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IV, 10

## The translocation of some viruses in *Physalis floridana* RYDB.

### Introduction

As is stated by several authors (SAMUEL, 1934; CAPOOR, 1949) a sap-transmissible virus remains in the inoculated leaf for some time after the inoculation. Once the virus has entered the stem of the plant, a rapid translocation starts along the stem of the plant both up- and downwards. In a number of experiments we have found the same for potato virus X in potato plants (BEEMSTER, 1954). To obtain more information concerning the interval between inoculation and entering the stem, several experiments were carried out with *Physalis floridana* RYDB. as a host. This plant proved to be useful because it is easy to obtain a set of uniform test plants and because it is a host for several viruses. In our experiments the following viruses were tested: potato leaf roll virus, potato virus X, potato virus Y and turnip mosaic virus, which was described recently (BEEMSTER, 1957). In this paper some of the results obtained will be given.

### Material and methods

Potato leaf roll virus was maintained in *Physalis floridana* and was inoculated using *Myzus persicae* (SULZ.), ten on each plant. The plants had to be inoculated on a special leaf and for this reason it was necessary to cage the aphids as described by WALRAVE (1951). The aphids stayed on the test plants for 24 hours after which they were killed. The potato viruses X and Y and the turnip mosaic virus were maintained in *Nicotiana glutinosa* and were inoculated mechanically using carborundum as an abrasive.

The idea of the experiments was as follows: in order to determine the lapse of time between the introduction of a virus into a leaf and the translocation from that leaf to the stem of the plant, the inoculated leaves were removed at varying intervals after inoculation in a series of test plants. Conclusions regarding the speed of translocation from the leaf to the stem could easily be drawn by watching the symptoms produced in the plants.

The experiments were performed in aphid-free glass-houses at a temperature of about 22—24° C under natural light conditions in the months from April till October, in the other months supplementary light was given using high pressure mercury lamps. The inoculations were performed on the third true leaf of a plant. The sap-transmitted viruses were inoculated at about five o'clock in the afternoon. The inoculation feeding period for potato leaf roll virus started at 2—3 o'clock in the afternoon.

The trials were conducted over a period of fifteen months, comparing the rate of movement of all four viruses in almost each trial.

The symptoms, caused by the viruses, used in our experiments, are as follows:

**Potato leaf roll virus:** about 10 days after the inoculation an interveinal chlorosis in the younger leaves appears, which gradually becomes more and more pronounced. Moreover the plants become somewhat stunted.

**Potato virus X:** 8—10 days after the inoculation a weak vein-clearing is visible in the younger leaves, about 3 weeks after the inoculation the plants show a faint mosaic in the younger leaves of the plants.

**Potato virus Y:** 10—12 days after the inoculation a very pronounced vein-clearing is visible in the younger leaves. Some days later the veins of these leaves become fully necrotic and the leaves drop. About 12 days after the inoculation brown necrotic lesions appear on the inoculated leaves.

**Turnip mosaic virus:** 10—12 days after the inoculation small yellowish spots appear on the younger leaves, some of them becoming necrotic after some days. The younger leaves of the plants show some malformation but afterwards new leaves develop with only mosaic symptoms.

### Results and discussions

From October 1955 till January 1957 almost every month an experiment was carried out in which the lapse of time between inoculation and entering the stem was estimated for the four viruses, already mentioned. The results of these experiments are given in the tables 1, 2, 3 and 4 for potato leaf roll virus, potato virus X, potato virus Y and turnip mosaic virus, respectively.

