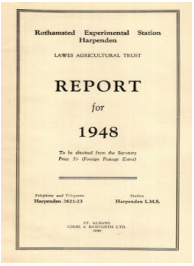
ROTHAMSTED RESEARCH

Report for 1948

Full Table of Content



Special Review : Review of the Work on the Serological Reactions of Plant Viruses

E. M. Crowther

E. M. Crowther (1949) *Special Review : Review of the Work on the Serological Reactions of Plant Viruses ;* Report For 1948, pp 120 - 127 **- DOI: https://doi.org/10.23637/ERADOC-1-70**

REVIEW OF WORK ON THE SEROLOGICAL REACTIONS OF PLANT VIRUSES

The value of serological techniques in the study of plant viruses is amply demonstrated by the steadily increasing number of laboratories where they are now used. They were introduced into Rothamsted by Birkeland in 1934, soon after Purdy Beale (1929, 1931) showed that plants infected with tobacco mosaic virus differed antigenically from uninfected plants. Since then specific antigens have been demonstrated in plants infected with several other viruses, and their study has strengthened the likelihood that they are the viruses themselves. During the last 15 years, serological tests, particularly the precipitin reaction in one form or another, have been regularly employed at Rothamsted, not only qualitatively to determine the presence or absence of specific viruses, but in quantitative assays which have been invaluable aids in work on the purification and properties of viruses. Although the main reason for studying the serological reactions of plant viruses has been to gain information about the viruses themselves, considerable work has also been done on certain basic serological problems for which the viruses have proved particularly convenient antigens.

Identification and grouping of viruses

To identify viruses from symptomatology is time-consuming and difficult, for unrelated viruses often produce similar symptoms in the same hosts whereas strains of one virus may produce symptoms of widely differing types. Even when a wide range of differential hosts is used, identification may still be uncertain. Serological tests identify with much greater certainty, they also cost less than infectivity tests and provide results in minutes instead of in days or weeks. The value of serology for identifying and grouping viruses was indicated by Birkeland's (1934, 1935) results, which showed that viruses with such different general properties as tobacco mosaic, tobacco ringspot and cucumber mosaic, were serologically unrelated, whereas the strains of tobacco mosaic virus that caused such diseases in tomato as mosaic, aucuba and streak, were all precipitated by one antiserum. That strains of one virus contain common antigens whereas viruses with different physical and chemical properties do not, has since been amply confirmed with many different viruses, and one of the main applications of serology to the study of plant viruses has been the identification of clinically distinct viruses as related strains. For example, cucumber viruses 3 and 4 which have no known hosts in common with tobacco mosaic virus, were found to share antigens with it (Bawden and Pirie 1937a). Also, potato viruses B and C, which were first described as separate viruses, were shown to be strains of viruses X and Y respectively (Bawden and Sheffield 1944), and outbreaks of apparently new diseases in tulip (Kassanis 1949a) and French bean were diagnosed serologically as caused by previously described tobacco necrosis viruses.

https://doi.org/10.23637/ERADOC-1-70

Serological tests (Bawden and Van der Want 1949) are applicable to different plants, and irrespective of whether plants show symptoms. An antiserum prepared against a virus propagated in one species will react with sap from any other infected speciedd, provided only that the virus-content is adequate, and it is as easy to diagnose the presence of a virus in a symptomless carrier as in a host that reacts in some characteristic manner. The value of the precipitin test for identifying unknown viruses lies in the fact that the test is group specific; the identification is not precise but relates the unknown to known types. For example, if sap from a tomato plant is precipitated by antiserum to tobacco mosaic virus, the presence of some strain of this virus is established, but the test will give no information on the identity of the particular strain, because, with few exceptions, all strains precipitate in essentially the same manner with their own or with each other's antisera.

Their similar behaviour in precipitin tests does not mean that strains are necessarily antigenically identical and, by extending serological methods to serum absorbtion tests, differences can be detected between them. Virus particles are not unit antigens, but contain a number of antigenic groups, each of which stimulates in animals the production of its separate specific antibody. Strains which have one or more antigenic groups in common will be precipitated by each other's antisera, but this precipitation will not affect antibodies for which the strains have no corresponding antigenic groups. Hence a serum that has been fully absorbed with some strain other than the one used in its preparation, may still precipitate its homologous strain. Cross absorption experiments using various strains of tobacco mosaic virus (Bawden and Pirie 1937a) and of potato virus X (Bawden and Sheffield 1944) and their respective antisera, showed that the strains contained specific as well as common antigens and that some strains were very similar antigenically and others shared fewer antigens. Close serological relationships were reflected in similarities of certain other properties, but not in type of symptoms caused.

