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Methods of quality assessment of seed potatoes

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Introduction

The yielding capacity of the potato depends largely on the quality of the seed. The quality is determined by a number of factors such as absence of pathogens (viruses, fungi, bacteria, nematodes), physiological stage of development, place of origin, authenticity and purity of variety. In addition size-grading and the prevalence of defects, such as malformed or damaged tubers, may affect yielding capacity.

Not all the factors mentioned are of equal importance. Since freedom from pathogens counts very much in quality assessment of seed, methods and difficulties in determination of the presence of pathogens will be discussed extensively; whereas size-grading and defects in tubers, caused by handling, effect of cold or frost, green tubers, and necrotic spots caused by spraying herbicides will be excluded from discussion. Neither will the ways of sampling be discussed. One should keep in mind, however, that sample size is determined by the criteria which the seed material must fulfil. For instance, if one diseased tuber is found in a sample of 6600 or 600 tubers there is a 95% probability that the batch of potatoes will not contain more than 0.1% or 1% diseased tubers, respectively. It is clear that samples of a few hundred tubers/ha are too small to give reliable information on the real level of infection. In practice, however, checking of large samples of potato tubers is very difficult. A survey is given of methods of disease assessment in the field during growing season and after lifting. Only the most important diseases are treated. Some remarks are made on the effect of physiological stage of development and of place of origin of seed on yield. Methods to check authenticity are mentioned briefly. Finally, it is pointed out which methods should be developed and improved (see Conclusions).

For requirements of the officially recognized grades (basic and certified) of seed in the European Economic Community, see Jacobsen (1973).

Before discussing methods of assessment of diseases caused by pathogens it must be pointed out that such assessment has to reckon with: 1. The danger of transmission of the disease by the seed tubers to the next crop. 2. The influence of the pathogens on the keeping quality of the seed tubers. The greater the danger of transmission or (and) the influence on keeping quality, the more important the assessment. The danger of transmission is great if the disease is only or mainly tuber-borne as with most virus and several bacterial and fungal diseases. This danger may still be of importance if

the disease is tuber-borne as well as soil-borne, but less so if the disease is mainly soil-borne.

The keeping quality is decreased by bacterial and fungal pathogens, either by causing rots or by decreasing the germination capacity of the tubers. Also the appearance of the tubers may be affected. Most potato viruses do not have such an influence on keeping quality.

Methods of assessing diseases during growing season

Viruses

Generally viruses are systemic. Viruses which are present in the foliage of a crop will also be present in the tubers (seed). Moreover, most viruses will induce fairly clear symptoms on the potato. This is especially true when plants are secondarily infected. Therefore a visual observation for the presence of virus symptoms during the growing season will give a good impression of the health of the crop as far as viruses are concerned. This field inspection may be repeated two or three times per growing season (Hiddema, 1968, 1972).

The inspector searches through the field to determine whether the health attains the figures for the highest grade the crop can reach. At four places in the field he examines 100 plants and counts how many are diseased. The figure for each disease is multiplied by a varying factor. The sum of the products (incidence $\times$ factor) is the disease index of the field (Dutch system, Hiddema, 1972).

Looking for symptoms caused by a pathogen and counting the diseased plants per unit is the oldest way of quality assessment, and actually is the basis of maintaining quality in each seed producing country. Although no exact figures are known it must be considered as a reliable method for producing certified seed.

The advantage of a field inspection is that the inspector has the possibility to observe many plants at the same time. Although he has to check thoroughly some hundreds of plants only, in fact he observes some thousands. Visual observation is a universal method. It is applied for all viruses. The field inspector has to be a trained specialist and knowledge of the occurrence of virus symptoms can be obtained only after a long experience. However, the appearance of symptoms, especially those induced by viruses, may be masked due to climatic conditions. Absence of virus symptoms may also be due to a late infection. In the latter case detection of the virus by its symptoms in the foliage will be very difficult, since a plant might be partially infected only. Hence, especially in countries where large masses of aphids are present at the end of growing season the assessment of virus infection according to visual observation will not agree with the real virus infestation.

During the growing season tubers on the plants are not checked for the presence of viruses. Virus symptoms can usually not be observed visually in tubers. Moreover, it would be too time-consuming and too unreliable.

When symptoms on the foliage of secondarily infected plants during growing season

are masked, various laboratory methods can be applied.