From these tables we perceive, that fairly great differences exist between the experiments taken at different times. These differences, however, are apparently not caused by seasonal influences. From the data we obtained it is hard to say which factor caused these differences. In all experiments of the same day, however, the same results could be observed viz. leaf roll and virus X enter the stem at about the same time. Some hours later the turnip mosaic virus does so and virus Y enters the stem only about 24 hours later than leaf roll and virus X. To bring the total result of the experiments in one figure we have summarized all the data and estimated the percentages of diseased plants at certain intervals which are given in the last row of each

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**Table 1.**

The interval between inoculation of the leaf and entering the stem of potato leaf roll virus in *Physalis floridana*.

| Date of inoculation | Inoculated leaves removed after (hours) |           |             |             |             |             |
|---------------------|---|-----------|-------------|-------------|-------------|-------------|
|                     | 15—20                                   | 24—30     | 40—45       | 46—50       | 64—72       | 92—96       |
| 19. 10. 1955        | 0/5 <sup>1)</sup>                       | 1/4       |             | 1/3         | 3/3         |             |
| 20. 12. 1955        |   | 0/5       |             | 2/5         | 2/5         |             |
| 25. 1. 1956         |   | 1/5       | 0/5         | 1/5         | 2/5         | 4/5         |
| 21. 2. 1956         |   | 1/5       | 1/5         | 5/5         | 5/5         | 5/5         |
| 21. 3. 1956         |   | 0/5       | 10/10       | 5/5         | 5/5         |             |
| 24. 4. 1956         |   | 0/5       | 0/5         | 10/10       | 5/5         |             |
| 22. 5. 1956         | 0/5                                     | 0/10      | 3/5         | 5/5         |             |             |
| 26. 6. 1956         | 0/5                                     | 0/10      | 0/5         | 1/5         |             |             |
| 23. 7. 1956         |   | 0/5       | 2/5         | 4/5         | 10/10       |             |
| 19. 9. 1956         | 1/5                                     | 0/10      | 1/5         | 7/10        | 5/5         |             |
| 1. 10. 1956         |   | 1/10      | 5/5         | 10/10       |             |             |
| 30. 10. 1956        | 0/5                                     | 1/5       | 4/5         | 9/10        |             |             |
| 4. 12. 1956         | 0/5                                     | 1/10      | 1/5         | 2/5         |             |             |
| 7. 1. 1957          | 0/5                                     | 0/10      | 1/5         | 3/5         |             |             |
| Total Percentage    | 1/35<br>3                               | 6/99<br>6 | 28/65<br>43 | 65/88<br>74 | 37/43<br>86 | 14/15<br>93 |

<sup>1)</sup> Numerator: Number of diseased plants.  
Denominator: Number of inoculated plants.  
(Holds for tables 1 to 7).

**Table 2.**

The interval between inoculation of the leaf and entering the stem of potato virus X in *Physalis floridana*.

| Date of inoculation | Inoculated leaves removed after (hours) |           |              |              |             |            |
|---------------------|---|-----------|--------------|--------------|-------------|------------|
|                     | 15—20                                   | 20—25     | 40—45        | 46—50        | 64—72       | 92—96      |
| 19. 10. 1955        |   | 0/5       |              | 5/5          | 10/10       | 5/5        |
| 20. 12. 1955        |   | 0/4       |              | 4/4          | 4/4         | 4/4        |
| 10. 1. 1956         |   | 0/5       | 8/8          | 5/5          | 5/5         |            |
| 25. 1. 1956         |   | 0/5       | 0/5          | 1/5          | 5/5         |            |
| 21. 2. 1956         |   | 0/5       | 0/10         | 1/5          | 5/5         |            |
| 21. 3. 1956         | 0/5                                     | 0/5       | 7/10         | 5/5          |             |            |
| 24. 4. 1956         |   | 0/5       | 0/10         | 8/10         |             |            |
| 22. 5. 1956         |   | 0/5       | 0/10         | 3/10         |             |            |
| 26. 6. 1956         |   | 0/5       | 0/10         | 2/5          | 3/5         |            |
| 23. 7. 1956         |   | 0/5       | 2/10         | 0/5          | 5/5         |            |
| 7. 8. 1956          |   |           | 10/40        | 15/20        | 18/20       |            |
| 4. 9. 1956          |   |           | 10/10        | 5/5          | 10/10       |            |
| 1. 10. 1956         |   | 1/5       | 10/10        | 5/5          | 5/5         |            |
| 30. 10. 1956        |   | 0/5       | 0/10         | 0/5          | 5/5         |            |
| 4. 12. 1956         |   | 1/5       | 10/10        | 5/5          | 5/5         |            |
| 7. 1. 1957          |   | 0/5       | 10/10        | 5/5          | 5/5         |            |
| Total Percentage    | 0/5<br>0                                | 2/69<br>3 | 67/163<br>41 | 69/104<br>66 | 85/89<br>96 | 9/9<br>100 |

