MAINTENANCE OF VIRUS X-FREE POTATOES[1]

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The relative importance of potato virus X has been much increased of recent years by the efficient control of potato virus Y and the leaf roll virus. According to Bawden *et al.* (5), virus X now probably causes more loss of the crop than the other two combined. Bald and Norris (4) also claim that the losses from potato virus X in Australia are as serious as the losses from all other viruses combined.

Although potato viruses Y and A may produce faint or barely distinguishable symptoms in certain varieties, it is generally the presence of potato virus X in potato stocks that creates doubt and uncertainty for the potato inspector and the experienced grower. Bald (1) suggests that those indefinite differences between plants within a crop are usually caused by a strain of virus X.

REDUCTION IN YIELD

It has been proven to the satisfaction of workers in several countries that mild strains of virus X can reduce the yield of potato crops. According to Bald (1) a loss in yield is inevitable when a plant becomes infected with a virus disease of the mosaic type, and in Australia he found that masked strains of virus X could reduce the yield of a crop by 12 per cent. Stapp (32) estimated the annual average reduction in yield in Germany from this source, as 10 per cent. In America, Schultz and Bonde (28) reported that from their experiments the loss in yield from virus X ranged from 9 to 22 per cent, and they recommended that varieties immune to virus X be developed. Smith and Markham (31) in England inoculated several varieties with a very mild strain, infection produced no leaf symptoms, but the yield of the inoculated plants was 12 per cent less than the controls.

Despite the evidence from many sources that mild strains of virus X significantly lower the yield of a potato crop, Clinch and McKay could not obtain that confirmation in their work (8,9).

They (8) carried out yield trials on the effect of mild strains of virus X, on two clones of the variety Up-to-Date. These results, on the whole, were indeterminate and they concluded that the reactions of different clones of the same variety to mild strains of virus X were variable.

The variety used by Clinch and McKay is a very late maturing one and the plants of most varieties that die down from early September onwards, in Ireland and in the British Isles, do so prematurely from the effects of *Phytophthora infestans* (Mont.) de Bary. Scott (36) found that the presence of a virus in a potato plant induced early ripening, and Bald

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(3) observed that when immature virus X-infected plants and virus X-free plants were harvested at the same time, there was no difference in yield. When, however, they were harvested after they had died down naturally, the X-free plants yielded significantly higher than the X-infected plants. When a crop with simple mosaic and a healthy contiguous crop of the same variety are infected simultaneously and then destroyed with late blight, the bulking of tubers within the virus infected crop is further advanced towards the ultimate yield than is the healthy crop. Muller and Munro (21) also found that the retarded fructification of late blight spores on virus infected foliage was such that an epidemic would move faster through a virus-free crop than through a virus-infected crop of the same variety growing under similar conditions. This quicker kill of the foliage, and the slower bulking of tubers in the virus-free plants would tend to cancel out any increased weight of crop over one with a slight or mild disease caused by a virus infection.

Strains of Potato Virus X

Careful roguing of seed potato crops over the past three decades seems to have eradicated most of the naturally occurring strains of potato virus X that cause severe diseases. The recent work on the effect of virus X on yield has been carried out largely with strains that caused a mild or no apparent disease on the varieties used. However, there is evidence that severe strains still occur naturally in the field. Ladeburg *et al.* (16) found and MacLeod (17) reported several virulent strains occurring naturally and commonly in potato crops. Clinch of Ireland (7) and several of her colleagues found a strain of X in several parts of the country that produced symptoms on certain varieties of potatoes typical of those caused by a severe strain of potato virus Y. This may be partially explained in that the effect of one strain upon a variety is not necessarily an indication of its effect on all varieties. Roberts *et al.* (27) could find no evidence that masked strains underwent rapid conversion into severe strains. Hutton (13) thought that separation of strains by potato seedlings may explain the origin of masked strains commonly found in potato varieties. It may also provide an explanation for the occurrence of apparently new virulent strains in relatively new varieties of potatoes which are susceptible to virus X (15). Hutton and Peak (14), suggest that in evolution it is possible that avirulent strains preceded virulent strains, and that avirulent strains could remain undetected in certain hosts until transferred to a host in which it reacts as a severe strain. But even in the same variety certified as free from visible virus diseases in one year, it is commonplace to find an appreciable number of plants showing simple mosaic in the following year. This is interpreted (5) as the result of the segregation of a strain more virulent to that variety from the mild one dominating the stock. On the whole, constant roguing has tended to eliminate those strains that cause obvious leaf symptoms in most of the popular varieties, but the symptoms induced in a potato variety or a seedling by any one strain should not be expected to follow the same general trend in all varieties.

