035<br>1037<br>1036 | Good growth, forming coarse, burly bristles in the center of the colonies. |
| 33°C. and 35°C. | No. 530<br>1080<br>1038 | No growth. | No. 1035<br>1037<br>1036 | Somewhat poor growth, forming thin bristles sparsely. |
| 38°C. | No. 530<br>1080<br>1038 | No growth. | No. 1035<br>1037<br>1036 | Very poor growth, forming small high colonies. |

On the malt extract agar at 5°C. *C. gramineum* began the growth and formed aerial hyphae, while *C. acremonium* did not grow at all. At 15°–20°C., the both fungi showed no great differences in the general appearance of the colonies. At 27°–30°C., *C. acremonium* grew very well and produced pretty thick hyphal layer, while *C. gramineum* showed very scant growth.

On the potato-dextrose agar at 5°C. *C. gramineum* formed bundles of aerial hyphae, or bristles, in the central part of the colonies. At 10°–15°C., *C. grami-*

*neum* showed vigorous growth and formed bristles, and *C. acremonium* also pretty good growth. At 20°–24°C. *C. gramineum* formed white cotton-like hyphal mass and dark-colored, somewhat twistled bristles as shown Plate XXII, Fig. 7. At these temperatures *C. acremonium* made also pretty good growth.

In short, the above stated results of experiments showed that the temperature relations to the growth *C. gramineum* and *C. acremonium* were quite different, the minimum, optimum and maximum for the growth of the former species were 5°, 20° and 30°C., while those for the latter were 10°, 30° and 38°C., respectively.

## VII. Pathogenicity to the Wheat Seedlings.

*Experiment I.* On the slant of the malt extract agar, *C. gramineum* and *C. acremonium* were grown at 20°C. When the colonies covered the greater part of the surface of the slant, surface-disinfected wheat-grains were placed 20 grains in a test-tube. The test-tubes, thus sown, were kept at 5°, 10°, 15° and 20°C. After the wheat grains germinated and the seedlings attained to the length of 1–3 mm. the seedlings were transplanted to soil in pots. The pots used, were of porcelain and 18 cm. high and in diameter. They were filled with fertile soil and covered with paper and autoclaved at 20 pounds pressures for one hour. After transplanting the pots were kept in a glass house and the wheat plants were grown under special care. On May 6, 1937, the striped and the healthy plants were counted, and the infection percentage were calculated. The results are given in Table XI.

Table XI.

**Result of the Inoculation Experiment of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda on Wheat.**

The inoculated seedlings were transplanted into soil in pots.

| Temperature, at which the wheat grains were inoculated | | 5°C. | | 10°C. | | 15°C. | | 20°C. | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. measured | Percentage | No. measured | Percentage | No. measured | Percentage | No. measured | Percentage |
| | | | % | | % | | % | | % |
| *C. gramineum* | Strain No. 530 | 26:87 | 29.9 | 32:96 | 33.3 | 27:76 | 35.5 | 14:64 | 21.9 |
| | „ No. 1038 | 37:101 | 36.6 | 31:95 | 32.3 | 24:89 | 37.0 | 16:88 | 18.2 |
| | „ No. 1080 | 31:87 | 35.7 | 38:82 | 46.4 | 32:82 | 39.0 | 17:93 | 18.3 |
| | Sum | 94:275 | 32.9 | 101:273 | 37.0 | 83:247 | 33.6 | 47:225 | 20.9 |
| *C. acremonium* | Strain No. 1035 | 0:87 | 0 | 0:96 | 0 | 0:77 | 0 | 0:67 | 0 |
| | „ No. 1036 | 0:100 | 0 | 0:88 | 0 | 0:79 | 0 | 0:72 | 0 |
| | „ No. 1037 | 0:94 | 0 | 0:80 | 0 | 0:78 | 0 | 0:101 | 0 |
| | Sum | 0:283 | 0 | 0:264 | 0 | 0:234 | 0 | 0:240 | 0 |

According to the results given in Table XI, *C. gramineum*, all of three strains tested, was able to attack the wheat seedlings. The seedlings germinated at 5°-15°C. were attacked by the characterized stripe disease up to 30-45 per cent. In those germinated at 20°C., the percentage of the infected seedlings was much smaller than those germinated at low temperatures and was 18-20 per cent. On the contrary *C. acremonium*, all the strains studied, was not able to infect the wheat seedlings and to cause the stripe disease at all.