table and plotted for the four viruses together in fig. 1. In this figure it is clearly demonstrated that there is no difference in the rate of movement between leaf roll and virus X. The turnip mosaic virus enters the stem somewhat later, and virus Y shows a striking lag with the three forementioned viruses.

In the case of the leaf roll virus we must consider that the inoculation with virus may require several hours. The three other viruses are introduced at one operation, which takes only a few seconds. As is known, the leaf roll virus is persistent in its vector and it may take some hours before a plant is infected after putting the aphids on

**Table 3.**

The interval between inoculation of the leaf and entering the stem of potato virus Y in *Physalis floridana*.

| Date of inoculation | Inoculated leaves removed after (hours) |           |           |              |             |           |
|---------------------|---|-----------|-----------|--------------|-------------|-----------|
|                     | 40—45                                   | 46—50     | 64—67     | 68—72        | 92—96       | 120       |
| 19. 10. 1955        |   | 0/5       | 0/5       |              |             | 4/5       |
| 20. 12. 1955        |   | 0/5       |           | 1/4          | 4/4         |           |
| 27. 12. 1955        |   | 0/10      |           | 10/10        | 10/10       |           |
| 25. 1. 1956         | 0/5                                     | 0/5       |           | 1/5          | 5/5         |           |
| 21. 2. 1956         | 0/5                                     | 0/5       | 0/5       | 0/5          | 0/5         |           |
| 21. 3. 1956         | 0/5                                     | 0/5       | 1/5       | 5/5          |             |           |
| 24. 4. 1956         |   | 0/5       | 0/5       | 2/10         | 5/5         |           |
| 22. 5. 1956         |   | 0/5       | 0/5       | 0/10         | 1/5         |           |
| 26. 6. 1956         |   | 0/5       | 0/5       | 0/10         | 1/5         |           |
| 23. 7. 1956         |   | 0/5       | 0/5       | 0/5          | 1/10        |           |
| 7. 8. 1956          |   |           | 0/10      | 2/30         | 28/40       |           |
| 11. 12. 1956        | 0/5                                     | 0/5       | 0/5       | 2/5          |             |           |
| 7. 1. 1957          |   | 0/5       | 4/5       | 10/10        | 5/5         |           |
| Total Percentage    | 0/20<br>0                               | 0/65<br>0 | 5/55<br>9 | 33/109<br>30 | 60/94<br>64 | 4/5<br>80 |

**Table 4.**

The interval between inoculation of the leaf and entering the stem of turnip mosaic virus in *Physalis floridana*.

| Date of inoculation | Inoculated leaves removed after (hours) |              |             |             |             |              |
|---------------------|---|--------------|-------------|-------------|-------------|--------------|
|                     | 21—25                                   | 40—45        | 46—50       | 64—67       | 68—72       | 96           |
| 19. 11. 1955        |   | 0/5          | 0/5         | 5/5         |             | 5/5          |
| 20. 12. 1955        | 0/4                                     |              | 3/4         |             | 4/4         | 4/4          |
| 11. 1. 1956         | 0/4                                     |              | 0/4         |             | 1/4         | 4/4          |
| 25. 1. 1956         |   | 0/5          | 0/5         |             | 5/5         | 5/5          |
| 21. 2. 1956         |   | 0/10         | 0/5         | 0/5         | 3/5         |              |
| 21. 3. 1956         | 0/5                                     | 0/5          | 2/5         | 10/10       |             |              |
| 24. 4. 1956         |   |              | 2/5         | 5/5         | 10/10       | 5/5          |
| 22. 5. 1956         |   | 0/5          | 1/5         | 2/5         | 3/10        |              |
| 26. 6. 1956         |   | 0/5          | 1/5         | 3/5         | 8/10        |              |
| 23. 7. 1956         |   | 0/5          | 2/5         | 4/5         | 7/10        |              |
| 7. 8. 1956          |   |              | 9/40        | 19/20       | 18/20       |              |
| 30. 10. 1956        | 0/5                                     | 0/10         | 0/5         | 4/5         |             |              |
| 4. 12. 1956         |   | 4/5          | 5/5         | 5/5         | 10/10       |              |
| 7. 1. 1957          | 0/5                                     | 1/10         | 5/5         | 5/5         |             |              |
| Total Percentage    | 0/23<br>0.0                             | 14/105<br>13 | 40/83<br>48 | 61/75<br>81 | 51/68<br>75 | 23/23<br>100 |

