Neth. J. Plant Path. 72: 105–113

LOCALIZATION OF VIRAL ANTIGEN IN NARCISSUS LEAVES INFECTED WITH YELLOW STRIPE VIRUS, DETERMINED BY MEANS OF A FLUORESCEIN CONJUGATED ANTISERUM[1]

MARGARETHA C. CREMER and J. A. VAN DER VEKEN
Laboratory of Flowerbulb Research, Lisse

By means of a fluorescein conjugated antiserum, local aggregation of antigenic material in narcissus leaves infected with yellow stripe virus was demonstrated in the cytoplasm of epidermal and parenchyma cells. By comparing results obtained by fluorescent-microscopy with those obtained by light and electron microscopy it is reasonable to assume that the antigenic material consists of aggregates of virus particles.

INTRODUCTION

Fluorescent antibody staining of sections of virus infected plants has found only a limited application in the study of plant virus infection and the related phenomenon of localization, reproduction and movement of viral antigens. This work has so far been restricted to viruses such as tobacco mosaic virus (SCHRAMM & RÖTTGER, 1959; NAGARAJ, 1962) and wound tumor virus (NAGARAJ & BLACK, 1961), which can be highly purified and separated from host plant contaminants. Antisera prepared to these viruses thus contain only minor amounts of antibodies to normal plant constituents and this results in the conjugation of fairly specific virus antibodies with the fluorescein stain.

Since many viruses cannot be purified completely the corresponding antisera generally contain a large amount of antibodies to normal plant constituents that should be removed. The elimination of these antibodies presents a difficult problem.

It should be borne in mind that the nature of the antigenic material in the sections reacting with fluorescent antibodies has not yet been definitely identified. In the present investigation fluorescent antisera were used as stains for sections of healthy and virus-infected narcissus leaves. The results were compared with those obtained with conventional light microscopy and with the electron microscope in order to identify the nature of the stained antigen.

Since in narcissus plants infected with the yellow stripe virus often the more common mosaic virus is simultaneously present there exists some confusion about these diseases in the literature. To avoid further difficulties they are defined here in short.

The characteristic symptoms of yellow stripe virus on narcissus leaves are yellow-green longitudinal stripes or regions of varying length (VAN SLOGTEREN & DE BRUYN OUBOTER, 1946). Often these stripes or regions show a roughened surface (CALDWELL & JAMES, 1938).

The characteristic symptoms of mosaic virus on the leaves are more or less severe mosaic patterns (VAN SLOGTEREN & DE BRUYN OUBOTER, 1946).

It is often difficult to distinguish the two virus diseases visually on the different

[1] Accepted for publication 28 February, 1964.

varieties of narcissus. To make a reliable diagnosis electron microscopical and serological investigation of the viruses are necessary. Both viruses investigated by electron microscopy are found to consist of elongated particles. They differ in size. The particles of yellow stripe virus have a normal length of about 800 mμ and those of mosaic virus of about 600 mμ. Antisera against both viruses were prepared in this laboratory; so far it has not been possible to demonstrate any serological relationship between the two.

In a previous paper (CREMER *et al.*, 1960), in which the present study is shortly referred to, the name "grey-disease" instead of yellow stripe was used. This "grey-disease" is the English translation of "grijs" used by the growers in the Netherlands for yellow stripe in narcissus.

MATERIALS AND METHODS

Plant material

In all experiments fresh young leaves were used from the narcissus cultivar 'Minister Talma'. The bulbs used as a source of yellow stripe virus were obtained from plants which were found to be free from narcissus mosaic virus by serology and electron microscopy in the previous season. The plants were also free from rattle virus (VAN SLOGTEREN, 1958).

The leaves of the diseased plants used, showed the above-mentioned yellow-green coloured rough regions on the leaf surface.

Preparation of the antiserum against narcissus yellow stripe virus

An antiserum against the yellow stripe virus was made by injecting rabbits with partially purified virus preparations made as follows. Narcissus leaves infected with yellow stripe virus which were free from mosaic virus were freeze-dried (VAN DER VEKEN, 1960). Two grams of the freeze-dried leaves were homogenized in a Waring blender with 200 ml cold chloroform at −15° C. The resulting suspension was filtered through a Buchner funnel and the chloroform sucked off. The remaining powder was extracted with chloroform, ethanol absolute, acetone and ether (ROZENDAAL & VAN SLOGTEREN, 1958). Finally the powder was vacuum-dried, suspended in 180 ml of phosphate buffer pH 7.2 (containing 0.3% KCN and 0.3% $NaHSO_3$) and filtered through cheese cloth. To one volume of this suspension 2/3 volume of a saturated $(NH_4)_2SO_4$ solution was added. The saturated $(NH_4)_2SO_4$ solution was brought to pH 7.2 by adding a 25% NH_3 solution. The precipitate was removed by centrifugation for 45 minutes at 3,000 rev/min, and suspended in a volume corresponding to two and a half times the original weight of the starting material. This suspension was dialysed against running tap water and finally 0.85% NaCl was added to obtain a physiological solution for injecting rabbits. The rabbits received 16 intraveneous injections three times a week in successive doses of 2–8 ml, with a rest period of ten days after the tenth injection. The antiserum produced showed a titre of 1280 to the virus but at the same time a titre of about 160 to normal plants constituents, as measured by the micro-precipitin test (VAN SLOGTEREN, 1955).

