A simplified serological test for the determination of virus X in potato plants

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(wpłynęło 21. IV. 1950)

Virus X is widely spread in potato cultures, being probably responsible for larger losses in potato crops than any other virus.

The control of this virus is not easy, as the symptoms of the disease are often masked. It is difficult therefore for the farmer to identify and to remove the infected plants from the field.

Since the discovery of the possibility of detecting the presence of this virus by serological methods, many workers in different countries have attempted to apply them for selection of virus-free potatoes.

In U. S. A. C h e s t e r (1937a) suggested the following test which can be carried out on a larger scale: 2 ml of crude, extracted sap are mixed with 2 ml of dilute antiserum in a test tube and left for some time. The results are read after 15 min. and 60 min.

As far as I know, the method has not become popular, probably because of the large amount of serum needed and difficulties in reading the results.

D u n i n and P o p o w a (1937) in U. S. S. R. developed a drop technique (kapelnij metod). The method consists in mixing 1 drop of crude plant sap with 1 drop of dilute antiserum on a glass slide. Aggregation of chloroplasts shows the presence of the virus. The procedure is quick and easy. It has a practical application in Holland, where v a n S l o g t e r e n (1945) uses this method with some modifications, for serological testing of potatoes on a large scale.

Although this method seems to be very useful, it has still some disadvantages. Handling large amounts of glass slides is fairly

tedious, and simultaneous examinations of the test and the control are difficult and consuming much time, as the observations are usually performed with the use of a microscope.

In Czechoslovakia Jermoliew and Hruska (1939) applied the drop technique, using for test the sap which was previously clarified by heating for 10 min at 55 centigrades and centrifuging off the clotted material. Observations are performed under microscope, with the help of dark field illumination. This technique has been further developed and applied in Germany by Stapp (1943), in Belgium by Rolland (1945) and in France by Limasset (1947).

The discussed method is sensitive enough to detect not only the presence of virus X, but also that of other potato viruses e. g. the viruses A, Y and G, provided suitable antisera are used. Unfortunately the method requires also some expensive laboratory equipment, a large power-driven centrifuge being indispensable for handling numerous samples.

In England Markham a. a. (1948) proposed the tube precipitation technique. The sap clarified by heating and centrifuging is mixed with dilute antiserum on a water bath. The authors propose an application of group testing, which makes possible an examination of samples from 10—20 plants in one test-tube.

Although this method is fairly laborious for testing single plants, it seems very useful for group testing and applicable in this way for field control.

In Poland the first to apply the serological technique for virus X determination was Kozłowska (1946), using the complement fixation test.

It seems obvious from the above review that in many countries there is a search for a serological technique suitable for a general application. All the methods devised so far failed to meet all the requirements.

The author has attempted therefore to work out a method simple enough to be used with poorest laboratory equipment, and on the other hand reliable enough to warrant the correct diagnosis.

The work has been started, at the Plant Virus Research Unit at Cambridge [1] and continued at the Potato Research Station (Badawcza Stacja Ziemniaczana S. G. G. W.) in Żelazna.

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[1] The author received there a course in research in 1948 having obtained a scholarship from the Polish Ministers Commitee for studies on plant virus diseases.

The drop method of D u n i n and P o p o w a (1937) has been the starting point. As the handling of glass slides seemed to be of little convenience, the author uses instead of them porcelain spot plates with 12 depressions (Fig. 1).