them. From experiments we have learned that four hours after the beginning of the inoculation feeding period about 90% of the plants got infected. From this we conclude that, in fact, the leaf roll virus moves slightly quicker out of the inoculated leaves than might appear from fig. 1. Yet it can be stated that it takes about 24 hours for the leaf roll virus to pass from the inoculated leaf to the stem. From this it may be concluded that the leaf roll virus is not brought directly into the sieve tubes by the aphids, but into some other parts of the phloem, e.g. the companion cells.

The experiments demonstrate that the interval of time between inoculation and entering the leaf is specific for each virus. Before a virus enters the stem, some time is needed during which the virus, except possibly the leaf roll virus, has to be translocated from the parenchyma cells to the phloem.

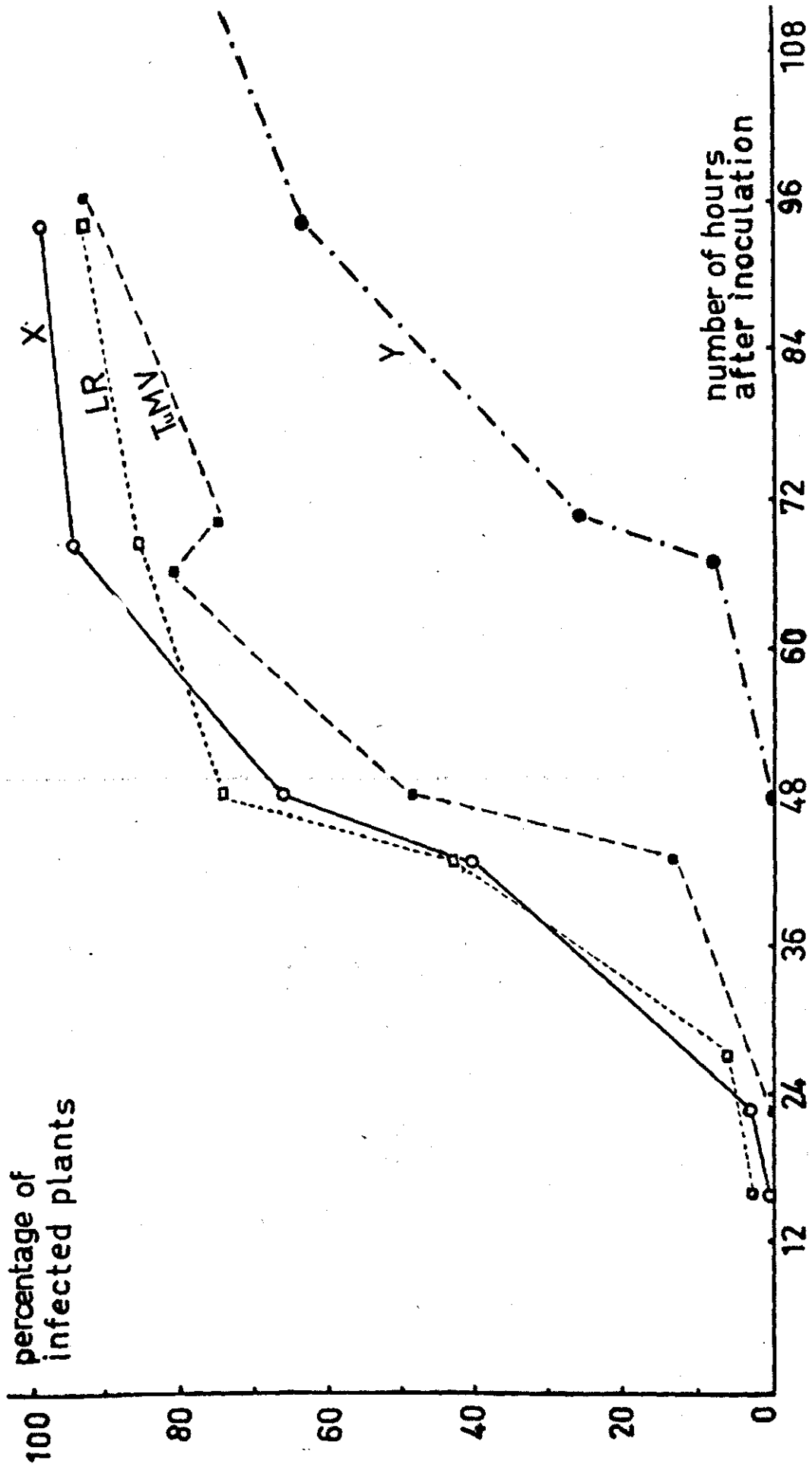


Fig. 1. The interval between inoculation of the leaves and entering the stem of potato leaf roll virus, potato virus X, potato virus Y and turnip mosaic virus. (Derived from the tables 1, 2, 3, and 4).

Table 5.

The interval between inoculation of the leaf and entering the stem of the potato viruses X and Y in *Physalis floridana*, when inoculated together.

| Date of inoculation | Inoculated leaf removed after (hours) |           |             |           |              |           |              |            |
|---------------------|---------------------------------------|-----------|-------------|-----------|--------------|-----------|--------------|------------|
|                     | 24                                    |           | 40—48       |           | 64—72        |           | 88—96        |            |
|                     | X                                     | Y         | X           | Y         | X            | Y         | X            | Y          |
| 19. 10. 1955        | 0/5                                   | 0/5       | 5/5         | 0/5       | 5/5          | 0/5       | —            | —          |