*Experiment II.* As stated in the previous experiment, wheat grains were surface sterilized and inoculated with *C. gramineum* and *C. acremonium*. The seedlings germinated were transplanted in the soil in the frame. The wheat plant were grown usually. On May 18, 1938, the wheat plants were studied and the number of the healthy and the striped culms were counted. The results are shown in Table XII.

Table XII.

**Result of the Inoculation Experiment of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda on Wheat.**

The inoculated seedlings were transplanted into soil in frames.

| Temperature, at which the wheat grains were inoculated | | 5°C. | | 10°C. | | 15°C. | | 20°C. | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. measured | Percentage | No. measured | Percentage | No. measured | Percentage | No. measured | Percentage |
| | | | % | | % | | % | | % |
| *C. gramineum* | Strain No. 530 | 40:146 | 27.4 | 46:127 | 36.2 | 6:33 | 18.2 | | |
| | „ No. 1038 | 62:147 | 42.2 | 82:130 | 63.1 | 39:81 | 48.2 | | |
| | „ No. 1080 | 69:179 | 38.5 | 43:138 | 31.2 | 24:85 | 35.4 | 7:40 | 17.5 |
| | Sum | 171:472 | 36.2 | 171:395 | 43.2 | 69:199 | 34.7 | | |
| *C. acremonium* | Strain No. 1035 | 4:172 | 2.3 | 10:147 | 6.8 | 8:137 | 5.8 | | |
| | „ No. 1036 | 6:143 | 4.2 | 14:141 | 9.4 | 4:129 | 3.1 | 0:48 | 0 |
| | „ No. 1037 | 4:141 | 2.8 | 7:139 | 5.0 | 3:85 | 3.5 | | |
| | Sum | 4:456 | 3.1 | 31:427 | 7.3 | 15:351 | 1.4 | | |

The results given in Table XII showed that *C. acremonium* were able to attack the wheat seedlings and cause the wheat stripe disease. On this point, considerations will be given later.

*Experiment III.* In the same way as stated above, the wheat grains were inoculated with pure cultures of *C. gramineum* and *C. acremonium*. They were kept at 5°, 10°, 15° and 20°C., respectively, and the germinated seedlings were, on December 16, 1937, transplanted into soil in the porcelain pots. The pots used were 15 cm. in diameter and five seedlings were transplanted in a pot. They were placed in the glass house and the wheat plants were grown in an ordinal way. On May 26, 1938, the results were studied, and the infection percentage was calculated. The results are given in Table XIII.

Table XIII.

**Result of the Inoculation Experiment of *Cephalosporium gramineum* Nisikado et Ikata and *C. acremonium* Corda on Wheat.**

The inoculated seedlings were transplanted into soil in pots.

| | Temperature, at which the wheat grains were inoculated | 5°C. | | 10°C. | | 15°C. | | 20°C. | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. measured | Percentage | No. measured | Percentage | No. measured | Percentage | No. measured | Percentage |
| *C. acremonium* | Strain No. 1035 | 0:7 | % 0 | 0:9 | % 0 | 2:8 | % 25.0 | 1:7 | % 14.3 |
| | „ No. 1036 | 1:8 | 12.5 | 1:8 | 12.5 | 1:9 | 11.1 | 0:8 | 0 |
| | „ No. 1037 | 1:10 | 10.0 | 2:8 | 25.0 | 3:17 | 17.6 | 0:4 | 0 |
| | Sum | 2:35 | 5.7 | 3:25 | 12.0 | 6:34 | 17.7 | 1:19 | 5.3 |
| *C. gramineum* | Strain No. 530 | — | — | 15:15 | 100 | — | — | — | — |
| | „ No. 773 | — | — | 7:9 | 77.8 | — | — | — | — |
| | „ No. 795 | — | — | 12:12 | 100 | — | — | — | — |
| | „ No. 796 | — | — | 16:16 | 100 | — | — | — | — |
| | „ No. 801 | — | — | 9:9 | 100 | — | — | — | — |
| | „ No. 1038 | — | — | 13:13 | 100 | — | — | — | — |
| | „ No. 1080 | — | — | 14:14 | 100 | — | — | — | — |
| | Sum | | | 86:88 | 97.8 | | | | |

According to the above given results of the second and the third experiments, all the strains of *C. gramineum* studied, attacked the wheat seedlings and caused the stripe disease at or near 100 per cent. As to *C. acremonium*, only a few of the wheat plant inoculated were infected by the stripe disease.