Preparation of labelled specific antibodies from the antiserum

The globulin fraction from the above-mentioned antiserum was precipitated

at 4° C by adding an equal volume of cold (4° C) saturated $(NH_4)_2SO_4$ at pH 7.2. After centrifugation (15 min, 10,000 rev/min) the precipitate was resuspended in a 0.85% buffered saline solution to give a final protein concentration of 10 mg/ml. The protein concentration was measured by means of refractometric procedure. These globulins were conjugated by the method of MARSHALL *et al.* (1958) with 0.05 mg of fluorescein *iso*thiocyanate per mg protein. The conjugated antiserum was dialysed against the above-mentioned buffered saline solution for eight days to eliminate free fluorescein. The buffered saline consisted of 1 part of 0.1 M phosphate buffer pH 7.2 and 3 parts of NaCl 0.85%. To remove aspecific staining material, COONS (1952) recommended the use of liver protein, a method which was also used by SCHRAMM & RÖTTGER (1959). As our results using this technique were unsatisfactory, a plant powder preparation was used instead of liver powder.

The plant powder was obtained by homogenizing 100 g leaf material of healthy narcissus in 500 ml cold acetone (4° C) in a Waring blender. By filtering the suspension through a Buchner funnel the acetone was sucked off. The remaining plant material was vacuum-dried and powdered.

The unwanted antibodies to the normal plant antigens and material causing aspecific fluorescence were absorbed by shaking the antibody protein suspension for one hour at 4° C with 0.0125 g of the plant powder preparation per ml antibody protein suspension. After shaking, the suspended material was removed by centrifugation for 15 minutes at 10,000 rev/min and the whole treatment was repeated. The solution obtained was ready for use and could be stored in freeze-dried condition. For best results the freeze-dried material was redissolved in distilled water in half of the volume obtained after absorption and centrifuged 10 minutes at 10,000 rev/min. For control tests a normal rabbit serum was treated and conjugated with fluorescein *iso*thiocyanate just as described for the antiserum.

Preparation of the leaf tissue sections

From leaves obtained from plants showing virus symptoms as well as from healthy leaves, small pieces about 5 × 10 mm were embedded for sectioning, using the method described by SCHRAMM & RÖTTGER (1959). Transverse sections of 12–18 μ were cut at –18° C on a Jung microtome placed in a Linde Cryostat.

The sections were mounted on a glass slide coated with a glycerol-gelatin mixture. "Merck's" extrafine white gelatin (Extra Gold-Label) gave excellent results when used for embedding and coating. Many other brands of gelatin appeared to produce an aspecific fluorescence.

The sections were stored in a refrigerator at 4° C for one night and then used in the staining procedure.

Staining technique

After dark storage in the refrigerator, the sections were incubated in darkness at room temperature for one hour with a drop of a fluorescent antibody suspension. They were then rinsed for 10–15 minutes with phosphate buffered saline of pH 7.2 to remove the excess of fluorescent material. As controls, other sections were similarly treated with a fluorescein conjugated rabbit normal serum.

The sections were then mounted in a glycerol-phosphate mixture, made by mixing 1 ml acid-free glycerol and 9 ml phosphate buffer pH 7.2. Other mounting media may also be used, but those which had an acid reaction were unsatisfactory because they destroyed the fluorescent properties of the materials used.

Fluorescent microscopy and photography

Observations were made under a Zeiss fluorescent microscope equipped with a mercury vapour lamp (Osram H.B.O. 200) and a filter-combination for inducing fluorescence in the range of 5500–6000 Å. We used for this purpose one or two filters, BG 12, filtering the incident light beam from the lamp before it irradiated the section. Residual ultraviolet light was removed by placing a filter combination OG 4 and OG 5 between the ocular lens and the section observed. The heat produced was absorbed by means of a heat absorption filter.

Sections were photographed by means of a Contax camera on Agfacolor film CT 18 and Isopan FF 13/10 DIN (black and white). In photographing the sections only one BG 12 filter was used. Both light and dark field illumination have given good results.

Histological techniques used for investigations by light microscopy

For the techniques and fluids used for fixation, dehydration and paraffin-embedding of the plant material reference is made to JOHANSEN (1940). Leaf tissue from narcissus with yellow stripe symptoms and comparable tissue from healthy narcissus were fixed in the so-called "strong" fixation fluid of FLEMMING. Fixation fluids named "FAA", "Carnoy", and "Nawashin" (according to RANDOLPH) were also used in checking the material for artefacts. After fixation, dehydration with ethanol-xylol followed and finally the material was embedded in paraffin.

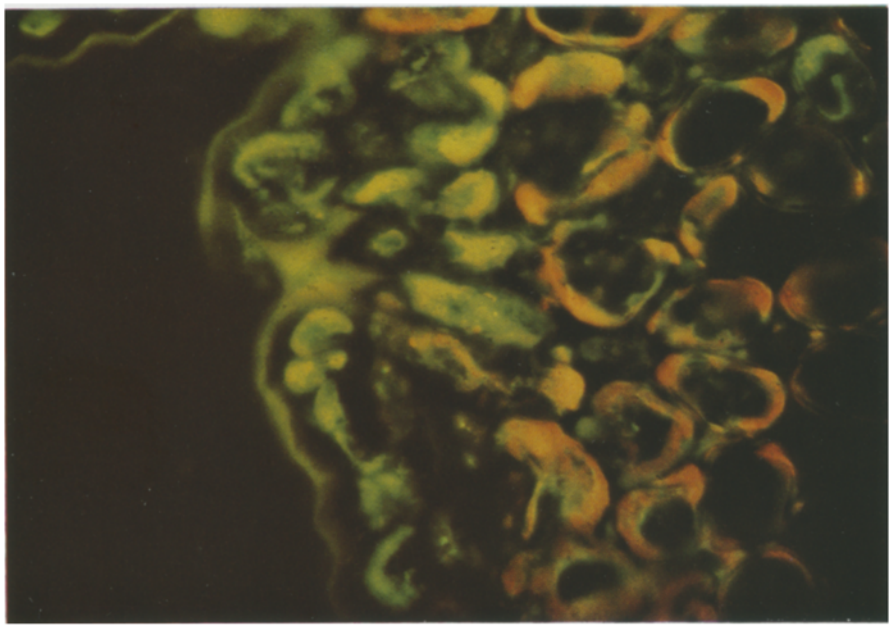
Cross sections (7 μ) were examined by phase-contrast microscopy. For histochemical research on proteins, nucleic and amino acids the sections were stained by means of the "Diazo" reaction according to DANIELLI (cf. PEARS, 1953) and the "Azan" staining according to HEIDENHAIN (1916).