Serology has been mostly used to show that viruses which cause different diseases, or have different host ranges, are related strains, but it has also been valuable in showing that what appeared to be one disease might have different causes. Serological tests helped to identify and group the various viruses that cause potato-top-necrosis (Bawden 1936, Bawden and Sheffield 1944) and provided the first evidence that symptoms of tobacco necrosis might be caused by more than one virus. After demonstrating that serologically unrelated viruses could be obtained from different sources, and that individual plants were often infected with mixed cultures (Bawden 1941), several viruses were isolated and their properties studied (Bawden and Pirie 1942, 1945a). It was found that those which shared antigens also resembled one another closely in other properties, usually differing detectably only in the type of crystals formed, whereas those which were serologically unrelated differed widely in other properties, having particles of different sizes and inactivating under different conditions.

Serological studies on different isolates of the bacteria (*Rhizobium* sp.) that cause nodules on the roots of leguminous plants, allowed these also to be arranged into groups, the individual members

https://doi.org/10.23637/ERADOC-1-70

of which share antigens whereas the groups are serologically unrelated. As with the tobacco necrosis viruses, serological relationships were not reflected by any biological characters (Kleczkowski and Thornton 1944).

Unfortunately serological methods are not applicable to all plant viruses and serology alone cannot provide a basis for a complete classification. At Rothamsted we have produced antisera against some viruses and virus strains, but have failed with others. So far twelve serologically distinct groups have been identified; tobacco mosaic, potato X, potato Y, henbane mosaic (Hyoscyamus virus 3), tobacco etch, tomato bushy stunt, soy-bean mosaic, broad bean mosaic, sugar beet yellows and 3 groups of tobacco necrosis viruses. Attempts to produce antisera against potato leaf roll, potato paracrinkle, dandelion yellows, lettuce mosaic, pea mosaic, sugar beet mosaic and strawberry crinkle viruses were all unsuccessful. Birkeland (1935) produced antisera against cucumber mosaic virus, but attempts to repear this in recent years have failed, despite the use of many different strains propagated in different hosts. Probably the commonest reason for failure is the low concentration at which the viruses occur in the sap; sometimes it may be that antisera are produced when sap is injected into rabbits, but there is insufficient antigen to produce a visible reaction with antiserum in vitro. An example of this kind was encountered with the tobacco etch viruses (Bawden and Kassanis 1941); antiserum produced by injecting rabbits with sap from plants infected with severe etch virus precipitated such sap but not that from plants infected with mild etch virus. Nor did the latter precipitate with the serum of a rabbit injected with it, although this clearly contained antibodies because it precipitated sap from plants infected with severe etch virus. To get a positive precipitin reaction with sap requires a virus concentration of at least 1 mg./1, and there is much evidence that many viruses occur more dilute than this. Serological techniques are most easily applied to viruses which are readily transmitted by inoculation and which give high dilution end-points. However, ready inoculability is not essential, for the methods have been highly successful with sugar beet yellows virus (Kleczkowski and Watson 1944), which has not until recently been transmitted by inoculation and is so transmitted only with difficulty (Kassanis 1949b).

Other reasons than a too small virus content may also prevent the use of precipitin tests. From strawberry plants, for example, no extracts could be made suitable for use as antigens, because their high tannin content precipitates all the proteins (Bawden and Kleczkowski 1945). It may be that some viruses are not antigenic, though there is no evidence suggesting this and all that have been obtained in suitable quantities and conditions have produced and reacted with antibodies. Sometimes however, virus occurs in forms that does not give the precipitin test. Tomato bushy stunt virus released from tomato leaf fibre by fine grinding occurs associated with chromoprotein, and although the complex combines with virus antibodies the combination does not lead to precipitation (Bawden and Pirie 1944). A similar phenomenon may account for the failure of some lots of sap from sugar beet with yellows to be precipitated by antiserum (Kleczkowski and Watson 1944).

https://doi.org/10.23637/ERADOC-1-70

Complement fixation is more sensitive than the precipitin test and gives positive results with one-tenth the concentration of virus (Bawden and Kleczkowski 1942). It can also be applied to non-precipitating virus complexes. Using it may extend the application of serological techniques to additional viruses, but unfortunately it is not easily used with untreated plant extracts, which contain materials that absorb complement. Another technique applicable to virus preparations too dilute to give precipitation is neutralization of infectivity. This, however, can be applied only to those viruses that produce countable local lesions and the results are also difficult to interpret. The infectivity of virus preparations is affected by the addition of many substances, including normal serum, and detailed quantitative tests are needed to distinguish between unspecific effects and neutralization caused specifically by antibodies. Kassanis (1943) found that in freshly prepared sera the unspecific effects are so great so to obscure specific neutralization. The unspecific effects decrease as the sera age, and serological relationships are determinable by neutralization of infectivity only by comparing the effects of sera stored for the same time and under similar conditions. The results of such tests suggest the same relationships as do precipitin tests, though it seems that different antibodies are concerned in the two reactions.