In the first experiment, undertaken in 1936–37, the wheat plants inoculated with *C. acremonium* were not affected by the stripe disease at all, in the second and the third experiments, they were attacked by the stripe disease. To ascertain if *C. acremonium* is able to attack wheat seedlings and cause the stripe disease, experiments were carried out. The striped culms of the wheat plants, developed from the grains inoculated with *C. acremonium* were cut into pieces, five culms being used from each lot. The cut pieces were then surface-disinfected and placed on agar plates, and the fungus colonies, appeared around the cut pieces, were studied. The fungus culture, thus reisolated, were certainly similar to those of *C. gramineum* and not *C. acremonium*, in the coloring of the colonies and temperature relations to the mycelial growth and the other characteristics. Therefore the reisolated cultures must be originated from the soil, which were contaminated with *C. gramineum*, or the seed grains, attacked by the fungus, and not from the inoculation.

In short, the results of these inoculation experiments showed that *C. acremonium* was not able to infect the wheat seedlings and to cause the stripe disease, although *C. gramineum* infected the wheat plants and caused the stripe disease.

## VIII. Conclusions.

The senior writer had, in 1933, found a new disease of wheat plant and named it "stripe disease". The causal fungus of the new disease was resembling with *C. acremonium* morphologically and pathologically. Especially the symptoms of the blackbundle disease of corn caused by *C. acremonium* resembled to those of the stripe disease of wheat caused by the writers' fungus. According to YOUNG (1926), *C. acremonium* was able to attack wheat seedlings when inoculated. However the both fungi were not similar in the following points: (a) The conidia of *C. acremonium* were somewhat smaller than those of *C. gramineum*. (b) The colonies of *C. acremonium*, grown on the media containing carbohydrates, were orange brown, while those of *C. gramineum* were colorless. Thus the causal fungus of the stripe disease of wheat plant had been ascertained to be new to science at that time, and a new name *C. gramineum* NISIKADO et IKATA was given. After than, Prof. WESTERDIJK kindly advised him to compare his fungus with *C. acremonium*, because the both fungi were somewhat resembling morphologically. She was so kind to send him the pure cultures of three strains of *C. acremonium*, secured from Holland, Germany and Italy, respectively.

The results of the comparative studies of the present writers on the both fungi in pure culture revealed that *C. gramineum* and *C. acremonium* were certainly different from each other chiefly in the following points: (1) the size of the conidia, (2) coloring of the colonies, (3) temperature relations to the fungus growth and (4) the pathogenicity to the wheat seedlings.

It is a wonder how the stripe disease appeared suddenly and caused such a serious damage to the wheat industry in the coast districts of the Inland Sea of Japan. As the disease is prominently systemic, very serious, and can not be confused with the other, it might have been detected and reported by Europian or American pathologists, if the disease occurred in Europe or in America. From the temperature relations to the growth of the present fungus, the disease could not be originated from tropical regions where the temperature is too high for the growth of the causal fungus of the stripe disease and also for the wheat growing.

In view of these facts it should be supposed that the causal fungus originated from some wild members of the grass family in Japan and attacked the wheat plant, as its cultivation increased greatly in recent years in Japan. Otherwise it may be suspected that the causal fungus was imported from the Asiatic Continent. The disease survey in the North China and Manchurian districts seems to be needed in order to solve the problems.

## IX. Summary.

1) The present paper deals with the comparative studies of *C. graminenm* NISIKADO et IKATA which causes the stripe disease of wheat plant, and *C. acremonium* CORDA.

2) The pure cultures of *C. acremonium* Corda were three strains, isolated in Germany, in Italy and in Holland, and sent to the senior writer from Prof. Westerdijk. And those of *C. gramineum* Nisikado et Ikata were isolated from diseased culms of wheat and *Alopecurus agrestis* L. in Okayama, Japan.

3) In the size of the conidia produced on the potato-dextrose agar at 20°C., *C. gramineum* Nisikado et Ikata was 4 – 12 $\mu$ long (1.22 $\mu$ in average), 1.0 – 3.3 $\mu$ wide (2.09 $\mu$ in average), while *C. acremonium* Corda was 3 – 11 $\mu$ long (5.67 $\mu$ in average), 1.0 – 3.0 $\mu$ wide (2.09 $\mu$ in average). Thus the conidia of the former species were much larger than the latter. Biometrically the differences in the average length of the conidia were 3 – 13 times of the errors, and those in the average width were 5 – 14 times of the errors.