Histological techniques used for investigations by electron microscopy

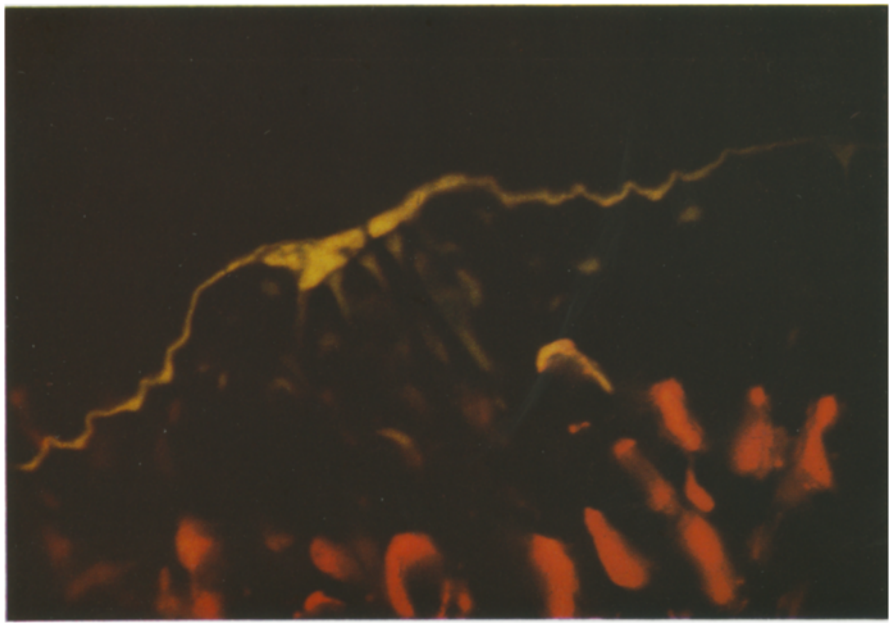
From young leaves showing virus symptoms as well as from healthy leaves small pieces about 2×5 mm were fixed in a 1% buffered OsO_4 solution, dehydrated in ethanol and embedded in methacrylate according to WALTER (1957).

The following modification was made to WALTER's method: after OsO_4 fixation and dehydration the leaf pieces were transferred from the liquid monomer into fresh monomer that was prepolymerized to be "syrupy" (BORYSKO, 1956), after which polymerization progressed for 18 hours at 48° C. For purposes of comparison, besides OsO_4 fixation, "FAA" and "Carnoy" fixatives were used (JOHANSEN, 1940). For studying membrane structures of endoplasmic reticulum the 5% $KMnO_4$ fixation (unbuffered, 75 min at 22° C) according to MOLLENHAUER (1959) was employed.

Ultra-thin transverse sections were cut with the aid of glass knives on an ultramicrotome according to the principle of Philips-Haanstra. The sections



A



B

FIG. 1. Transverse sections, 15 μ thick, through frozen tissue of a narcissus leaf infected with yellow stripe virus, × 300.
A. After treatment with fluorescent antiserum.
B. After treatment with fluorescent normal serum.

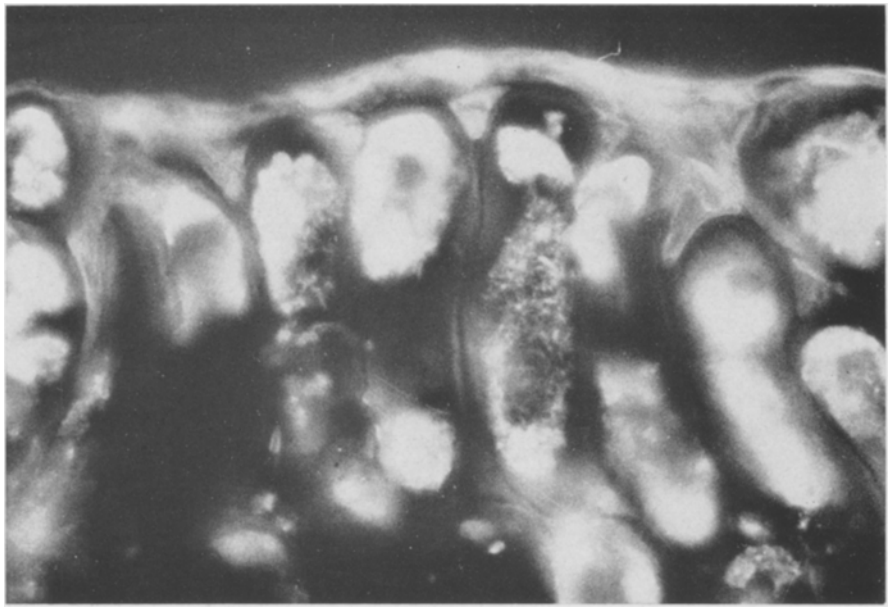


FIG. 2. Transverse section, 15 μ thick, through frozen tissue of a narcissus leaf infected with yellow stripe virus, after treatment with fluorescent antiserum. × 500. Homogeneous and filamentous specific fluorescence in the cytoplasm of the cells can be observed.