The Need for Virus-Free Potato Stocks

That there is a need for X-free potatoes in many countries is evident by the sustained popularity of certain old varieties, and the constant demand for them in the higher seed grades. New varieties are being introduced yearly with justified claims to disease resistance coupled with good quality and heavy cropping propensities, yet the older varieties like King Edward and Majestic in Great Britain, Up-to-Date in Australia and Green Mountain in America still retain their old popularity despite the difficulties of finding these varieties, even in small quantities, in a virus-free state. Many years ago Salaman exclaimed that he didn't really know what made a variety popular in the eyes of a grower, as many of the traits of the most popular varieties seemed so much at variance with the accepted qualities desired. Up-to-Date and King Edward in Britain are the two varieties more susceptible to blight than any other varieties grown there. Majestic, the most popular variety among growers in that country seems to have little or no resistance to any of the common potato viruses, yet there have been more demands for virus-free Majestic than for all other varieties put together.

The often suggested plan to infect a stock with a mild strain to prevent further infection is not a substitute for virus-free stocks. It has been assumed that potatoes infected with a mild type of virus X are protected against infection with a severe strain, but investigations by Ladeburg *et al* (16) have clearly demonstrated that this is not always the case, and work by Matthews (19) indicated that he also had found like results. Obvious single stem infections of mild or severe strains on otherwise healthy looking plants occur perhaps more frequently than is appreciated. It seems that until we are able to produce varieties that are field-immune (hypersensitive) or immune from virus X, and which are also acceptable to the growers, there will be a need to maintain or to raise virus-free stocks of potatoes.

Perhaps even more important than the maintenance of old varieties in a virus-free state is the need to raise and maintain promising potato seedlings free from virus infection. Seedlings that are raised as late blight resisters and with other highly desirable qualities are too valuable to risk exposure to infection with virus X in the early stages of their multiplication. No matter how desirable in most respects, a new variety should not be released in a condition that reveals any of its weaknesses, however slight they are. It is difficult to observe a newly introduced and boosted variety with an unbiased eye. Claims of many desirable features within a variety are often taken to mean an absence of all weaknesses, and it is possible to multiply a seedling up to the stage of release in a virus-free state if care is taken. Seedlings may be raised and multiplied in a virus-free condition without difficulty if infected breeding material is kept in isolation or off the station altogether. Roberts (26) reported that of the many thousands of plants that have been raised in isolation plots as virus-free since 1946, in only one case has virus-X been found. Old varieties that are used as controls or late blight infectors should be either virus-free or field-immune to virus X.

Variations

Ladeburg *et al.* (16) thought that the chief disadvantage of such an undertaking as raising virus-free stocks would be the difficulty of preventing infection during the period of increase and the constant renewal of virus-free stocks for commercial seed growers; whereas the real difficulty lies largely in the replenishment of new selections of virus-free material from commercial stocks to replace the virus-free stocks that are changing from desirable to less desirable types. Experience has shown that it is much more difficult to obtain and maintain a desirable type of a variety free from variations within that variety than it is to keep it virus-free. Dr. Salaman's nucleus stocks of virus-free varieties, now in the care of the National Institute of Agricultural Botany, Cambridge, were collected during the nineteen-thirties. Replacements of certain stocks within this collection began in 1948 shortly after it was taken over from the Plant Virus Research Station at Cambridge. At this time there were three distinct clones of the variety Majestic in the collection that had been multiplied continuously under glass for many years. These clones were wholly free from virus but when grown under conditions of good potato culture, not only were there obvious foliage differences between clones, but there were also marked differences in cropping capacities in favor of a good commercial type that had been recently introduced. Their foliage differences were largely an appearance of lowered vigor with reduced size and slight distortion of the leaflets.