Although difficult to apply to plant viruses, neutralization of infectivity can be used reliably with bacteriophages. These are unaffected by components of normal sera, and are completely neutralized by specific antibodies. Those that attack *Rhizobium* sp. occur too dilute to give a precipitin reaction, but can be distinguished serologically by testing effects of antisera on infectivity (Kleczkowska 1946).

Quantitative Estimations

Quantitative assays for plant viruses are usually made by infectivity tests, local-lesion counts being assumed to be correlated with virus content. From its nature the method is applicable only to viruses that produce discrete local lesions; with these it is reasonably accurate only when the inocula being compared are similar in all respects except virus content and do not have widely different virus contents. Even then tests must be done with care, and call for comparisons with inocula at various dilutions, many replications and statistical analysis of the results. Also, results obtained at different times cannot be compared directly, unless a constant and standard control is run each time because different batches of plants may vary widely in their susceptibility; neither are results directly interpretable in absolute quantities of virus. If preparations being compared vary in other respects than virus concentration, the interpretation of results is very uncertain, for many factors other than relative virus contents can affect infectivity.

The precipitin test provides a quantitative method free from many of the disadvantages and uncertainties inseparable from lesion counts. Results are obtained more rapidly, those obtained at different times can be compared directly, and they can usually be translated into relative virus concentrations regardless of how widely the preparations being compared differ in concentration. With a knowledge of the behaviour of known weights of purified virus,

https://doi.org/10.23637/ERADOC-1-70

the results can also be interpreted in absolute quantities. Two methods can be used, to determine the greatest dilution at which a preparation precipitates (precipitin titre), or to determine the antigen/antibody ratio that first precipitates when one is kept constant and the other varied (optimal proportions). Finding optimal proportions is more rapid, but the results are less accurate than precipitin titres. On many preparations quantitative estimations by lesion counts give results similar to those obtained from precipitin tests (Bawden 1935), but this is far from being generally true. If a relationship always held, there would be much to recommend the replacement of lesion counts by serological tests, and indeed for many kinds of work serological methods are much to be preferred. However, the two methods are not simple alternatives, for they often give conflicting results; they are best regarded as complementary and the application of both methods can provide much more information than can be gained from either used alone.

Lesion counts indicate the relative infectivities of preparations, whereas precipitin titres estimate the total amount of material capable of reacting with virus antiserum. Often these two are correlated, but far from always. There are many treatments that rob viruses of their infectivity without affecting their antigenicity. With all the plant viruses so far studied, appropriate treatments with formaldehyde, hydrogen peroxide and nitrous acid, or irradiation with X-rays or ultra-violet, render preparations non-infective but leave unimpaired their ability to produce antibodies and to react with them (Bawden, Pirie and Spooner 1936, Bawden and Pirie 1937b, 1938a, 1938b). Infectivity can apparently be lost because of changes within the particles that leave gross structure and physico-chemical properties unaltered, whereas disruption of the particle is needed to destroy serological specificity. Certain treatments destroy infectivity and antigenicity more or less simultaneously with some viruses but not with others. Potato virus X and tobacco mosaic virus, for example, remain infective when heated until they also begin to denature and suffer loss of serological activity, whereas tomato bushy stunt and tobacco necrosis viruses become non-infective when heated at temperatures far below those needed to denature and affect serological reactions (Bawden and Pirie 1938a Bawden 1941). With most viruses denaturation by heating destroys all serological specificity, but denatured bushy stunt virus can still combine with some virus antibodies. Ageing *in vitro* with viruses such as potato X also leads to loss of serological activity when infectivity decreases, but old crystalline preparations of tobacco necrosis viruses may be largely non-infective but fully active serologically (Bawden and Pirie 1945a).

Serologically active but non-infective particles are not peculiar to virus preparations that have been subjected to extensive treatments in the laboratory, but they occur in freshly extracted sap. Combining serological assays with quantitative tests for infectivity on preparations of tobacco mosaic virus (Bawden and Pirie 1945b), and the Rothamsted tobacco necrosis virus (Bawden and Pirie 1945a), has shown that similar quantities of serologically-active material may differ widely in their infectivities.