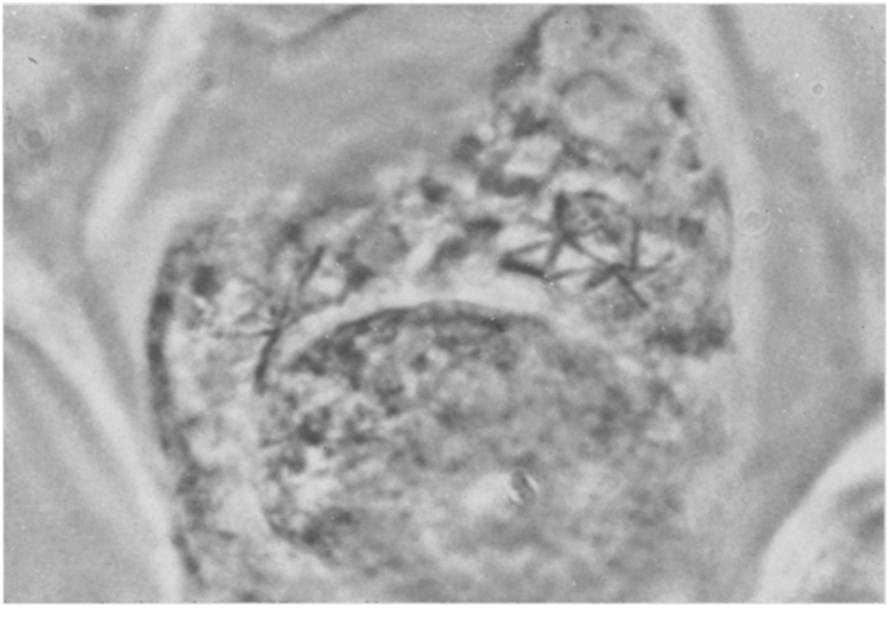


FIG. 3. Parenchyma cell from tissue of a narcissus leaf infected with yellow stripe virus. × 3300. (This is the same cell as indicated by arrow in Fig. 4A.) "Starlike" and oblong inclusions in the cytoplasm can be observed.

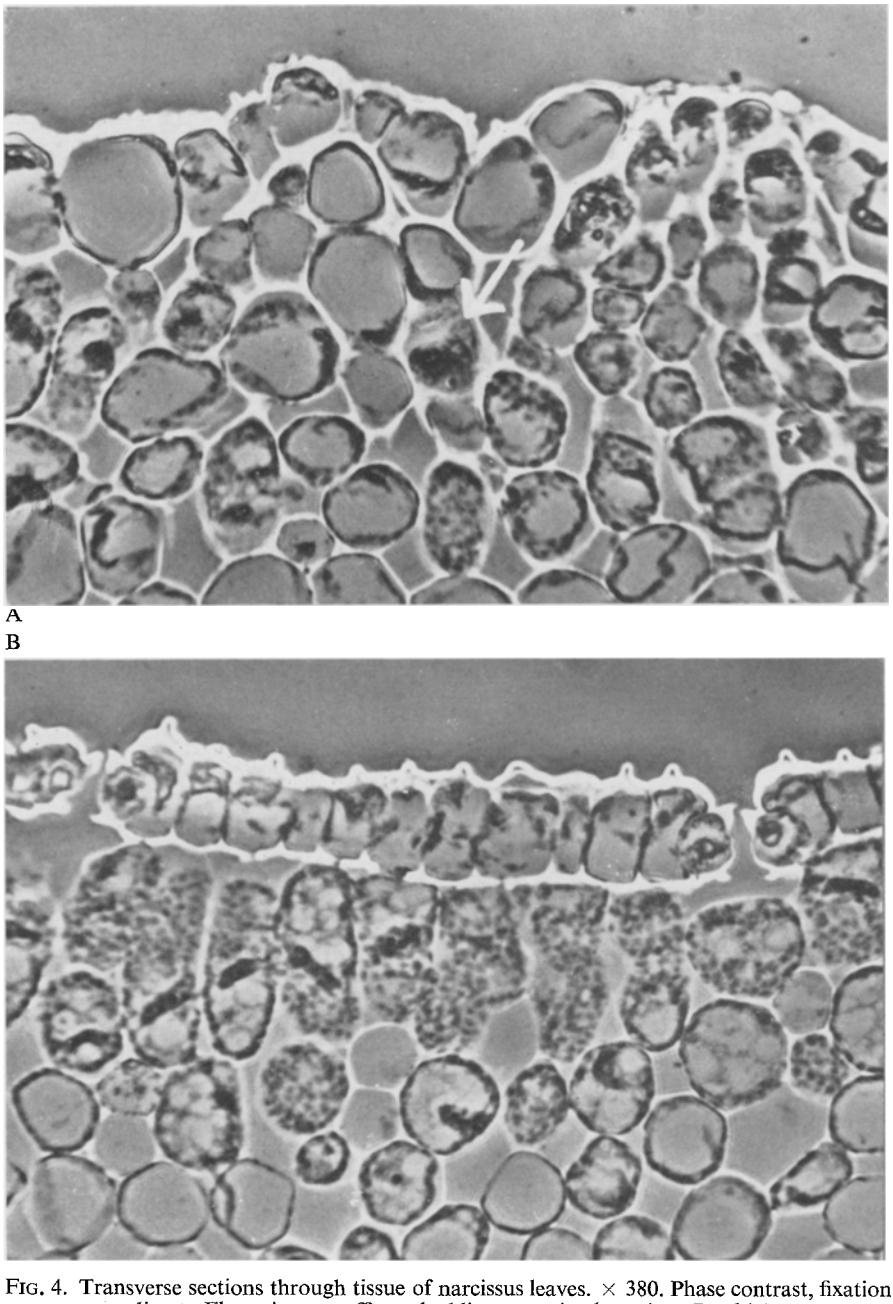


FIG. 4. Transverse sections through tissue of narcissus leaves. × 380. Phase contrast, fixation according to Flemming, paraffin embedding, unstained, sections 7 μ thick.
A. Infected with yellow stripe virus; "tumorous" growth of the parenchyma layers and irregular growth and destruction of epidermal cells can be seen. The cell indicated by arrow is reproduced at a higher magnification in Fig. 3.
B. Healthy leaf tissue.