When Bald (2) made his virus-free selections of Up-to-Date he had thirteen clones. Of these thirteen clones one was discarded because of bolter characteristics, and there were definite agronomic differences amongst the other twelve. Roberts (25) made selections only from the most desirable types of his chosen varieties when he started his virus-free propagation scheme in Perthshire, Scotland.

Scott's work (36) with a feathery variation of the variety Kerr's Pink showed that this healthy variation gave a yield in total weight of 21.5 per cent less than the normal commercial type, but 84 per cent less in weight of tubers that would not pass through a two-inch square mesh. The variety Kerr's Pink was introduced in 1917 and about twenty years later there were at least twenty known variations from the desirable type of that variety. Scott also reported (36) that wildings and other variations may reduce a yield from 50-95 per cent of salable produce.

The investigations that have been made to determine the frequency with which variants arise in pure stocks have shown that in Arran Pilot, one of Britain's most popular early varieties, about three in every thousand normal plants annually assume the semi-bolter condition under the environmental conditions existing around Edinburgh (10). Semi-bolter variations are taller and later in maturity than the normal plants of a variety, and they are most commonly found in early maturing varieties.

Freedom from virus in a variety (25) is doing much to evaluate clonal variations, and in this evaluation the choice of desirable types as laid down by an authority such as the Scottish Department of Agriculture for Scotland, is being confirmed.

Maintaining Stocks Virus-Free

Great Britain. The National Institute of Agricultural Botany, Cambridge, is one of the official bodies that operate a virus-free potato scheme in Great Britain. Selections are made in the best seed growing areas and they are multiplied initially in a greenhouse at Cambridge.

Further stages of multiplication are made in cool humid wind-swept areas of Northern Ireland with a final multiplication, before commercial distribution, in the stock seed areas of Scotland. The production of virus-free potatoes is not vested in any one body. Recognized stock seed growers in Scotland who are in suitable areas for such work, and who have the required facilities for virus testing, may apply for virus-tested certification of their stocks. There are several such growers regularly producing virus-free stocks for commercial distribution. Although such potato stocks are officially named Virus-Tested, it is not certified as of this grade if any virus is revealed to be in the stock by the official check tests that are made by the serological and plant indicator methods.

As most of the virus-free material in Great Britain is raised from single-plant selections, virus tests by the growers are made by the plant indicator method because of its simplicity. In the early stages of multiplication all plants are tested once or twice each year. In the later stages up to and including the final stage immediately before commercial distribution, only random tests are carried out.

The only official certification scheme for virus-free stocks in Great Britain is conducted by the Department of Agriculture for Scotland (11). The scheme was initiated in 1950 with four growers and a total of thirty-one acres. This year there are seven growers and an expected acreage in excess of seventy. According to MacIntosh (18) the scheme is successful and only an occasional crop has been failed through virus X. Leaf-roll was a major trouble in the early years when roguing was not allowed, but roguing-out of up to ten plants per acre is now permitted.

Crops grown from Virus-Tested stocks up to 1952 have been marked with a star in the Potato Stock Seed Register. Until the introduction of Virus-Tested Stocks, Stock Seed was the highest grade of seed available in Great Britain. However as these starred Stock Seed Crops have been found to be so much superior to the common run of the Stock Seed grade, a new and higher grade called Foundation Seed, has been introduced this year (18). Only stocks grown from Virus-Tested seed and Starred Stock Seed of 1952 are eligible for certification in the new grade. The maximum permitted tolerance of virus disease in this grade is two plants per acre of leaf roll and ten plants per acre of mosaic due to potato mosaic viruses.

