BEAN STIPPLE-STREAK CAUSED BY A TOBACCO NECROSIS VIRUS

Met een samenvatting:
Het stippelstreep van de boon, veroorzaakt door een tabaksnecrosevirus

BY

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In the peaty soils between Amsterdam and Leiden, pole beans (*Phaseolus vulgaris*) are extensively grown and considerable losses sometimes occur because of a necrotic disease, known as stipple-streak and caused by a soil-borne virus (HUBBELING, 8, VAN DER WANT, 13). The beans are sown in frames at the end of April or the beginning of May and are transplanted to the field when two to three weeks old. The disease usually occurs first early in June, when scattered dark brown or black spots and streaks appear on stems, petioles and veins. Often some shoots only of a plant are affected, and lesions may occur on one part of a leaf while the remainder appears normal. The necroses increase in number and size, and adjacent leaf tissues become chlorotic. Later whole stems or individual leaves wilt, and necrotic spots, rings or streaks also appear on the pods.

When sap from necrotic tissue is inoculated to healthy bean seedlings under glass, the rubbed leaves develop darkly coloured local lesions in three to four days. Within a week, black streaks appear in the petioles and stems and necrotic lesions develop on or near the veins of uninoculated young leaves. The affected plants remain stunted, have twisted and chlorotic leaves, and usually shrivel and die within a few weeks. In the winter, inoculated bean seedlings react rather differently. Local lesions again appear first as discrete spots, but, particularly if a concentrated inoculum is used, they soon coalesce and rubbed leaves rapidly shrivel. Severe necrosis develops in the stem and the plants usually wilt, collapse and die before any necrotic lesions appear on uninoculated leaves. On tobacco (*Nicotiana tabacum*) the virus causes white necrotic local lesions, spots or rings, but does not become systemic.

SEROLOGICAL RELATIONSHIP WITH ROTHAMSTED TOBACCO NECROSIS VIRUS

The symptoms of stipple-streak in naturally infected plants differ from any previously described disease of field beans, but the reactions of inoculated tobacco and bean plants suggest similarities with tobacco necrosis, which was first described by SMITH and BALD in 1935 (12). This disease has since been found to be caused by several distinct viruses, which are serologically unrelated, have particles of different sizes and inactivate under different conditions (BAWDEN, 2, BAWDEN and PIRIE, 4, 5). Some of these viruses also occur in a number of strains, differentiable by their individual crystal forms, but all that have been extensively studied have been clinically indistinguishable and have shared some unusual host-parasite relationships. They have an extensive host range, but until recently no hosts have been known in which they cause systemic diseases, and symptoms in naturally infected hosts have been noted only in tobacco and *Nicotiana glutinosa*.

In these hosts symptoms are restricted to the lowest leaves of naturally infected plants and upper leaves both look normal and appear to be virus-free (SMITH, 10). Tobacco necrosis viruses occur in the roots of such plants, as they may also do in the roots of these and many other species whose foliage has shown no symptoms. This is not because the leaves are immune; when inoculated they become infected, but the viruses are usually localised and in most hosts they produce necrotic lesions. Exceptions are *Primula* sp. and *Polyanthus* sp., in which the viruses multiply locally without causing any symptoms (BAWDEN and KASSANIS, 3), and tulip in which they not only produce necrotic local lesions but also cause a lethal systemic disease (KASSANIS, 9); the disease may cause considerable losses in naturally infected tulips and this host is the first to be discovered in which the viruses are economically important.

In addition to its local effects on tobacco and French bean, the stipple-streak virus resembles the tobacco necrosis viruses in being soil-borne and it also has a similar high thermal inactivation point (VAN DER WANT, 13). The main difference seems to lie in the greater ability of the stipple-streak virus to invade bean plants (fig. 1), and it seemed possible that it might be a variant of one or other of the