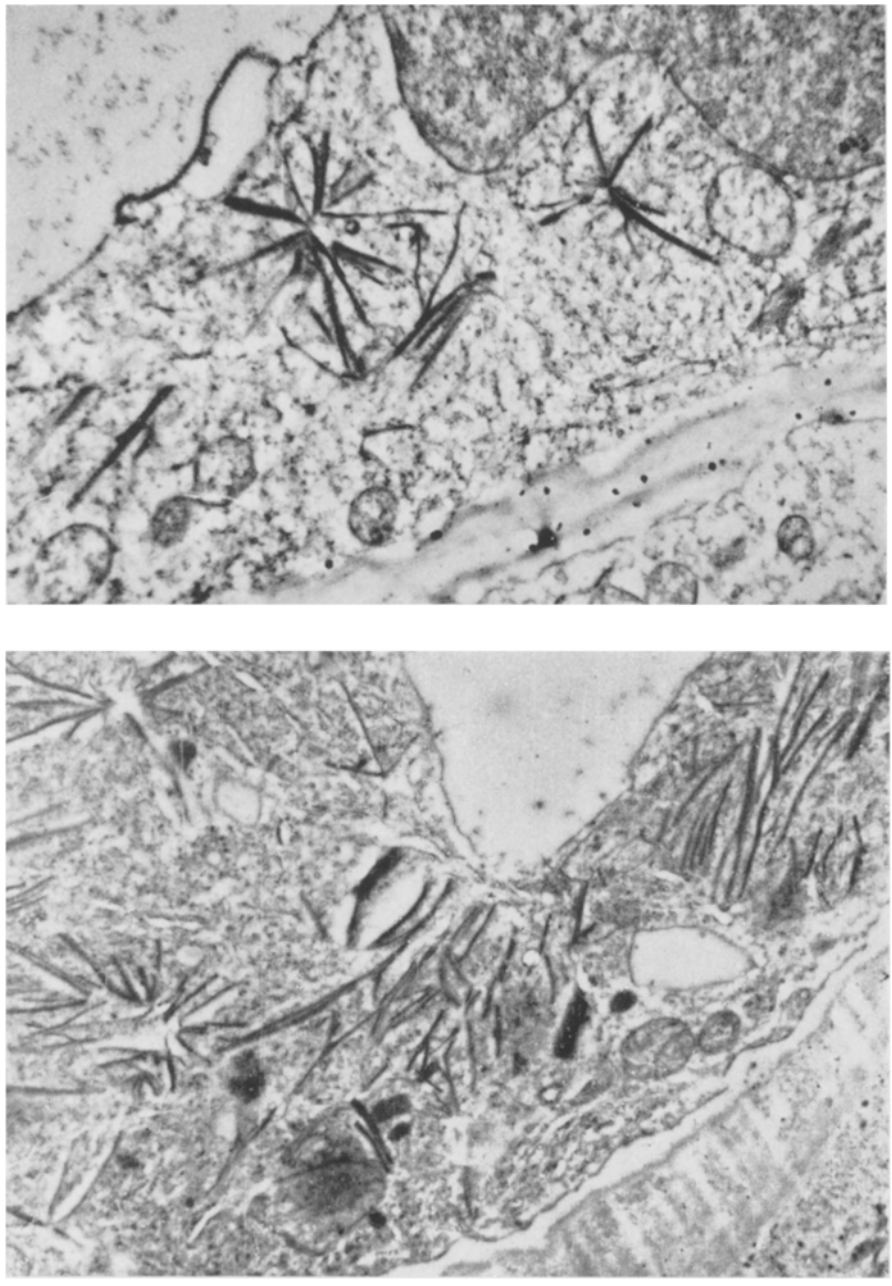


FIG. 5. Parts of parenchyma cells from tissue of narcissus leaves infected with yellow stripe virus. OsO_4 fixation, methacrylate embedding, sections 0.2–0.5 μ thick, electron microscope Philips E.M. 100.
A. "Starlike" and oblong inclusions in the cytoplasm are noticeable. × 11,000.
B. Cytoplasm packed with "starlike" and oblong inclusions. × 13,000.