4) The colonies of *C. acremonium* Corda grown on potato-dextrose agar under the diffused day light, showed orange yellow color, while those of *C. gramineum* Nisikado et Ikata showed no such coloring at all.

5) The minimum, optimum and maximum temperatures for the fungus growth of *C. gramineum* Nisikado et Ikata were 5°, 20° and 30°C., while those of *C. acremonium* Corda were 10°, 30° and 38°C, respectively.

6) *C. gramineum* Nisikado et Ikata was able to attack wheat seedlings and cause the characteristic, systemic, stripe disease, while *C. acremonium* Corda was not able to attack the wheat plant and to cause stripe disease.

7) As stated above, *C. gramineum* Nisikado et Ikata and *C. acremonium* Corda were quite different in the morphological, physiological and pathogenetic characteristics.

## X. References.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## Explanation of Plates XXI–XXV.

Figs. 1–6. Morphological comparisons of the conidia of *Cephalosporium gramineum* NISIKADO et IKATA and *C. acremonium* CORDA produced on the potato-dextrose agar after two weeks' culture at 24°C. (Magnification ×1000)

**Fig. 1.** Conidia of the strain No. 530 of *C. gramineum* NISIKADO et IKATA, isolated from wheat.

**Fig. 2.** Conidia of the strain No. 1080 of *C. gramineum* NISIKADO et IKATA, isolated from wheat.

**Fig. 3.** Conidia of the strain No. 1038 of *C. gramineum* NISIKADO et IKATA, isolated from a striped culm of *Alopecurus agrestis L.*

**Fig. 4.** Conidia of the strain No. 1035 of *C. acremonium* CORDA, isolated in Italy.

**Fig. 5.** Conidia of the strain No. 1036 of *C. acremonium* CORDA, isolated in Holland.

**Fig. 6.** Conidia of the strain No. 1037 of *C. acremonium* CORDA, isolated in Germany.

**Fig. 7.** Test-tube cultures of the strains No. 1035 of *C. acremonium* CORDA (A), No. 1080 (B) and No. 530 (C) of *C. gramineum* NISIKADO et IKATA, grown on the potato-dextrose agar for 2 weeks at 24°C., showing the characteristic growth, especially the formation of aerial hyphae and dark bristles.

Figs. 8–11. Test-tube cultures of *Cephalosporium gramineum* NISIKADO et IKATA and *C. acremonium* CORDA on the malt extract agar after 2 weeks culture at 10°, 20°, 27° and 30°C., respectively, showing the temperature relations to the mycelial growth.

(I) Strain No. 1080 of *C. gramineum* NISIKADO et IKATA, isolated from *Alopecurus agrestis L.*

(II) Strain No. 530 of *C. gramineum* NISIKADO et IKATA, isolated from wheat plant.

(III) Strain No. 1080 of *C. gramineum* NISIKADO et IKATA, isolated from wheat plant.

(IV) Strain No. 1035 of *C. acremonium* CORDA, isolated in Italy.

(V) Strain No. 1036 of *C. acremonium* CORDA, isolated in Holland.

(IV) Strain No. 1037 of *C. acremonium* CORDA, isolated in Germany.

**Fig. 8.** Fungus growth at 10°C.

**Fig. 9.** Fungus growth at 20°C.

**Fig. 10.** Fungus growth at 27°C.

**Fig. 11.** Fungus growth at 30°C.

Figs. 12–17. Morphological comparisons of the conidia of *Cephalosporium gramineum* NISIKADO et IKATA and *C. acremonium* CORDA produced on the potato-dextrose agar after 2 weeks' culture at 24°C. (×2000)

**Fig. 12.** Conidia of the strain No. 530 of *C. gramineum* NISIKADO et IKATA, isolated from barley.

**Fig .13.** Conidia of the strain No. 1080 of *C. gramineum* NISIKADO et IKATA, isolated from barley.

**Fig. 14.** Conidia of the strain No. 1038 of *C. gramineum* NISIKADO et IKATA, isolated from *Alopecurus agrestis* L.

**Fig. 15.** Conidia of the strain No. 1035 of *C. acremonium* CORDA, isolated in Italy.

**Fig. 16.** Conidia of the strain No. 1036 of *C. acremonium* CORDA, isolated in Holland.

**Fig. 17.** Conidia of the strain No. 1037 of *C. acremonium* CORDA, isolated in Germany.

# PLATE XXI.

Fig. 1.