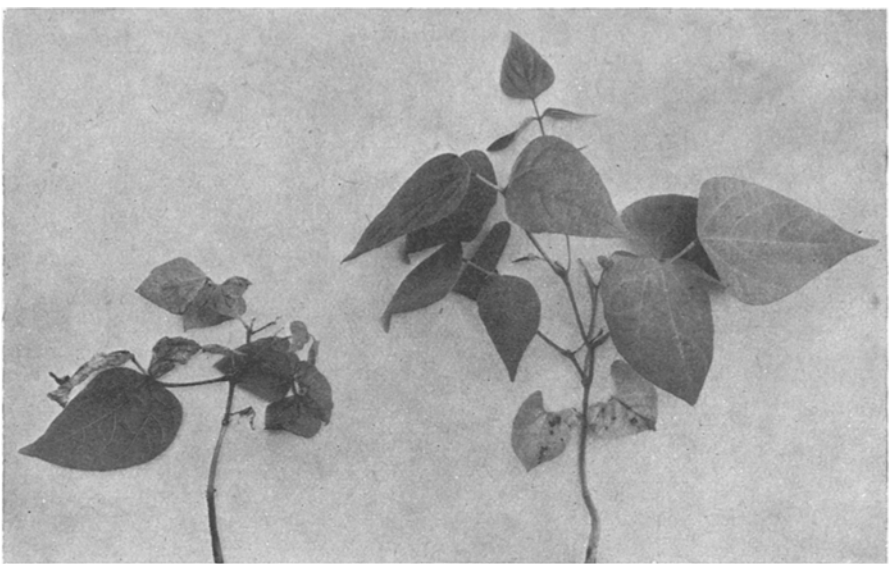

Fig. 1. Bean plants inoculated on the first leaves on the same day.
Right: with a tobacco necrosis virus obtained from Dr K. M. Smith (Cambridge); only local symptoms have developed.
Left: with the stipple-streak virus (inoculated leaves fallen off); both local and systemic symptoms have developed.
(Photografed by J. Boekhorst.)

Bonenplanten, waarvan de enkelvoudige bladeren op dezelfde dag geïnoculeerd werden.
Rechts: met een tabaksnecrosevirus, afkomstig van Dr K. M. Smith (Cambridge); er ontwikkelden zich slechts locale symptomen.
Links: Met stippelstreepvirus; zowel locale als verspreidingssymptomen zijn opgetreden.

previously known tobacco necrosis viruses. To gain information on its possible relationships with these, the stipple-streak virus was propagated in tobacco to obtain preparations suitable for use in serological tests. Sap from tobacco leaves bearing many local lesions was frozen, thawed and centrifuged; the virus was twice precipitated with quarter-saturated ammonium sulphate solution, and the second precipitate was resuspended in a volume of water equal to one-fifth that of the original sap. After centrifuging to free it from insoluble material, the supernatant fluid was titrated against antisera to the three serologically unrelated groups of tobacco necrosis viruses which BAWDEN and PIRIE (4, 5) called potato, tobacco and Rothamsted cultures. The preparation precipitated specifically and strongly with antiserum prepared against the Rothamsted culture, but not with the other two.

Although this positive serological reaction suggested strongly that stipple-streak is not a „new" virus but a strain of one already known, it was not by itself conclusive. The virus culture used had been propagated for some months in tobacco, a plant that is frequently infected naturally with tobacco necrosis viruses, and the virus responsible for the positive precipitin reaction might have been acquired as a contamination during this time. Additional experiments were therefore made to provide information on the homogeneity of the culture and to identify with greater certainty the stipplestreak virus. First, the preparation was further purified by ultra-centrifugation, which immediately showed further similarities with the Rothamsted tobacco necrosis virus. The pellets sedimented at 40,000 r.p.m., were crystalline, closely resembling pellets from the Rothamsted culture itself, and after re-solution in water the sedimented material gave a precipitin titre similar to that given by highly purified preparations of the Rothamsted culture. A visible precipitate was produced when 1 ml of diluted antiserum was added to 1 ml containing 0.005 mg of the dissolved pellets, showing that the material serologically related to the Rothamsted tobacco necrosis virus formed the bulk of the preparation and was not a minor constituent. This fact was further confirmed by examination with the electron microscope. The particles of purified preparations of the Rothamsted tobacco necrosis virus are spheres, about 17 mμ in diameter, and are smaller than other known plant viruses. Most particles in the purified preparation of stipple-streak were also about 17 mμ in diameter, but there were some larger ones (fig. 2).