Among both farmers and merchants there is a growing appreciation of the commercial value of virus-free stocks which is being reflected in the increasing demands for the popular varieties in this condition.

West Germany. The private potato breeding farms of Luneberger, Heide, make greater use of virus-testing and virus-free multiplication methods than any other area in West Germany. Their seedlings on trial and under multiplication in the field are maintained virus-free by serological tests for virus X, and by *Solanum demissum* L. indicator plant tests for virus A. Leaf-roll virus and virus Y infections which are said to be rare or slight in this province, are controlled by visual roguing.

Each farm has its own staff of technicians and a well equipped laboratory. Tests for virus X are made by the Stapp serological method that requires the use of a centrifuge, an incubator and a microscope. Petri dishes are used in the detached leaf method with *Solanum demissum* for virus A tests.

Stapp *et al.* (33, 34, 35) developed an efficient mass serological method of testing for virus X in potatoes that could be carried out by simply-trained technicians on these plant breeding farms. They adapted the serological precipitin drop test method initiated by Dounin and Popova (12) and the sera drying method of Mez (34) to produce paper wafers impregnated with virus X antiserum (35). These wafers, four miltimetres square, are cut from sheets of thin white paper on which virus X-immune rabbit antiserum has been spread on both sides at the rate of about one millilitre per sixty square centimeters and dried over calcium chloride in a desiccator at room temperature. For controls, normal rabbit serum is treated in the same way. To make a test, a wafer of antiserum is placed at one end of a microscope slide and a wafer of normal serum at the other end; to each is added a drop of physiological saline solution and a drop of clarified sap of the plant to be tested. The slide is incubated at 23° C for 20 minutes and then examined under a microscope in a dark field at a magnification of 50X.

Holland. In Holland some half-million serological tests are carried out each year for the elimination of virus X from the Foundation stocks of the Dutch varieties (30). Potato virus X antiserum is produced in horses at the Laboratory for Flowerbulb-Research, Lisse, Holland, under the direction of Prof. van Slogteren. The supervision and administration of these tests, which are carried out for stock-seed growers, is under the control of the Netherlands Inspection Service (N.A.K.) (37). Holland is divided into thirteen provinces and at a centre or a joint centre for each province, a well equipped laboratory has been established by (N.A.K.). The virus X antiserum is distributed from Lisse to each of these laboratories where the trained staff receive and test hundreds of samples daily during the growing season (20).

The method of testing used in Holland is also by that of micro-serology. The details of procedure are similar to those of Stapp except that drops of antiserum and normal serum are used and not impregnated paper wafers, and the expressed potato sap is not centrifuged.

Union of South Africa. Virus X-free seed potato production in South Africa is centered at Riet River Settlement, an area protected by legislation for the production of virus-tested stocks (24). The area is isolated and disease-carrying aphids are rare in its hot dry climate. To maintain adequate isolation, regulations made under the Act are used to prohibit the entry of all unauthorized potatoes. The prohibited area includes the Settlement and all land within 10 miles of it, and this in turn is surrounded by dry grazing country.

Since the inception of the scheme until the present time, virus testing of stocks has been carried out at Pretoria. Facilities for virus testing on the spot are now being arranged at Riet River. These tests will be made on the foliage, instead of the tubers as is practiced at Pretoria (23). The State still maintains a nucleus of virus-free stocks for the renewal and replacement needs of the growers in the settlement.

Canada. The primary work of this type is to maintain our seedlings and nucleus sources of the two varieties, Canso and Keswick, virus-free, and to this end a mass serological slide drop-test method initiated by Bradley in 1951 (6) was introduced in 1952. It is fairly efficient without being costly.

The drop slide methods of Van Slogteren of Holland and Stapp of Germany require a certain minimum of equipment, consequently in each case the simplicity of the tests, and the number that can be carried out in any given time, is much reduced. The tests can therefore only be made in a laboratory.