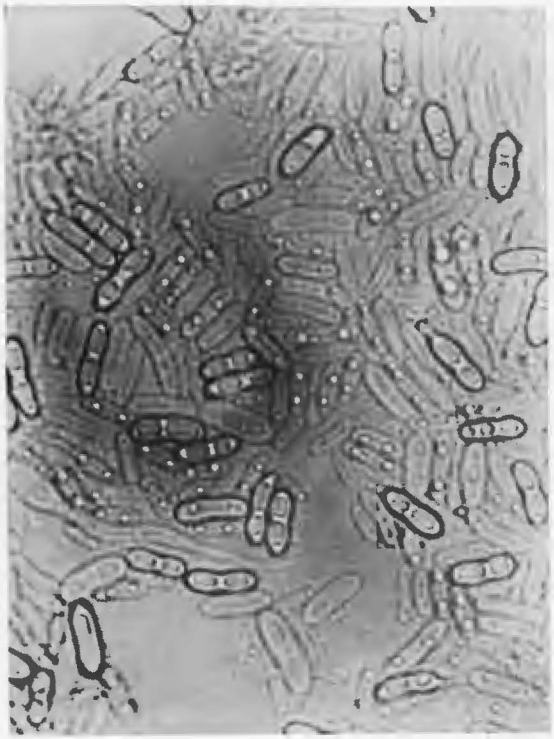


Fig. 2.

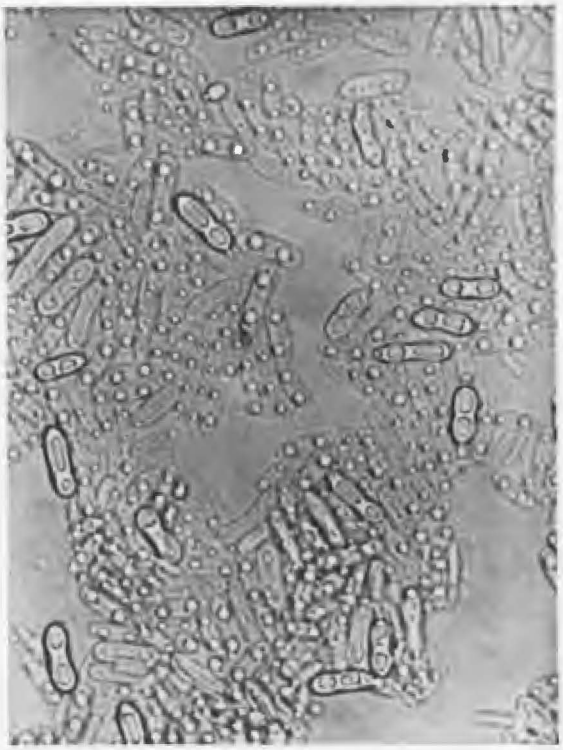


Fig. 3.

Fig. 4.

# PLATE XXII.

Fig. 5.

Fig. 6.