Some purified preparations of the Rothamsted tobacco necrosis virus that have been examined in the ultra-centrifuge have also been found to consist predominantly of small particles but to contain some larger ones (BAWDEN and PIRIE, 4). Whether the two represent different forms of one virus or are two different viruses is unknown, for preparations of the virus readily lose infectivity and the relationship between the characteristic crystalline material and infective virus remains uncertain. To determine whether the large or small particles are specifically concerned in the production of stipple-streak, the virus was passed through beans and recovered from systemic lesions. The first-formed leaves of French bean seedlings were inoculated with the purified preparation, and when lesions developed in the stems, the necrotic stem tissue was macerated separately and used as inoculum to infect new tobacco plants. On repeating the purification procedure with sap from these plants, ultracentrifugation again produced the characteristic crystalline pellets and the preparation again gave the customary precipitin reaction and titre with antiserum to the Rothamsted virus, while failing to react with

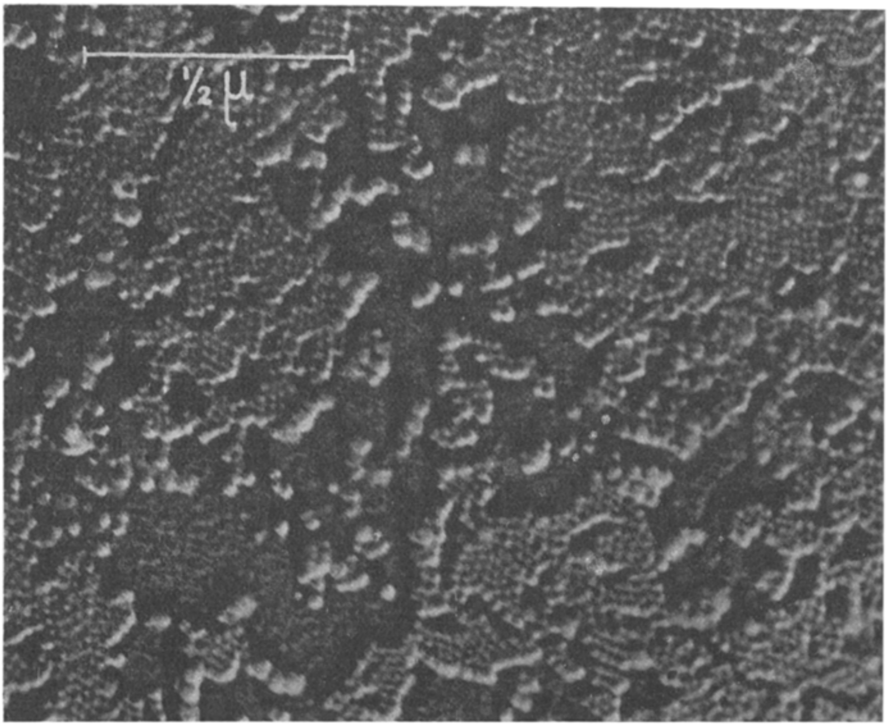

Fig. 2. Electron microgram of purified preparation of bean stipple-streak virus, shadow-cast with nickel. Most of the particles are about 17 mμ diameter, but some are larger. (Microgram made by H. L. Nixon).
Electronenfoto van gezuiverd stippelstreepvirus, „shadow-cast" met nikkel. De meeste deeltjes zijn ongeveer 17 mμ in diameter, maar sommige zijn groter.

antisera to the other tobacco necrosis viruses. This established that a virus causing stipple-streak and capable of invading uninoculated bean tissues is serologically related to the Rothamsted culture, but it did not settle the problem raised by the occurrence in the first preparation of particles of two sizes. When the second preparations were examined with the electron microscope, they resembled the first ones and, although spherical particles of approximately 17 mμ predominated, there were again some larger particles. To decide whether there are two viruses with particles of different sizes that can cause stipple-streak or whether the two particles are alternative froms of one virus, will require extensive studies on isolates propagated from single local lesions, and this has not yet been attempted.

The Rothamsted culture differs from other tobacco necrosis viruses in not crystallizing from ammonium sulphate solution in the cold; crystals do develop, however, from concentrated salt-free solutions allowed to stand for long periods. The stipple-streak virus similarly produces only amorphous precipitates with ammonium sulphate, but insufficient material has yet been obtained to know whether concentrated solutions in water would form crystals.