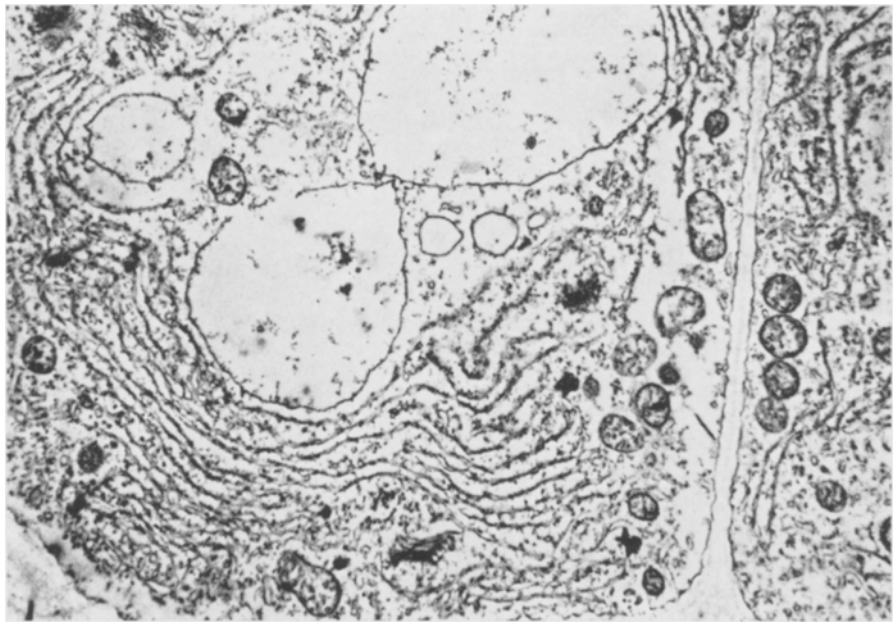


FIG. 6. Part of palissade parenchyma cell from tissue of a narcissus leaf infected with yellow stripe virus. × 9000. Endoplasmic reticulum membranes in the cytoplasm may be seen. OsO_4 fixation, methacrylate embedding, section 0.2–0.5 μ thick, electron microscope Philips E.M. 100.

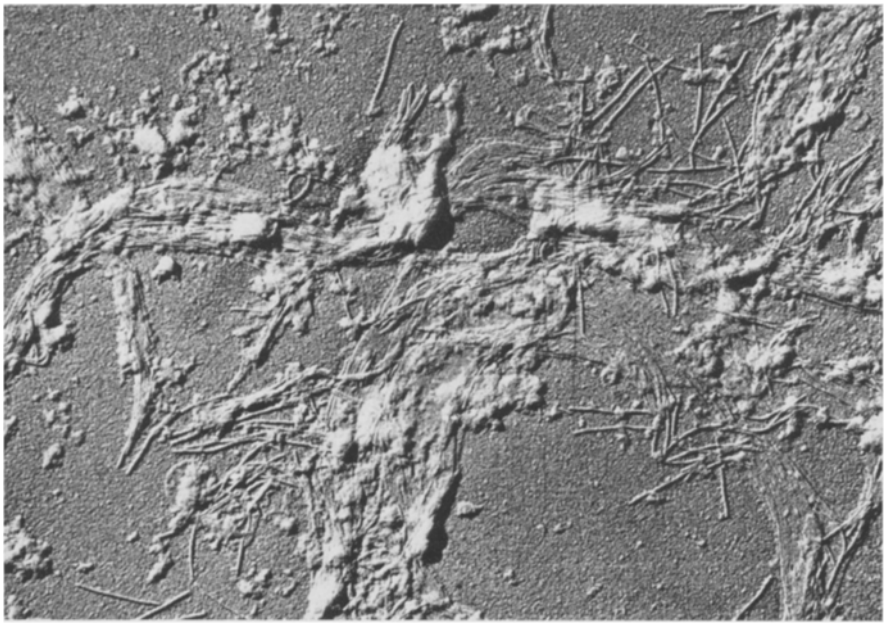


FIG. 7. Cell content from leaf tissue of narcissus infected with yellow stripe virus, obtained by application of the dip method. × 16,000. Aggregates of elongated virus particles in the shape of either strings or mats can be observed. Shadow-casted with gold-palladium, electron microscope Philips E.M. 100.

mounted on formvar covered grids were examined with a Philips E.M. 100 electron microscope.

For sectioning and mounting we used the methods described by PEASE (1960). Electron-diffraction (HALL, 1953) was applied on sections from the leaf material fixed with "FAA" and "Carnoy". These experiments were done by Miss Drs. C. VAN DER SCHEER, Physico-Technical Service for Agriculture, Wageningen.

RESULTS

Results obtained by fluorescent microscopy

As the antisera used in this work contained a high proportion of antibodies to normal plant material we had to deal with two types of aspecific fluorescing material, viz. the fluorescein conjugated antibodies against normal plant constituents and the aspecific material normally present in fluorescein conjugated antisera. In the sections of healthy leaves treated with fluorescein conjugated antisera, after preliminary shaking with liver powder, a slight green fluorescence in the tissue cells could be observed. This was apparently due to the antibodies of normal plant proteins, for the sections of healthy and virus diseased leaf tissue treated with the fluorescein conjugated normal serum did not show this fluorescence.

Treatment of the fluorescein conjugated sera with acetone extracted plant powder instead of liver powder resulted in absorption of antibodies produced by host antigens as well as the removal of aspecific staining material. The results with the thus absorbed fluorescein conjugated antiserum and fluorescein conjugated normal serum are shown in Figs. 1a, 1b and 2.

The disappearance of the red autofluorescence of the chlorophyll in the parts of the plant showing the external symptoms of the virus disease should be noted in these pictures. In fact, this bleaching of the chlorophyll improves the observation of the specific bright green fluorescence. The specific fluorescence in the sections of the diseased plants was restricted to the area of the external symptoms, while the staining treatment using normal serum did not demonstrate any specific fluorescence; only a yellow-green autofluorescence of cuticle and cell walls was observed. In addition, the bright green specific fluorescence often had a filamentous appearance (Fig. 2) and seemed to be restricted to the cytoplasm of epidermal and parenchyma cells in the region of the symptom area.