| 7. 11. 1955         | —                                     | —         | 10/10       | 0/10      | 5/5          | 0/5       | 5/5          | 1/5        |
| 1. 12. 1955         | 0/5                                   | 0/5       | 4/5         | 0/5       | 5/5          | 0/5       | 5/5          | 2/5        |
| 20. 12. 1955        | 0/5                                   | 0/5       | 5/5         | 0/5       | 5/5          | 1/5       | 5/5          | 5/5        |
| 18. 3. 1957         | 0/10                                  | 0/10      | 30/30       | 0/30      | 30/30        | 2/30      | —            | —          |
| Total Percentage    | 0/25<br>0                             | 0/25<br>0 | 54/55<br>98 | 0/55<br>0 | 50/50<br>100 | 3/50<br>6 | 15/15<br>100 | 8/15<br>55 |

Why this time is not the same for each virus, is not clear. It is possible that a certain rate of virus multiplication must be reached before translocation to the phloem occurs. The rate of virus multiplication for a given virus may depend on different conditions and in our experiments the virus-host relation may have been decisive. Perhaps in other hosts the differences found here might be different. On the other hand it is possible that the virus transport from cell to cell may depend on characteristics of the virus particles. By comparing the results in different hosts and of more viruses it is perhaps possible to find out which of the above mentioned possibilities is most probable.

#### Inoculation of one leaf with two viruses

In a number of experiments the viruses X and Y were inoculated together to find out whether the viruses have any influence on each other. The results of the experiments are given in table 5 and from this we learn that each virus passed from the inoculated leaf to the stem within the time that is normally needed. Here we have a method of separating virus X from a mixture of both virus X and Y. Maybe this method offers possibilities for separating virus mixtures which are hardly to separate.