Most viruses that have been studied in any detail have been found to be not single units but to compromise many different variants. Such variants or strains usually have similar physical, chemical and serological properties, but are distinguishable from one another because they cause different symptoms in one or more hosts. The tobacco necrosis viruses previously studied have been anomalous in this respect, for with one exception all the cultures studied have been clinically indistinguishable, although different ones have been found to differ widely in other characters. The exception was mentioned by SMITH (11), who found that one culture, after continuous passage through French bean for more than a year during which it consistently produced only local lesions, began to invade systemically, producing lesions on the young uninoculated leaves and killing whole plants. This may well have been similar to, if not identical with, the bean stipple-streak virus. Unfortunately this variant was not further studied, but it is known that SMITH's cultures contained a mixture of viruses and included forms serologically related to the Rothamsted one (BAWDEN, 2), from which the invasive form may have arisen.

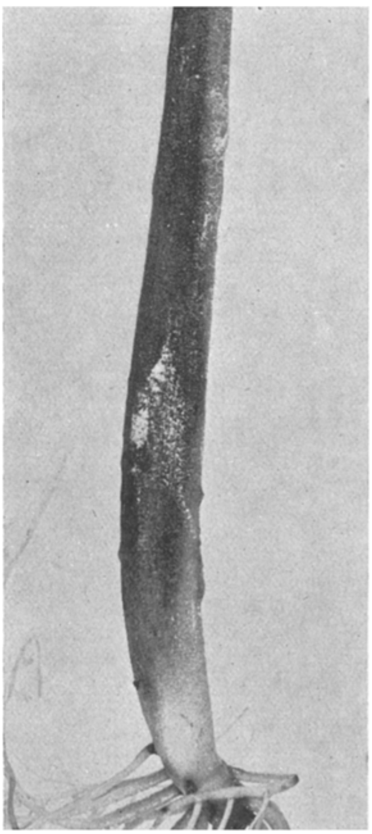

Fig. 3.
Necrotic lesion spreading up the stem of a French bean seedling, inoculated with stipple-streak virus in the roots.
(Photografed by V. Stansfield.)
Voortschrijdende necrotische vlek op de stengel van een bonenplant, waarvan de wortels met stippelstreepvirus werden geïnoculeerd.

The stipple-streak and Rothamsted culture of tobacco necrosis virus can be distinguished from one another by their effects on beans, but not by any other tests yet made. Both produce local lesions in beans at about the same time and at first these are very similar, but leaves rubbed with the stipple-streak soon become wholly necrotic and shrivelled whereas those rubbed with the Rothamsted virus collapse less rapidly, the lesions remaining separate for longer and the spread of necrosis along the veins occurring more slowly. Their subsequent behaviour separates them most definitely. The Rothamsted virus rarely moves from inoculated leaves into the petioles and main stems, and only occasionally does an isolated lesion occur on uninoculated leaves. By contrast, most of the plants rubbed with the stipple-streak virus develop stem lesions and either die rapidly or the young leaves become infected and produce lesions. In tobacco the two behave in the same manner; in most plants lesions occur only on rubbed leaves, but both occasionally cause a ringspot lesion to develop on or near a vein of an uninoculated leaf.

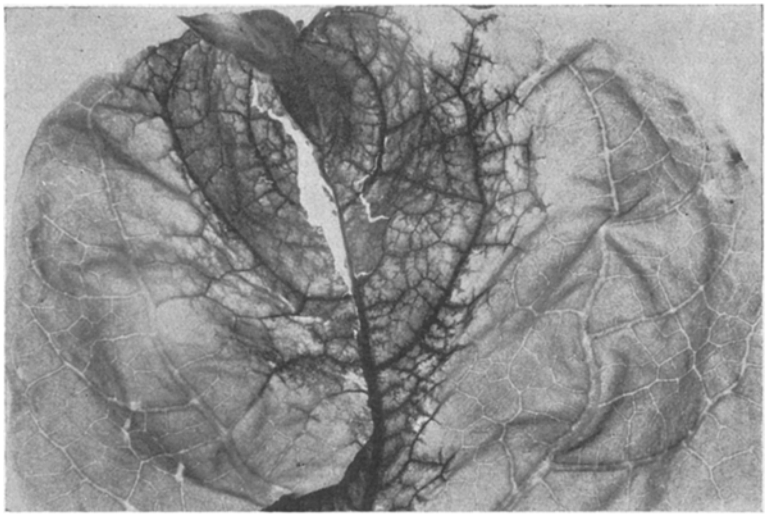

Fig. 4. Necrosis spreading along and from the veins of a bean leaf on a plant whose roots were inoculated with stipple-streak virus.
(Photografed by V. Stansfield.)
Necrose, die zich uitbreidt langs nerven en aangrenzend bladmoes bij een bonenplant, waarvan de wortels met stippelstreepvirus werden geïnoculeerd.