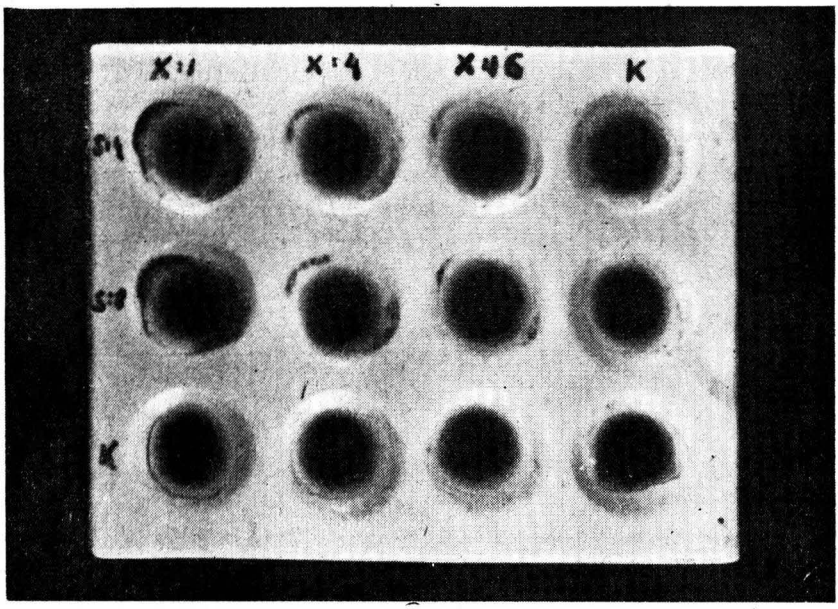

Fig. 1.

The detection of virus X in leaves of infected tobacco. From the left follow: undiluted sap, sap diluted 1:4, sap diluted 1:16 and sap of healthy tobacco used for preparing the dilutions.
Each test is performed with X-antiserum diluted 1:4 (the uppermost longitudinal row), X-antiserum diluted 1:16 (the central row) and „healthy serum" diluted 1:4 (the lowest row).

Drops of crude sap when mixed on the spot-test plate with the antiserum show a typical agglutination in case the sap is from virus-infected plants.

It has been found later that slow balancing of the plates induced the clotted material to aggregate at the edges of the drop-mixture. The material aggregates in the same way also in cases when the agglutinated particles are too small to be observed in the fluid with naked eye.

To prevent the drying out of the examined mixture the plates are covered with a small glass pane, or, if several plates are used at once, they are put one upon another, the top-most one being covered with a glass pane.

The tests are performed as follows:

The crude sap from the collected leaf samples is extracted by means of a mortar or with special forceps, described by Stapp (1943).

At least 10—15 drops of sap are desired. The sap from each sample is collected in a separate small test tube.

Before each subsequent extraction the forceps (or mortars) are cleaned by dipping them into water, removing the green residue and wiping with a dry towel. These precautions are sufficient as the method is not sensitive enough to detect very small amounts of the virus.

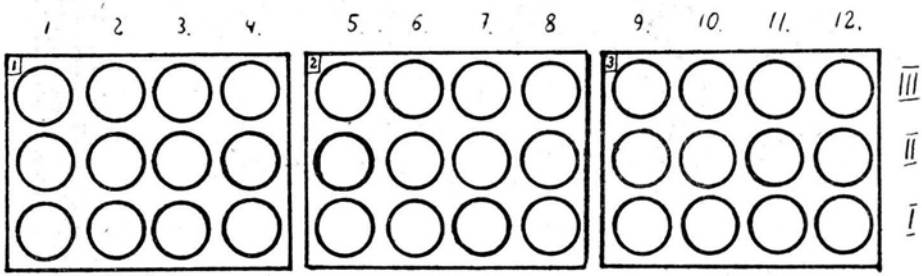

Fig. 2.
Arrangement of the spot plates. 1—12 Arab figures show single tests. I—III Roman figures show tests treated respectively: I with „healthy serum", and II and III with X-antiserum in two different dilutions.

For one test we usually take 3—4 clean, dry plates and arrange them in the order presented on Fig. 2. Each plate has its own number.

3 drops of the tested sap are taken by means of a pipette and put into each of the three depressions of the first transverse row. The pipette should not be put too deep into the sap, as some plant fragments settle down on the bottom of the test tube. The size of the drops may be various. The author usually used pipettes which gave 25—35 drops from 1 ml.

After filling the first row, the pipette is cleaned by rinsing it 3—4 times with water, and then the sap is taken from the next test-tube and the second row filled.