Results obtained by light microscopy

Unstained transverse sections from the paraffin embedded leaf tissue of diseased and healthy leaves were compared, using phase contrast. In the symptom areas of the diseased leaves "tumorous" tissue could be seen in the parenchyma layers, i.e. tissue with abnormal growth to "giant" cells and a stimulus to cell division. In the epidermal layer, cells having abnormally different sizes could be observed. In association with the "tumorous" growth of the parenchyma layers there was often complete elimination of the epidermal cells (Figs. 2 and 4). The rough-feeling protuberances on the leaf surface are due to the histological modifications mentioned. Furthermore there was a diminution in the number of chloroplasts, due either to their inhibition or destruction, which resulted in discoloration of the affected regions.

By using phase contrast at high magnification "starlike" and oblong bodies were observed in the cytoplasm of many parenchyma as well as epidermal cells,

as shown in Fig. 3. These inclusions were absent from the cytoplasm of comparable cells from healthy material.

In examining the nature of the inclusions, histological staining tests for proteins, nucleic and amino acids were applied. Due to the great optical density of the inclusions it was impossible to obtain a differential staining reaction which would indicate their chemical composition. In well stained tissue the inclusions were always observed as black structures.

Results obtained by electron microscopy

Ultra-thin sections from leaf tissue from comparable parts of healthy and diseased leaves fixed in OsO_4 and embedded in methacrylate were investigated. The same histological modifications were observed in the symptom areas as described in the previous section. Moreover, the shape of the "starlike" and oblong inclusions in the cytoplasm of the cells in the symptom area was also confirmed as shown in Fig. 5A. In some cells cytoplasm could be seen almost wholly filled with inclusions as in Fig. 5B. Here, also, the inclusions were extremely dense. The great density of the inclusions was also observed when the fixatives "FA" and "Carnoy" were used (cf. FARVARD & CARASSO, 1957). The possibility of the inclusions having an inorganic crystalline structure was eliminated by the negative results of electron diffraction experiments.

Since in many cells in the symptom area there was a large amount of endoplasmic reticulum membranes (Fig. 6), also $KMnO_4$ fixation of the leaf material was used (MOLLENHAUER, 1959). This method of fixation gave rather poor results compared with OsO_4 fixation; furthermore the "starlike" and oblong inclusions seemed to be strongly affected and sometimes even a total destruction was observed.

Preparations from healthy and diseased leaves were also made using the dip method of BRANDES & WETTER (1959). In these preparations the cells from the diseased areas were seen to contain aggregates of the elongated virus particles, in the shape of either strings or mats as shown in Fig. 7.

DISCUSSION

Hitherto, results using fluorescent antibodies for detecting plant virus antigen have not been compared with results obtained by electron and light microscopy. Accordingly, the nature of the antigenic material in the cells showing fluorescence has not been demonstrated. A more significant interpretation of the results from fluorescent antibody staining seems to require a comparison of these results with those obtained by light and electron microscopy.

In considering the results of this study it is tempting to assume that the bright green specific fluorescent material in the cells is in reality the complex of virus aggregates and fluorescent antibodies. Such an assumption would appear to gain support from the observation of the occasional filamentous appearance of the specific fluorescent material shown in Fig. 2. This filamentous material may be identical with the "starlike" and oblong inclusions in the cells as shown in Figs. 3 and 5A. The more homogeneous-looking specific fluorescence may be due to the over-irradiation caused by inclusions densely packed in the cytoplasm of cells, as shown in Fig. 5B.

The "starlike" and oblong inclusions in the cells shown in Figs. 3 and 5 are

believed to be composed of strings or mats of aggregated virus particles, as revealed by Fig. 7. In this connection it should be emphasized that suspending the contents of a cell by dip-preparation always involves a flattening of the three dimensional structures. Unfortunately a chemical resemblance between the inclusions shown in Figs. 3 and 5 and the virus material in Fig. 7 could not be proved by differential staining techniques, probably due to the great optical density. However, the possibility that the inclusions are of an inorganic crystalline nature is excluded by the negative results of electron diffraction experiments. The results obtained greatly support the assumption of identity between the inclusions and the viral material.

As regards the histological modifications in the leaf tissue from plants infected with yellow stripe virus it is to be noted that our results with different techniques substantially confirm the results of CALDWELL & JAMES (1938) obtained by using only light microscopy, viz. a "tumorous" growth of the tissue in the parenchyma layers ("giant" cells and a stimulus to cell division); irregular abnormal growth and destruction of epidermal cells; diminution in the number of chloroplasts due to inhibition or destruction; and presence of "starlike" and oblong inclusions in the cytoplasm of epidermal and parenchyma cells. Though they mentioned the presence of cell structures resembling inclusion bodies, in the present investigation these bodies appeared to be composed of coagulated cytoplasm, in which the "starlike" and oblong bodies were embedded (Fig. 3). This coagulated cytoplasm was often restricted to an area around the nucleus.

Based on the histological modifications mentioned, CALDWELL & JAMES (1938) suggested a complex of different viruses, but in view of our present knowledge of yellow stripe this seems to be most unlikely.

Notwithstanding the positive results obtained in our work, the application of the fluorescent antibody technique seems likely to be restricted to a very small area of plant virus research. This conclusion is based on the following considerations. First, only those viruses which are present in high concentration seem to be detectable by this technique. Our efforts to find a similar bright green fluorescence in leaves of tobacco plants infected with potato virus Y yielded no results, even when a very concentrated antiserum was used. Secondly, even a high virus concentration in the host does not ensure success as indicated by our negative results using potato virus X.