For the detection of potato leafroll virus (PLRV) the callose test is used. The test is mainly used for detection of PLRV in potato tubers, but in addition it could be applied on the stems of field grown plants as a help to the field inspector's work. However, due to practical difficulties, checking for the presence of excessive callose in sprouts or stems of potato has not yet been included in routine procedures. This is the more remarkable since Igel & Lange (1953) developed the test especially for checking stems and sprouts. Sprau (1957), however, mentioned that reliable testing was possible on stems but not on sprouts. Kratchanova, de Bokx & Bakker (unpublished) found that excessive callose could be detected reliably in stems of 'Bintje' and 'Eersteling' infected with PLRV. This was true for plants grown in greenhouses and in the open, even when the plants did not show leafroll symptoms. Sprau (1957) mentioned that sieve tubes in the petioles of leafroll-infected potato plants showed more callose then those in leafroll-infected stems. This was confirmed by Foschum et al. (1973). They found that using the petioles of plants in tuber indexing for detection of PLRV was highly reliable. From more than 3100 leafroll-infected plants only 1.5% were not diagnosed correctly Although they did not make a comparison between results of testing leafroll-infected tubers and petioles it may be concluded that checking petioles for the presence of callose is as reliable as or even more reliable than checking tubers. The test can be carried out quickly (see p. 417). However, the cut pieces of the stems will curl which makes observation under the microscope difficult.

Checking for the presence of potato aucuba mosaic virus (PAMV), potato virus A (PVA), potato virus M (PVM), potato virus S (PVS), potato virus X (PVX) and potato virus Y (PVY) can be carried out with serological methods (see p. 418). Wetter (1957) carried out experiments with PVS. He found that PVS could be detected reliably using the precipitin test when just unfolded top leaves were tested at the time the plants started flowering. De Bokx (1967b) obtained similar results with 'Eersteling' plants infected with PVS and PVX. Also test plants can be used (see p. 419). Both methods are highly reliable, but will take more time than the visual observation. Additional testing with these tests is only done for foundation stock.

Bacteria and fungi

Assessment of jungal diseases in the field is less frequently carried out than assessment of virus diseases, as the fungal diseases are not systemic and the haulm of the potato often is not or only slightly attacked. Assessment of most bacterial diseases of the potato in the field is necessary as bacterial pathogens often attack the aboveground as well as the underground parts of the plant.

Inspection is carried out by visual examination, though in doubtful cases laboratory methods are needed for identification of the disease (see p. 421).

The symptoms caused by black leg (*Erwinia carotovora* (Jones) Bergey et al. var. *atroseptica* (van Hall) Dye) are not always typical, but may resemble those of other potato diseases (Anon., 1971a). As has been mentioned laboratory methods are needed

then to identify the disease. The degree of attack by black leg may be expressed as a percentage of affected plants.

The symptoms of ring rot (*Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh.) usually appear late in the season, but can be obscured by the symptoms of blight or drought (Dounine, 1961). The disease has not been found in several west-European countries. If it does occur in west Europe, the incidence is generally low. According to Nilsson (1961) the finding of ring rot in a growing crop is very rare in such a case and assessment of the disease in tubers much more important as the symptoms in tubers are much easier to detect.

Brown rot (*Pseudomonas solanacearum* (E. F. Smith)) does not occur in the temperate zones of Europe, but may be imported. The symptoms of brown rot in the field are described by Granhall (1961). However, as with black leg and ring rot, in cases of doubt, identification is only possible with the help of laboratory methods.

If ring rot or brown rot is found in a potato field, this field is disqualified for the production of seed as these dangerous diseases are transmitted by the tubers.

Late blight (*Phytophthora infestans* (Mont.) De Bary) and stem canker and black scurf (*Thanatephorus cucumeris* (Frank) Donk (stat. myc. *Rhizoctonia solani* Kühn)) are two fungal diseases which cause symptoms in the aboveground parts of the potato plant. Often these symptoms are typical, but identification may sometimes be difficult. In such cases laboratory methods may be used to isolate and identify the causal pathogen. These methods may not always be successful because the pathogen is not present any more in a live state in the diseased tissue. However, failures in identification with late blight or stem canker and black scurf are not so important as with diseases, such as ring rot, which have to be eliminated. For assessment of degree of attack by late blight, descriptive keys may be used, e.g. the 'BMS' scale (Anon., 1947). However, tuber infection may be independent of the severity of the haulm attack (Lapwood, 1965), and therefore disease assessment for late blight in seed tubers after harvest is more important than assessment on the haulm. The same is true for stem canker and black scurf.