Precipitin tests can also be used quantitatively on virus preparations containing substances that inhibit infectivity, such as

https://doi.org/10.23637/ERADOC-1-70

trypsin (Bawden and Pirie 1936, 1937b), ribonuclease (Kleczkowski 1946, Bawden and Kleczkowski 1948) and a glyco-protein from *Phytolacca* sp. (Kassanis and Kleczkowski 1948). The presence of such substances has little effect on precipitin titre, but may entirely destroy any relationship between virus content and infectivity.

Viruses as somatic and flagellar antigens

Bawden and Pirie (1938b) noted that the type of precipitate produced by viruses with their antisera depended on particle shape, spherical viruses producing compact, dense floccules, and rod-shaped ones producing fluffy, translucent floccules. The first resemble precipitates formed by bacterial somatic ("O") antigens and the latter those produced by flagellar ("H") antigens. The serological behaviour of the viruses with different shapes were also found to resemble that of "O" and "H" antigens in other respects. The one most studied has been the effect of heat on the antisera.

It has long been known that antisera to "O" antigens lose their power to precipitate when heated at lower temperatures than antisera to "H" antigens, a difference that has been taken as showing that the two types of antigen stimulate antibodies with different heat-stabilities. Kleczkowski (1941b, 1941c) found that antisera to spherical and rod-shaped viruses behaved on heating like "O" and "H" antisera respectively, but his results showed that the differences lie in the antigens and not in their antibodies. Precipitating antibodies undergo at least two changes when heated. During the initial stages of denaturation, they form complexes with other proteins in the serum while still retaining their ability to combine specifically with their antigens, whereas with further heating they lose their serological specificity. Antibodies to both "O" and "H" antigens behave in the same manner, and form similar complexes. Those formed with euglobulin behave much like unchanged antibodies and still precipitate their antigens normally, but those formed with other serum proteins, particularly albumin, do not precipitate their antigens after combining with them. The presence of such non-precipitating antibody-complexes interferes with the precipitation of antigen by unchanged antibodies, the interference being great with "O" antigens (or spherical viruses) and slight with "H" antigens (or rod-shaped viruses). The fact that "O" antisera lose their precipitating power with less heating than do "H" antisera is not because the antibodies differ, but because fewer antigen-albumin complexes are need to prevent the precipitation of "O" than of "H" antigens.

This conclusion, that differences between the two types of serological behaviour lie in the antigens and not in antibodies, was confirmed by studies on the serological behaviour of tobacco mosaic virus in different states of aggregation, when all the features normally associated with "O" and "H" serological behaviour were produced using the same antiserum (Bawden and Pirie 1945b). The virus was separated by differential ultra-centrifugation into fractions containing particles of widely different average sizes. The smallest were approximately spheres and the others rods of various lengths. By various treatments the particles in all types of preparation could be caused to aggregate linearly to produce greatly elongated rods, and such aggregation, particularly with the smaller particles,

https://doi.org/10.23637/ERADOC-1-70

caused striking changes in serological behaviour. The unaggregated particles in every respect behaved like a somatic antigen, whereas after linear aggregation, the same material tested against the same antiserum behaved in all respects like a flagellar antigen.

Essentially similar results were also obtained with potato virus X aggregated to various extents (Bawden and Crook 1947). The serological behaviour of viruses that aggregate linearly to form rods of various lengths is affected so greatly by the average particle-length that, in making quantitative assays by the precipitin test, it is essential to ensure that preparations being compared are in comparable states of aggregation. Extensive aggregation may more than double the precipitin titre and alter the point of optimal precipitation by a factor of 50. With tobacco mosaic and potato virus X, heating sap to 60° C. and centrifuging causes extensive aggregation and brings particles to a condition comparable with those of purified preparations and in which estimations are made satisfactorily (Bawden and Pirie 1945b).

Like antibodies, antigens can also combine with other proteins during the initial stages of heat denaturation and the resulting complexes have changed serological behaviour. Complexes between serum albumin and tomato bushy stunt virus still combine and fix complement with virus antiserum, but they are not precipitated by such combination. Their presence interferes with the precipitation of uncombined virus. They are still antigenic and when injected into rabbits produce apparently normal antisera to bushy stunt virus; the antisera precipitate the virus but not the virus-albumin complex with which the rabbit was injected (Bawden and Kleczkowski 1941a, b, 1942). Digesting the complexes with pepsin releases the virus in a form precipitable by antibodies (Kleczkowski 1945a). Such non-precipitating complexes occur only when the virus is heated in the presence of excess albumin, and presumably they do not precipitate because only a small part of the complex particle is affected by antiserum to the virus (Kleczkowski 1945b). The complexes formed between heated tobacco mosaic virus and albumin neither combine with virus antibodies nor interfere with the precipitation of free virus (Kleczkowski 1949). Salts are needed in the heated solution for complexes to be formed, the efficiency of the salts at pH7 increasing with increasing valency of cations according to Hardy's law (Kleczkowski 1943).