The tests at Fredericton are made simply by squeezing a drop of sap from a potato leaflet on each end of a microscopic slide. To one drop is added a drop of suitably diluted virus X rabbit antiserum and to the other a drop of diluted normal rabbit serum. The drops on each slide are stirred with opposite ends of a wooden toothpick which is then discarded. Because infected sap causes a precipitation in the antiserum drop within ten seconds of stirring, the stirred drops can be examined almost immediately. The precipitation can be easily and clearly seen without the aid of a microscope, simply by holding it to the junction of a white light fluorescent tube and the edge of attached black paper or board. However the greatest advantage of this method of testing is that it can be carried out with equal efficiency in the field. During the summer months our tests are conducted on the headlands of potato fields with the aid of a small specially constructed hand-wagon or in the potato crop itself with a smaller easily maneuvered aluminum hand-wagon as shown in figure 1. This latter wagon can be folded up into a bulk of 41 x 28 x 5 inches for easy conveyance by automobile to different parts of the province.

Efficiency of Methods of Testing

The Stapp and Bercks (35) and the van Slogteren (29) serological methods are very easy and effective ways for plant breeders and stock seed growers to test and rogue for potato virus X on the same day. They only require simple equipment and the developed technique is one in which technicians can be easily trained. The prime disadvantages are that they are not field tests and the number of tests that can be carried out in a day is much less when centrifuging, incubating and microscopic readings are necessary. However the gain to the plant breeder of the paper wafer test is still immense because it is the only way that he can effectively test large numbers of his seedlings and varieties. The incentive to plant breeders in West Germany to raise their own varieties in a virus-free state is much increased by the law that permits only the plant breeder in that country to be eligible for the highest official seed potato grade for his own varieties.

There may be no claims by van Slogteren and Stapp to absolute accuracy with their methods, but they do maintain that it is close to 100 per cent. The method used at Fredericton is not so efficient as those used in continental Europe, but the ease and speed with which large numbers of tests can be completed must far exceed any other known method. About one hundred tests can be made in thirty minutes by four technicians, either in the laboratory or in the field, exclusive of the time required

FIGURE 1.—Testing for potato virus X in the field. Note aluminum wagon with equipment which can be converted into small bulk for transport.

to collect the samples. Four men can do about 800 tests a day continuously over long periods, including the collecting of samples and allowing for fatigue. A car mirror is used as a source of light to observe the slides in our outdoor tests.

On the whole we find that this method of testing is about as efficient as tuber testing on to plant indicators. With each lot of antiserum from a rabbit we make serial dilutions with physiological salt solution, and each dilution is tested with freshly extracted crude sap from upper leaves of virus X-infected Green Mountain potato plants. The dilution that causes a precipitin reaction almost immediately is the one chosen for our test work. Dilutions of X antiserum for use in the field and in the laboratory are usually made up twice a day and on each occasion a check test is made with young leaves of a known infected plant.

In our experience with the simple field drop test method for the detection of potato virus X, check tests with *Datura stramonium* L. have always confirmed the positive slide results; but we do on occasion get up to 20 per cent of the infected plants giving negative results on the slides. This was found to be due, in part, to the slow formation of the precipitate and the consequent discard of the slide before it appeared. Despite the

precaution of delayed readings of series of slides, there seems to be occasional laggard reactors within most of the varieties and seedlings that we have tested so far. Similar experiences may have guided both Stapp and van Slogteren to make the refinements in their methods that are lacking in ours. Eventually our comparatively crude method may have to be improved before virus X can be wholly eliminated from our stocks. The method that we employ may only be of real value when a stock is infected with a high percentage of the virus, but much less so when only occasional infected plants remain in the stocks.

In the production of seed potatoes the most certain way of avoiding loss is to avoid virus infection (1), and it is only when healthy potato plants are grown in proximity to virus X-infected plants that there is any likelihood of a breakdown in virus-free stocks. To quote van der Plank (22) freedom from virus X is an elementary requirement for good seed, and seed free from virus X should be available as the ordinary article.

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