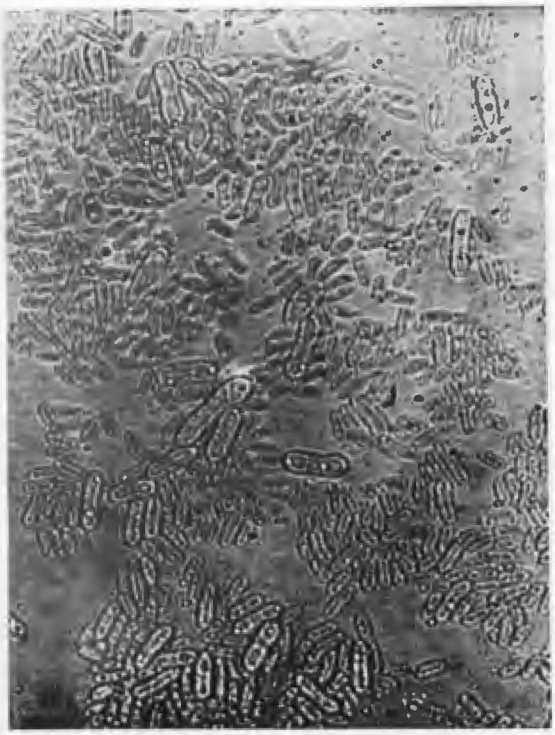
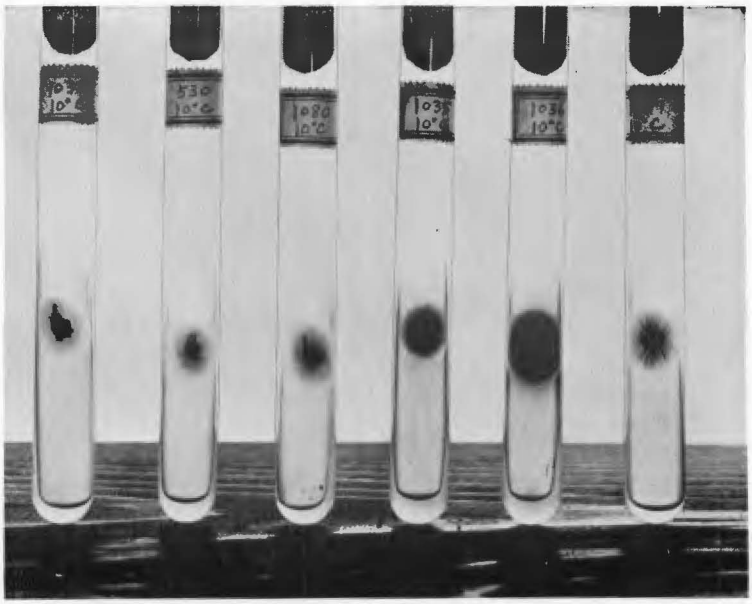


Fig. 7.

A B C

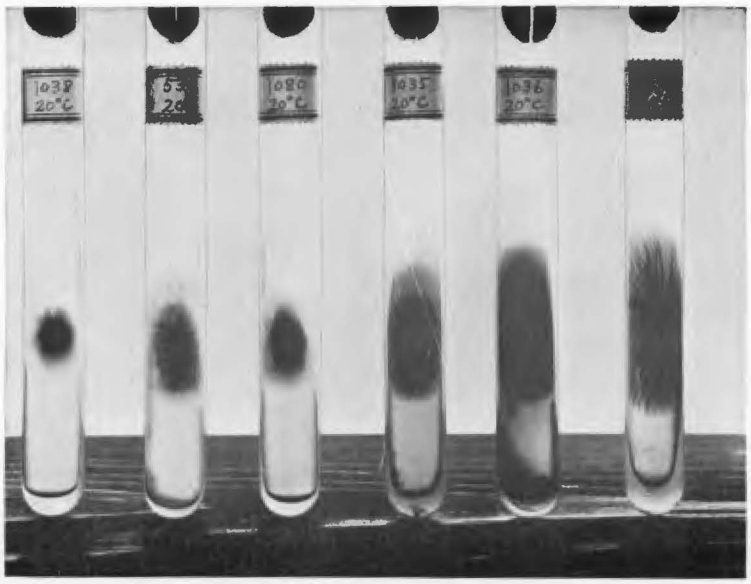
# PLATE XXIII.