When all the 12 samples have been taken, we use another pipette for adding the antiserum. The applied dilution of the anti-

serum depends on its strength. The author used commonly rabbit-antiserum prepared by 3 injections of 1,5—2 ml. of clarified infective tobacco sap in weekly intervals. Such antiserum has been used in dilutions 1:4 and 1:8. „Healthy serum" was used for the controls; it was obtained from rabbits immunised in the same way against the sap of healthy tobacco, and diluted 1:4.

At first we put one drop of „healthy serum" into each of 12 depressions of the Ist longitudinal row (Fig. 2). In the same way antiserum 1:8 is added to the depressions of the IInd row, and antiserum 1:4 to the IIIrd row.

After adding the antiserum the pipette is throughly [2] cleaned by repeated rinsing it with physiological solution, whereupon we start at once to shake the plates to avoid the drying out of the fluid.

Each plate is shaken separately until the mixtures look quite homogeneous. The plates are then put one upon another, the topmost one is covered with a glass pane, and the set-up is balanced slowly for 5 min.

Fig. 1. illustrates the results of virus X detection in a tobacco plant, infected with this virus.

The undiluted sap (first on the left), sap diluted in healthy sap 1:4 and 1:8 respectively, and the sap of healthy tobacco, used for control, are analysed for the presence of the virus. Each test sample is mixed with 2 different dilutions of antiserum, „healthy serum" serving again as a control.

The undiluted sap produces symptoms usual for high concentrations of the virus. The diluted sap produces symptoms common in low concentration. No symptoms can be seen in the controls. The narrow edges, which may be seen in Fig. 1 in the control tests are of a quite different nature and are brought about by slight drying out of the fluid. As it may sometimes obscure the results, the plates with the samples should be always kept covered with glass panes, eventually moistened by slight breathing on them.

The tests have been always performed at room temperature, and it seems, that the serum, after being brought from the cold, should be kept for some time at that temperature before being used for testing.

To evaluate the suggested method several experiments have been made to test the sensibility of the method, its reliability and the time needed for performing the tests.

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[2]) Instead of thorough cleaning different pipettes may be used for „healthy serum" and for the antiserum.

The sensibility of the method has been tested by examining series of dilutions of the sap of infected plants, the sap being diluted in the sap of healthy plants to avoid the change of the concentration of chloroplasts.

Several tests of this kind indicate that the virus can be detected in the leaves of infected potato plants up to a dilution 1:8 to 1:32, and in diseased tobacco sap up to a dilution 1:16 to 1:64.

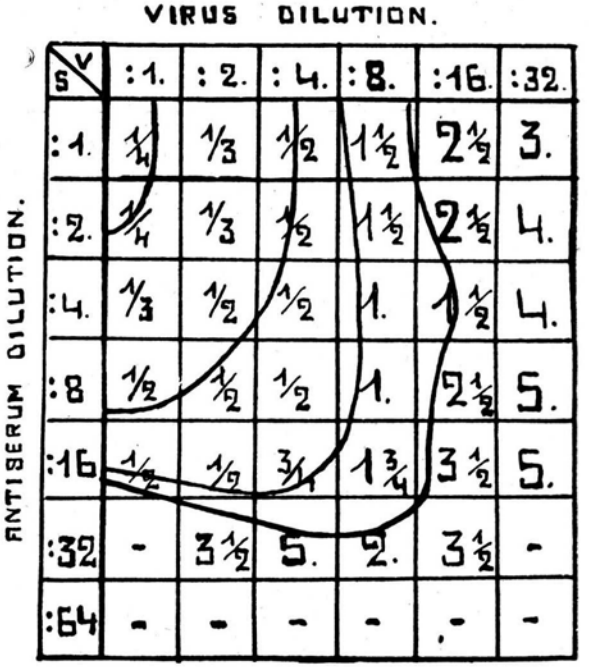

Fig. 3.

The rate of appearance of symptoms while using various dilutions of infective sap (extracted from potato leaves) and various dilutions of X-antiserum. The time of appearance is noted in minutes and the curves connect places where the symptoms appear after: 1/4 min, 1/2 min, 1 min, and 2 min. respectively.