#### Influence of darkening the plants

Some experiments with virus X were executed to investigate whether there is any difference between

plants kept in the dark from the inoculation till the moment of removing the leaf and plants kept under normal light conditions. The results of the experiments are given in table 6, which shows that in a number of plants the translocation of virus X is as quick as in plants under normal conditions. It seems, however, that the number of plants that become infected does not increase very much. Apparently the plants use up their stored material in the beginning but afterwards, when all those materials are exhausted, there is only little transport from the leaves to the stem.

#### Transport of virus X in plants already infected with leaf roll virus

Two experiments were carried out to find out whether the transport in plants, already infected with leaf roll virus, is delayed. We would expect this because leaf roll virus causes a necrosis of the phloem and this necrosis might delay the transport. In these experiments *P. floridana* plants were used which had been inoculated with leaf roll virus in a young stage about three weeks earlier; as a control plants of the same age were used, which were of course in a better state of health. The results of the experiments are given in table 7 and we see that virus X transport from the inoculated leaves to the stem is slightly slower in the case the plants are already infected with the leaf roll virus. Perhaps we can say that in the greater part of the plants

Table 6.

The interval between the inoculation of the leaf and entering the stem of potato virus X in *Physalis floridana* plants which are kept in the dark.

| Date of inoculation | Inoculated leaf removed after (hours) |           |              |              |             |             |             |             |
|---------------------|---------------------------------------|-----------|--------------|--------------|-------------|-------------|-------------|-------------|
|                     | 24—30                                 |           | 40—45        |              | 46—50       |             | 64—72       |             |
|                     | N*                                    | D         | N            | D            | N           | D           | N           | D           |
| 30. 1. 1956         | 0/5                                   | 0/5       | 0/10         | 0/10         | 0/5         | 0/5         | 4/5         | 0/5         |
| 6. 3. 1956          | 0/5                                   | —         | 4/10         | 3/5          | 4/5         | 3/5         | 4/5         | 2/10        |
| 27. 3. 1956         | 0/5                                   | 0/5       | 4/10         | 8/10         | 5/5         | 5/5         | —           | 9/10        |
| 30. 1. 1957         | 0/5                                   | 0/5       | 15/15        | 3/15         | 5/5         | 1/5         | 5/5         | 2/10        |
| 12. 2. 1957         | 0/5                                   | 0/5       | 15/15        | 2/30         | 5/5         | 4/5         | —           | 2/5         |
| 5. 8. 1957          | —                                     | —         | 40/40        | 20/40        | 20/20       | 7/20        | —           | —           |
| Total Percentage    | 0/25<br>0                             | 0/20<br>0 | 78/100<br>78 | 35/110<br>33 | 39/45<br>87 | 20/45<br>44 | 13/15<br>87 | 15/40<br>38 |

\* N = Normal light conditions.

D = Darkened from the inoculation till the removing of the leaf.

Table 7.

The interval between the inoculation and entering the stem of potato virus X in *Physalis floridana* after inoculation on healthy and on leaf roll diseased plants.

| Experiment number<br>Inoculated with leaf roll<br>Inoculated with virus X | 1                          |           | 2                         |           |
|---|----------------------------|-----------|---------------------------|-----------|
|   | 23. 2. 1956<br>13. 3. 1956 |           | 2. 5. 1956<br>25. 5. 1956 |           |
|   | Healthy                    | Leaf roll | Healthy                   | Leaf roll |
| Leaf removed after 24 hours   | 0/5                        | 0/5       | 0/5                       | 0/5       |
| Leaf removed after 40 hours   | 4/5                        | 2/5       | 0/5                       | 0/5       |
| Leaf removed after 44 hours   | 5/5                        | 1/5       | 2/5                       | 0/5       |
| Leaf removed after 48 hours   | 5/5                        | 0/5       | 2/5                       | 0/5       |
| Leaf removed after 64 hours   | 5/5                        | 5/5       | 4/5                       | 0/5       |
| Leaf removed after 68 hours   | 5/5                        | 4/5       | 5/5                       | 1/5       |
| Leaf removed after 72 hours   | 5/5                        | 3/5       | 5/5                       | 3/5       |
| Leaf removed after 88 hours   | 5/5                        | 5/5       | 5/5                       | 3/5       |

already infected with the leaf roll virus, virus X passes out of the inoculated leaves 24 hours later than in plants without leaf roll.