Methods to assess diseases in tubers after lifting

Visual examination

Viruses. Batenburg & Goettsch (1960) found that tubers of varieties Bintje, Eigenheimer, Furore and Urgenta showed light spots in the flesh when infected with PVY^O or PVY^C. So far this type of symptom has not been used for identification yet.

Martin & Quemener (1956) observed that tubers infected with mosaic viruses (PVX or PVY) could be recognized by the irregular spotty coloration which, after exposure to light, developed on sprouts grown in the dark. If the symptoms are very marked (heavy crinkle or streak), especially with mixed X and Y infections, necroses appear on the sprouts in addition to or instead of the coloured spots, especially at their base (Wenzl, 1959, 1962a, 1962b). Experiments were performed with varieties

Falke, Prof. Broekema, Fina, Eersteling, Delos and Sirtema. Wenzl suggested, when examining potato tubers for mosaic virus infection, that necroses could be regarded as an additional symptom to the irregular spotty coloration.

Hiddema & de Bokx (unpublished) found the method unreliable when applied to Dutch potato varieties.

Bacteria and fungi. Assessment of seed tubers by visual examination for bacterial and fungal diseases is important, as all bacterial and fungal pathogens attack the tubers and/or contaminate them. Often distinct symptoms are produced. The inspector will take random samples for examination from the seed tuber lot, e.g. one sample from one bag out of twenty-five. He may also inspect the seed lot during grading. If necessary, tubers are cut through to examine them for internal symptoms. Sometimes, e.g. with skin spot (*Oospora pustulans* Owen & Wakef.) or silver scurf (*Helminthosporium solani* Dur. & Mont.), the tubers should be washed before examination. Symptoms may be so characteristic that they are sufficient for identification of the disease, but often this is not the case. For identification it is necessary then to make use of laboratory methods (see p. 421).

The degree of attack may be assessed as a percentage of affected tubers, as is usual in the case of tuber rots. If the tuber is attacked only superficially, pictorial keys or scales, representing different degrees of attack, may be used, as e.g. with common scab (*Streptomyces scabies* (Thaxter) Waksman & Henrici). Bacterial soft rot is often the result of infection by *Erwinia carotovora* (Jones) Bergey et al., or *E. carotovora* var. *atroseptica*. Other bacteria, e.g. *Pseudomonas* spp. and *Bacillus* spp., although isolated occasionally, are not thought to be of general importance (Boyd, 1972). There is no clear difference in symptoms between soft rot caused by *E. carotovora* or by *E. carotovora* var. *atroseptica*, though var. *atroseptica* may cause hard rot under certain circumstances (Logan, 1964). Identification of soft rot caused by var. *atroseptica* is important, as black leg is transmitted by the seed tubers (Boyd, 1972). For identification use must be made of laboratory methods. Black leg is also transmitted to the crop if the seed tubers are contaminated, but remain free from infection (Pérombelon, 1969, 1972). Methods by which contamination can be detected are not applied yet in practice, but would be very useful, especially if it is the aim of a seed certification programme to reduce black leg (see p. 423).

Granhall (1961) gives a description of the symptoms of ring rot in tubers. The tubers should be examined by cutting them across near the heel end. However, it still is difficult to detect the disease in the tubers, as often the symptoms are not typical. At least 600 tubers of 10 tonnes of potatoes should be cut and examined (Nilsson, 1961). Even more tubers could be necessary (Simonsen, 1968). Techniques for rapid identification of *C. sepedonicum* are very important therefore.

Common scab (*S. scabies*) causes a superficial attack, which does not affect the keeping quality of the tubers, except when infection is serious. The disease is generally considered to be predominantly soil-borne. Transmission of common scab by seed tubers is only important on newly cultivated land (Labruyère, 1971, p. 23). Neverthe-

less the disease is important as many countries have strict regulations regarding the occurrence of scab on imported seed. Several types of scab are recognized such as superficial scab, deep scab and russet scab. For disease assessment pictorial keys may be used, which illustrates different grades of infection for different types of scab. Large & Honey (1955) used a key based on the proportions of the surface area of the tubers affected.