Fig. 3 presents the rate of appearance of the symptoms while using various dilutions of the antiserum. This figure presents one of many experiments of this kind performed on various plants.

The sensibility of this method has been compared with that of other methods.

On the whole, the testing of clarified sap in test-tubes, proved to be considerably more sensitive than the suggested method.

On the other hand, the drop method of Dunin and Popowa proved to be slightly less sensitive, the dilution end point of infective potato sap for this method being usually 1:4 to 1:16.

As the reliability of the method is concerned, it has to be pointed out that three tests are made for each plant, viz. two tests with the specific X-antiserum and one with control serum.

Simultaneous observations can be made on all three tests, what makes the comparing much easier.

The test is considered positive if both tests with the specific antiserum give a clear positive result, and the control one produces no aggregate. The test is negative if all three tests do not show any aggregate. If only slight symptoms are obtained with the specific antiserum, or only one test with antiserum gives a positive reaction, or a slight aggregation in the control test is noted, the test is doubtful and must be repeated. By proper work, the repetition often does not solve the problem, the result remaining doubtful owing to the properties of the sap.

The sap agglutinating spontaneously is often a great nuisance, bringing about a similar aggregate in the control test, as in the specific antiserum test and making the reading of the results impossible. The same fact has been reported by other workers: Chester (1937b), Muskin (1942). In the last season about 15% of samples tested at our station agglutinated spontaneously. The author's observations seem to indicate that the way of taking the sample from the plant is of great importance. Young, but already developed leaves are most suitable, as the sap from mature leaves, though they look green and healthy, often clots spontaneously. On the other hand very young leaves are also undesirable, the virus concentration being considerably smaller in such leaves.

The suggested test may be performed fairly quickly, the greatest amount of time being needed for extraction of sap from the leaf samples.

The samples being collected, one person with an assistant to extract the sap, can make more than 200 single tests daily.

The amount of serum needed is not high. Supposing, a rabbit gives 50 ml of antiserum and 1 ml consists of 30 drops and we use the dilutions 1:4 and 1:8, serum from one rabbit is enough for 4000 tests.

During the last season more than a thousand tests were performed by the use of this method with satisfactory results.

The method is designed primarly for field work.

For experimental work it seems of little value, being considerably less sensitive than the precipitation methods.

The method is best suited for testing leaf samples from plants growing in the field or in the glasshouse. If needed, the samples may be sent by post for testing. A few experiments have been performed with sprouting potatoes in the spring. The results seem to indicate that the method is not adapted for testing small sprouts or very young leaves. Only when the leaves of the young plant reach the length of 5—8 cm, and the leaf blades the diameter of 3—4 cm, it is possible to make reliable tests.

The method is simple enough to be used in most primitive conditions. It must be warned however that, as all serological methods, it is a delicate one. Only minute, careful and clean work may warrant satisfactory results.

I am very much indebted to Prof. E. Malinowski and Dr Kenneth, M. Smith for advice, encouragement and providing facilities, which made possible the work on this problem.

I am also much obliged to Dr J. Szuleta for help in preparing Fig. 1, to Mgr. M. Zamojska for preparing Fig. 2 and , and to Mgr. K. Raczyńska-Bojanowska for stylistical correction of the text.

SUMMARY

A simple technique suitable for detecting virus X in potato plants is described.

The test is based on mixing a drop of crude sap, extracted from a leaf sample, with a drop of dilute antiserum on a porcelain spot test plate. The mixing is performed by shaking, and then the plate is slowly balanced. After 5 min. the results are read. Green aggregate appearing on the edges of the mixture indicates the presence of the virus. When no virus is present, the mixture remains transparent and no aggregate is visible.

The proposed method may be very easily performed requiring neither a microscope nor a power-driven centrifuge nor any expensive equipment.

The sensibility of the method is greater than that of other agglutination methods, although it is below the sensibility of the precipitation methods.

The tests are performed quicker than by any other method, tried by the author, and the ease of carrying out parallel control tests make the results reliable.

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