Combining ratios of antigens and antibodies

Precipitation occurs only over a limited range of antigen/antibody proportions, the range being greater for "H" than for "O" antigens, and precipitation is usually more strongly inhibited by excess of antigen than by excess of antibody. In making qualitative tests for the presence or absence of viruses, particularly those that may occur at high concentrations, a range of antigen/antibody ratios should be covered and serum should not be too dilute.

The ratio of antibody to antigen in a precipitate depends on the proportions in which the two are mixed, but at the equivalence point, i.e. when all the antigen and antibody present occur in the precipitate, the ratio is characteristic of the particular antigen. The most important factor influencing the ratio is size of the antigenic

https://doi.org/10.23637/ERADOC-1-70

particle. The antibody/antigen ratio at equivalence for horse serum globulin is about 4, and for the much larger bacteria that cause root-nodules on peas is about 0·01. Plant viruses are intermediate in size between these two, and the combining ratios for tomato bushy stunt and tobacco mosaic virus are 0·3 and 0·2 respectively (Kleczkowski 1941a). To obtain inhibition of precipitation by excess antigen with bushy stunt virus, the ratio of antigen to antibody must be increased to at least 10 times that at equivalence point and with aggregated tobacco mosaic by more than 100 times. At equivalence point tomato bushy stunt virus particles combine with about 15 antibody particles and for precipitation to occur at all at least 3 antibody particles must combine with one virus particle. No similar calculation can be made for tobacco mosaic virus because of the great variations in length of the various particles.

REFERENCES

Bawdet, F. C. 1935. Brit. J. exp. Path., **16**, 435. 1936. Ann. appl. Biol., **23**, 487. 1941. Brit. J. exp. Path., **22**, 59.

Bawden, F. C., and Crook, E. M. 1947. Brit. J. exp. Path., **28**, 403.

Bawden, F. C., and Kassanis, B. 1941. Ann. appl. Biol., **28**, 107.

Bawden, F. C., and Kleczkowski, A. 1941a. Brit. J. exp. Path., **22**, 208. 1941b. Nature, **148**, 593. 1942. Brit. J. exp. Path., **23**, 169. 1945. J. Pomol., **21**, 2. 1948. J. gen. Microbiol., **2**, 173.

Bawden, F. C., and Pirie, N. W. 1936. Brit. J. exp. Path., **17**, 64. 1937a. *ibid.*, **18**, 275. 1937b. Proc. Roy. Soc., **B, 832**, 274. 1938a. Brit. J. exp. Path., **19**, 66. 1938b. *ibid.*, **19**, 251. 1942. *ibid.*, **23**, 314. 1944. *ibid.*, **25**, 68. 1945a., **26**, 277. 1945b. *ibid.*, **26**, 294.

Bawden, F. C., and Sheffield, F. M. L. 1944. Ann. appl. Biol., **31**, 33.

Bawden, F. C., Pirie, N. W., and Spooner, E. T. C. 1936. Brit. J. exp. Path., **17**, 204.

Bawden, F. C., and van der Want, J. P. H. 1949. Tijdschr. Pl-ziekten., **55**, 142.

Beale, H. P. 1929. J. exp. Med., **49**, 919. 1931. Contrib. Boyce Thompson Inst., **3**, 529.

Birkeland, J. M. 1934. Bot. Gaz., **95**, 419. 1935. Ann. appl. Biol., **22**, 719.

Kassanis, B. 1943. Brit. exp. Path., **24**, 152. 1949a. Ann. appl. Biol., **36**, 14. 1949b. *ibid.*, **36**, 270.

Kassanis, B., and Kleczkowski, A. 1948. J. gen. Microbiol., **2**, 143.

Kleczkowski, A. 1941a. Brit. J. exp. Path., **22**, 44. 1941b. *ibid.*, 22, 188. 1941c. *ibid.*, **22**, 192. 1943. Biochem. J., **37**, 30. 1945a. Brit. J. exp. Path., **26**, 33. 1945b. *ibid.*, **26**, 41. 1946, Biochem. J., **40**, 677. 1949. Biochem. J., **44**, 573.

Kleczkowski, A., and Thorntor, H. G. 1944. J. Bact., **48**, 661.

Kleczkowski, A., and Watson, M. A. 1944. Ann. appl. Biol., **31**, 116.

Kleczkowska, J. 1946. J. Bact., **52**, 25.

https://doi.org/10.23637/ERADOC-1-70