**Fig. 8.**



I II III IV V VI

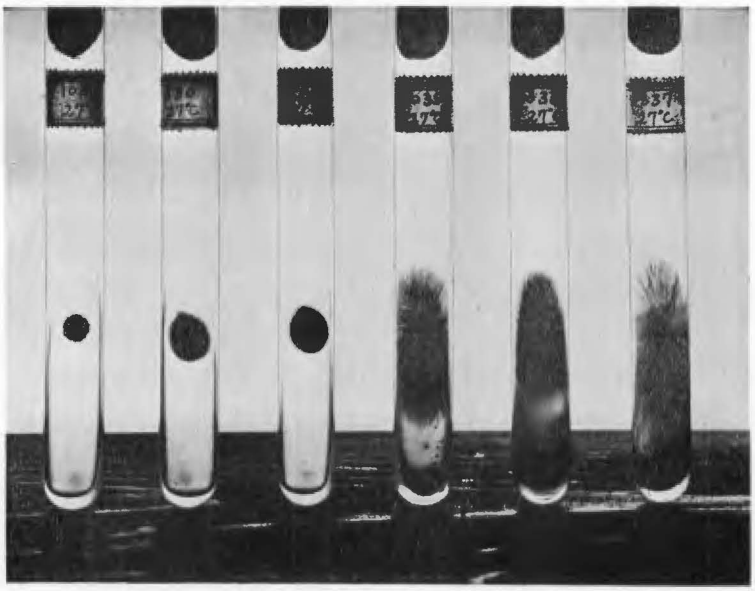
**Fig. 9.**



I II III IV V VI

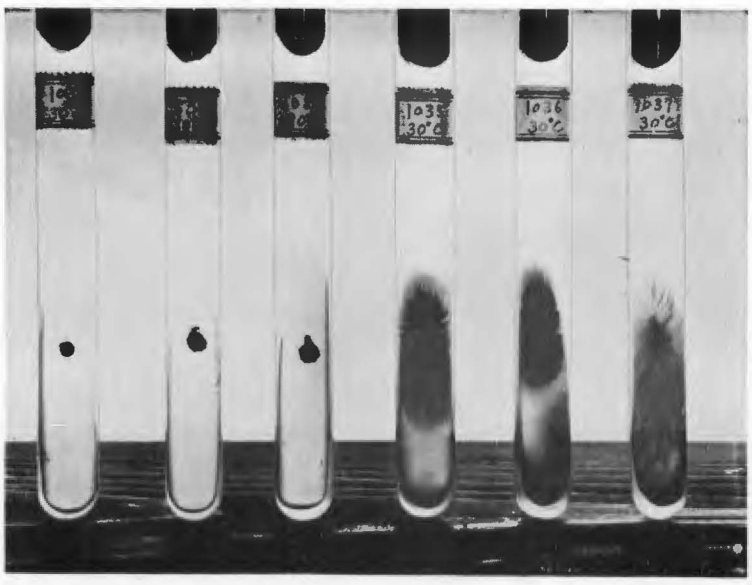
# PLATE XXIV.

**Fig. 10.**



I II III IV V VI

**Fig. 11.**



I II III IV V IV

PLATE XXV.

Fig. 12.

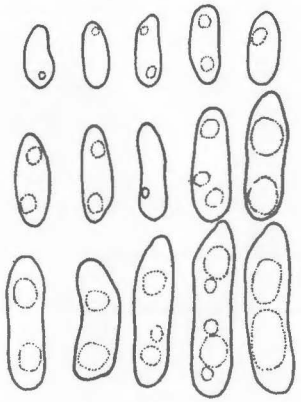


Fig. 13.

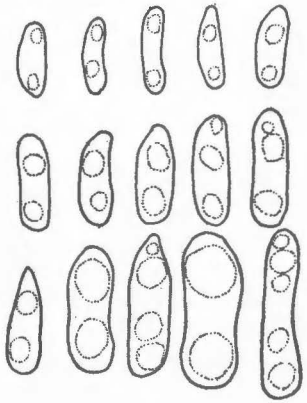


Fig. 14.

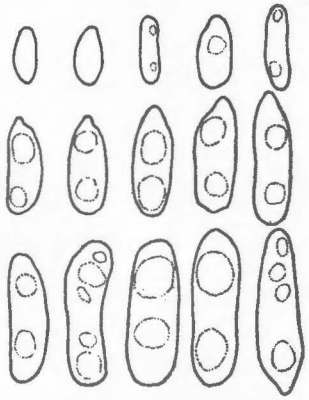


Fig. 15.

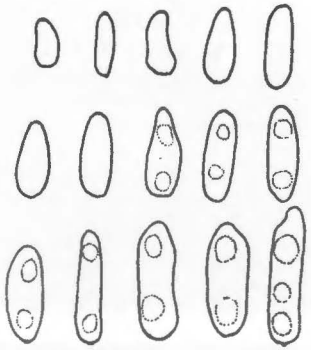


Fig. 16.

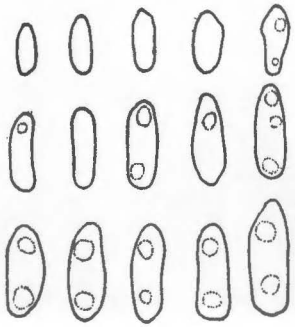


Fig. 17.