VAN DER WANT (13) has shown that the stipple-streak virus is soil-borne and that the disease can be prevented by soil sterilisation. There is also much evidence that the tobacco necrosis viruses are soil-borne, of which perhaps the most relevant is that plants commonly occur with infected roots although no virus is detectable in their stems and leaves. Hence outbreaks of stipple-streak probably occur because, unlike other tobacco necrosis viruses which remain localised around their entry points (BAWDEN and KASSANIS, 3), this virus can move from roots into stems and leaves. To test this, bean plants were grown in water culture solution, and when the first leaves were developing, the main roots were rubbed with infective sap containing a little kieselguhr to facilitate transmission. Six plants were rubbed with stipple-streak and six with the Rothamsted tobacco necrosis virus. All the rubbed roots showed brown spots within five days, but no other symptoms were produced by the Rothamsted virus in the four weeks the plants were kept under observation. By contrast, within a week of inoculation, brown lesions developed at the stem bases of five of the six plants inoculated with the stipple-streak virus and these spread gradually upwards (fig. 3). Young leaves on three of the plants also developed lesions, which started on a vein and spread to the nearby tissues, leading to their discoloration and collapse (fig. 4). In naturally infected plants in the field symptoms may not appear until some weeks after the beans have been transferred from contaminated to clean soil, suggesting that movement of the virus from infected roots to tops occurs more slowly than in the tests under glass. Much further work will be needed to understand the conditions that determine whether or not movement occurs, but it may be facilitated by high temperatures, which would account for the disease being prevalent in July and August.

Tobacco necrosis viruses show many unusual properties, but the most important difference between them and other viruses that have been studied is their general failure to cause systemic infections. The reasons for this are unknown, but it cannot be attributed entirely to the fact that they are localised because of the death of cells first invaded. The stipple-streak virus causes equally severe local lesions in bean leaves from which it moves as in those from which it does not, and there is nothing to suggest that it kills infected cells any less rapidly than do other tobacco necrosis viruses. In *Primula obconica*, too, systemic infection does not occur, though this host is a symptomless carrier (BAWDEN and KASSANIS, 3); the viruses multiply locally at their entry points into roots or leaves, but movement over distances occurs rarely and then leads to infection of only isolated pieces of tissue, a result comparable with the occasional lesions that develop on uninoculated tobacco leaves.

There is considerable evidence that viruses which cause systemic infections move from their initial entry points through petioles and stems in the phloem, and it may be that their ability to enter and leave the phloem freely depends on their ability to multiply in it. The tobacco necrosis viruses probably occasionally enter and escape from the vascular tissues, for this is the simplest explanation of the lesions sometimes produced at points remote from inoculation sites, and their inability to become fully systemic may reflect the fact that they cannot multiply in the phloem. If this is so, the greater invasiveness of the stipple-streak virus may reflect some difference from the other tobacco necrosis viruses in its requirements that allows it to multiply to some extent in phloem cells. The ability of all the tobacco necrosis viruses that have been inoculated to tulips to invade this species more thoroughly than other hosts, may also mean that conditions in the phloem of tulip are more favourable for multiplication. Little or nothing is known of the growth requirements of plant viruses, but the susceptibility of plants to infection with tobacco necrosis viruses, and the extent to which they multiply, does vary greatly with changes in the physiological condition of the host (BAWDEN and ROBERTS, 6, 7). Also, it is known that cultures of certain bacteria that are normally insusceptible to a bacteriophage become susceptible if tryptophane is added to the medium (ANDERSON, 1). Thus only slight differences in the constitution of cells may determine whether or not a virus can become established and multiply.