We assume, therefore, that besides the total virus concentration the occurrence of virus aggregates plays an important part in obtaining positive results using this method. This assumption derives support from comparing the positive results with narcissus yellow stripe virus and the negative results with potato virus X. Possibly more sensitive methods may be found that will give better results with viruses not detectable by the method used in the present study. In this connection the recent paper by WORLEY & SCHNEIDER (1963), dealing with fluorescent antibody staining of southern bean mosaic virus antigen in bean leaves, should be mentioned. Although the indirect method used by these authors might be responsible for the good results they obtained, the technique employed in treating the sections by freeze-drying and chloroform-fixation could be of importance.

Finally, it is to be noted that the use of plant powder obtained from acetone-extracted healthy leaf material, for absorbing antibodies against normal plant proteins, might also find application in other serological techniques.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Prof. Dr. Ir. J. P. H. VAN DER WANT, Director of the Department of Virology, Wageningen, for his stimulating advice; to Ir. D. H. M. VAN SLOGTEREN, Laboratory of Flowerbulb Research, Lisse, Dr. J. P. E. HERS, Hospital of Leyden University and Drs. W. C. DE BRUYN, Hospital "Dijkzigt", Rotterdam, for valuable suggestions and to Miss Drs. C. VAN DER SCHEER, Physico-Technical Service for Agriculture, Wageningen, for conducting the electron diffraction experiments.

They wish to express their appreciation to Miss J. C. M. LANGEVELD, Mr. M. MEIJERS, Mr. B. KUYPER and Mr. D. J. TIMMERMAN for technical assistance and to Dr. R. H. LAWSON, Oregon State University, Corvallis, U.S.A., for correcting the English text.

REFERENCES

BRANDES, J. &. C. WETTER, – 1959. Classification of elongated plant viruses on the basis of particle-morphology. Virology 8: 99–115.

CALDWELL, J. & A. L. JAMES, – 1938. An investigation into the 'stripe' disease of narcissus. Ann. appl. Biol. 25: 244–253.

COONS, A. H. & M. H. KAPLAN, – 1950. Localization of antigen in tissue cells II. Improvements in a method for detection of antigen by means of fluorescent antibody. J. exp. Med. 91: 1–13.

COONS, A. H., – 1958. Fluorescent antibody methods. In: DANIELLI, J. F., General cytochemical methods, Vol. 1, Academic Press, New York: 339–422.

CREMER, M. C., D. H. M. VAN SLOGTEREN & J. A. VAN DER VEKEN, – 1960. Intracellular virus inclusions in leaves of 'grey-diseased' narcissus. Proc. Eur. Reg. Conf. Electron Microscopy 2: 974–977.

FARVARD, P. & N. CARASSO, – 1957. Mise en évidence au microscope électroniques de granules protéiques régulier dans les plaquettes vitellines de l'oeuf de planorbe. Le problème de leur contraste aux électrons et de l'osmiophilie. C. R. Acad. Sci., Paris 2547: 245.

HALL, C. E., – 1953. Introduction to electron microscopy, McGraw-Hill Book Company, New York, Toronto, London.

HEIDENHAIN, N., – 1916. Über die Mallorische Bindegewebesfärbung mit Karmin und Azokarmin als Vorfärbung. Z. wiss. Mikr. 32: 361–372.

JOHANSEN, D. A., – 1940. Plant microtechnique. McGraw-Hill Book Company, New York, London.

MARSHALL, J. D., W. C. EVELAND & C. W. SMITH, – 1958. Superiority of fluorescein-isothiocyanate (Riggs) for fluorescent antibody technique with a modification of its application. Proc. Soc. exp. Biol., N.Y. 98: 898–900.

MOLLENHAUER, H. H., – 1959. Permanganate fixation of plant cells. J. bioph. biochem. Cyt. 6: 431–435.

NAGARAJ, A. N., – 1962. Fluorescent antibody staining of viral antigen in dissociated plant cells. Virology 18: 329–332.

NAGARAJ, A. N. & L. M. BLACK, – 1961. Localization of wound tumor virus antigen in plant tumors by the use of fluorescent antibodies. Virology 15: 289–294.

PEARS, A. G. E., – 1953. Histochemistry theoretical and applied. J. &. A. Churchill, London: 47.

PEASE, D. C., – 1960. Histological techniques for electron microscopy. Academic Press, New York, London.

ROZENDAAL, A. & D. H. M. VAN SLOGTEREN, — 1958. A potato virus identified with potato virus M and its relationship with potato virus S. Proc. 3rd Conf. Pot. Virus Dis., Lisse-Wageningen, 1957: 20–35.

SLOGTEREN, D. H. M. VAN, – 1955. Serological micro-reactions with plant viruses under paraffinoil. Proc. 2nd Conf. Pot. Virus Dis., Lisse-Wageningen, 1954: 51–54.

SLOGTEREN, D. H. M. VAN, – 1958. Ratelvirus als oorzaak van ziekten in bloembolgewassen en de mogelijkheden de infectie door middel van grondontsmetting te bestrijden. Tijdschr. PlZiekt. 64: 452–462.

SLOGTEREN, E. van & M. P. DE BRUYN OUBOTER, – 1946. Investigations on virus diseases of narcissus. Daffodil and Tulip Year Book, R. hort. Soc. 12: 3–20.
VEKEN, J. A. VAN DER, – 1960. Some applications of freeze-drying in virological research. Tijdschr. PlZiekt. 66: 1–11.
WALTER, F., – 1957. Studien zur Präparation pflanzlicher Objekte für die Elektronenmikroskopie in Verbindung mit einer einfachen Methode zur Herstellung von Dünnschnitten. Z. wiss. Mikr. 63: 227–235.
WORLEY, J. F. & J. R. SCHNEIDER, – 1963. Progressive distribution of southern bean mosaic virus antigen in bean leaves determined with a fluorescent antibody stain. Phytopathology 53: 1255–1257.