It is possible that this fact is caused by the necrosis of the phloem but on the other hand we have to keep in mind that leaf roll diseased plants having rather yellowish leaves are hampered in their physiological activities and this fact may influence virus multiplication or virus transport from cell to cell.

#### Summary

The rate of movement of four viruses out of inoculated leaves of *Physalis floridana* was compared.

It appeared that potato leaf roll virus and potato virus X entered the stem about 40 hours after the inoculation. The turnip mosaic virus did so some hours later and potato virus Y entered the stem about 36 hours later.

When virus X and virus Y were inoculated together on one leaf both viruses moved out of this leaf at the time they did when they were inoculated singly.

When the plants were kept in the dark, movement of virus X from the inoculated leaf was retarded in the greater part of the plants, but not in all.

When virus X was introduced in plants, already infected with leaf roll virus, the translocation from the leaf to the stem seemed to be retarded by about 24 hours.

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#### Diskussion

MELCHERS (Deutschland): Die Frage, ob die „Wanderungsgeschwindigkeit“ etwas mit Diffusion oder einem

anderen Leitungsmechanismus oder aber mit der Vermehrungsprobe zu tun hat, kann doch durch Bestimmung der Konzentration in verschiedenen Teilen der Pflanze zu verschiedenen Zeiten geklärt werden. Wurden solche Versuche schon begonnen?

BEEMSTER: Versuche zur Konzentrationszunahme sind für das X-Virus mittels Lokalläsionstest an *Gomphrena globosa* durchgeführt worden. Da die Resultate nur vorläufige waren, wurde darüber nicht gesprochen. Für das Blattrollvirus war eine Konzentrationsbestimmung bisher unmöglich. Es wäre wichtig, gerade hierüber Naheres zu wissen.

SILBERSCHMIDT (Brasilien): I asked about the occurrence of local lesions on *Physalis floridana*, according to A. F. ROSS, by potato virus Y. The reaction of this species to potato virus Y depends very much on external conditions.

BEEMSTER: Under the conditions used we always got rather the same symptoms with potato virus Y on *Physalis floridana*. As described the temperature was rather the same in all experiments as well as the stage of growth at the time of inoculation of the test plants. The light intensity may have been different in the different experiments due to daily and seasonal fluctuations in this respect.

MUNDRY (Deutschland): Haben Sie Erfahrungen über den Einfluß der Temperatur auf die Ausbreitung und die Symptomausprägung bei X- und Y-Virus?

BEEMSTER: Experimente in dieser Hinsicht wurden nicht durchgeführt.

NIENHAUS (Deutschland): Die Ausbildung der Lokalläsionen auf *Physalis floridana* nach der Infektion durch Y-Virus ist temperaturabhängig, und zwar konnte ich nur Läsionen beobachten bei konstant 16° C, während sie bei 18° C und höher nicht mehr deutlich auftraten. Auch die Temperatur vor der Inokulation hat einen entscheidenden Einfluß auf die Empfänglichkeit, ebenso eine Dunkelperiode, deren Effekt durch kurze Lichteinflüsse wieder rückgängig gemacht werden kann. Alle Versuche müssen also unter konstanten Bedingungen durchgeführt werden, wenn ein Vergleich mit anderen Viren angestellt werden soll.

BEEMSTER: In unseren Versuchen wurde immer mit unverdünntem Preßsaft und unter gleichen Temperaturbedingungen gearbeitet. Ebenso war es für unsere Versuche ohne Interesse, ob es Lokalläsionen gab oder nicht. Wichtig war, daß die Inokulation gelang. Ob ein Zusammenhang zwischen dem Auftreten von Lokalläsionen und der Wanderungsgeschwindigkeit des Virus aus dem inokulierten Blatt besteht, ist eine interessante Frage, die experimentell gelöst werden soll.