Whatever the reasons for tobacco necrosis viruses remaining localised in most infected plants, the fact is of great economic importance. The viruses occur so widely in soil and have such extensive host ranges that, could they produce systemic infection in a range of plants, they would inevitably cause great losses. How they get introduced into uncontaminated soil, and how they are maintained there, remains undetermined, but their rapid re-entry into sterilised soil suggests that they may have as hosts some of the normal soil-inhabiting flora (BAWDEN and KASSANIS, 3). Once present in a soil, localised infections in the roots of their many host plants might suffice to perpetuate, or even increase, them, but the presence of plants such as tulip, which they invade more extensively, would obviously increase their numbers far more rapidly. The production of a strain that is more invasive than others towards certain hosts will lead to this being differentially favoured by the presence of these hosts. It seems that a strain unusually invasive in beans arose during prolonged passage in this host in Cambridge (SMITH, 10), and that the stipple-streak virus is probably another example of this occurring in some parts of the Netherlands where too frequent cultivation of beans has allowed it to accumulate to dangerous amounts.

Until now tobacco necrosis viruses have been studied largely because of their great academic interest, but the recent experiences of their serious effects in tulip and French bean show that they also deserve attention because of their potential economic importance. At present too little is known about how they are disseminated and cause infection for any certain control measures to be recommended, except that soil sterilisation gets rid of them temporarily and an adequate rotation of different kinds of crops might prevent actively pathogenic forms from increasing unduly.

SUMMARY

Stipple-streak of French bean, a serious disease in some parts of the Netherlands, is caused by a strain of the Rothamsted tobacco necrosis virus. The two viruses are serologically related, crystallize in the same manner and have particles of similar sizes, but differ in the manner in which they affect beans. The Rothamsted virus rarely spreads beyond inoculated leaves, whereas the stipple-streak virus does. Stipple-streak virus can also invade stems and leaves when introduced into roots.

SAMENVATTING

Het stippelstreepvirus van de boon veroorzaakt na inoculatie op bladeren van boon en tabak locale necrosen, die niet te onderscheiden zijn van de locale symptomen, door verschillende tabaksnecroseviren op deze planten teweeggebracht. Het stippelstreepvirus verspreidt zich evenwel in de bonenplant en doet na verloop van tijd ook afstervingsverschijnselen op de niet-geïnoculeerde delen van de plant ontstaan (fig. 1). Het virus dringt ook binnen in stengel en bladeren als de wortels van bonenplanten er mee geïnoculeerd worden (fig. 3 en 4). Dit in tegenstelling tot de tabaksnecroseviren, die slechts in zeldzame gevallen tot verspreidingssymptomen aanleiding geven. Op tabak blijven zowel het stippelstreepvirus als de tabaksnecroseviren gelocaliseerd.

Bij onderzoek van het stippelstreepvirus met de in het Rothamsted Experimental Station aanwezige antisera van tabaksnecroseviren, bleek het virus serologisch verwant te zijn aan het zgn. Rothamsted tabaksnecrosevirus. Verder werd geconstateerd, dat het stippelstreepvirus op dezelfde wijze uitkristalliseert als het Rothamsted virus en dat de deeltjes van beide viren even groot zijn (fig. 2). Daar beide viren overigens slechts verschillen in het vermogen om zich door de bonenplant te verspreiden, wordt geconcludeerd, dat het stippelstreepvirus een variant van het Rothamsted virus is.

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EXPERIMENTS WITH A MOSAIC OF SHALLOTS

Met een samenvatting: Proeven over een mozaiekziekte in sjalotten

BY

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„Soft shallots" have been known in Denmark for more than 30 years; before inspection of propagating material was established by the horticultural organizations, plots with more than 50 per cent of diseased plants were not rare.

The plants attacked by mosaic are small, the leaves flattened and corrugated, distorted, flaccid, and as a rule with yellow stripes (a coloured plate is to be found in GRAM & WEBER: Plantesygdomme, plate 9).

Experiments by H. RØNDE KRISTENSEN on the heat stability of the virus indicate that *Cucumis virus 1* cannot be the pathogen of this mosaic, which is probably caused by *Allium virus 1*. Treatment of the bulbs with dry heat, 24 hours at 40 °C or 8 hours at 45 °C, did not cure the disease.

In 1948 the „Onion Section of Jutland" inspected the shallots of about 225 growers who had planted 35000 kg of shallots for propagation; one third of the lots were entirely free from mosaic. Any lot which showed more than one half per cent of mosaic plants as a total of percentages found at first and second inspection, was rejected.

In some plots of shallots grown for experiments on the control of *Peronospora destructor* (BERK.) CASP. about 5 per cent of the plants were attacked by mosaic in 1945. On the 2nd July a series was lifted consisting of the first, second and third