Generally speaking, such keys are satisfactory for the purpose for which they are made as they enable reasonably accurate assessments of disease incidence to be recorded numerically. The symptoms of common scab are typical. Usually there is no need to isolate the causal organism in order to identify the disease.

Wart disease (*Synchitrium endobioticum* (Schilb.) Perc.) is now quite exceptional on seed potatoes (Noble & Glynne, 1970). The symptoms are quite typical (Anon., 1969), though similar symptoms may be produced by powdery scab (*Spongospora subterranea* (Wallr.) Lagerh.) and microscopical examination may be necessary to distinguish between the two diseases (Anon., 1970). Infection by wart disease can be so slight, that it is easily overlooked, however. At relatively high humidity infected eye tissue develops into warts, but in seed tubers, set to sprout in low humidity, infection may remain dormant until after the tubers are planted (Noble & Glynne, 1970). A physiological disorder, causing abnormal germination, resulting in wart-like symptoms may also be confused with wart disease (de Lint & Leeuwenburgh, 1967, p. 78) but microscopical examination will easily distinguish between the two diseases. Seed lots, contaminated with the wart disease organism, are not accepted for certification.

Late blight (*Phytophthora infestans* (Mont.) De Bary) is mainly tuber-borne under west-European conditions. However, if we except the situation in Norway (Førsund, 1960) it is not the aim at present of certification programmes to free seed lots from diseased tubers, as this is not feasible under the conditions in practice and because there are other sources of contamination, like infected tubers on cull piles. The symptoms of late blight in tubers may be confused with those caused by the tuber eelworm, *Ditylenchus destructor* Thorne, or by *Phytophthora erythroseptica* Pethyb. It is possible to identify late blight by storing infected tubers, cut lengthwise, under humid conditions for a week. After this time *P. infestans* will grow on the cut surface, provided living mycelium is still present in the infected tissues. Slight symptoms are dangerous as they may escape attention. If such symptoms are present the tubers should be placed for some time at a higher temperature to be able to recognize the symptoms better.

Dry rot (*Fusarium* spp.) is a typical storage disease, which is the more serious the longer the tubers are stored. The disease is soil-borne (Small, 1944; Foister et al., 1945) but also tuber-borne (Ayers & Robinson, 1956; Boyd, 1970). Various species of *Fusarium* are capable of causing dry rot, though *F. solani* var. *coeruleum* (Sacc.). Booth is the most common one in West-Europe (Boyd, 1972). Symptoms of infection with each of the *Fusarium* spp. show minor differences. Dry rot may be confused with gangrene (*Phoma exigua* Desm. var. *foveata* (Foister) Boerema) and with soft rot, if secondary soft rotting organisms hasten the decay of the tubers. Isolation of the causal pathogen may therefore be necessary to identify the disease. A difficulty with the

assessment of dry rot is that certification of seed tubers often takes place during the early time of storage, when dry rot may not have been developed or only to a slight extent. Disease assessment at that time does not give information on the keeping quality of the seed lot as affected by dry rot. A method by which this difficulty could be overcome would be very desirable (see p. 423).

Gangrene, caused by *P. exigua* var. *foveata* is tuber-borne, but may also be soil-borne (Boyd, 1972), though the role of soil-borne inoculum in the epidemiology of this disease is not clear. Like dry rot, gangrene is a storage disease. Its importance has increased very much during the last ten to fifteen years. Gangrene may also be caused by *P. exigua* var. *exigua* (Foister) Boerema, but this fungus is less dangerous. Isolation and identification of the causal pathogen is necessary to distinguish between gangrene and dry rot, and also to distinguish between rots caused by var. *foveata* or by var. *exigua*. Gangrene, caused by var. *foveata*, is transmitted to the next crop by infected seed tubers, but also by contaminated tubers which are symptomless (Todd & Adam, 1967; Khan & Logan, 1968). As visual assessment only takes into account tubers with gangrene symptoms it is not possible to guarantee freedom from contamination by seed certification. Also, as with dry rot, disease assessment at an early time during storage, when gangrene has not been developed, or only to a slight extent, does not give information on the keeping quality of the seed lot as affected by gangrene. A method by which these difficulties probably could be overcome, is mentioned on p. 423.

Skin spot (*Oospora pustulans* Owen & Wakef.) causes superficial spotting of the tubers and killing of the eyes. Like dry rot and gangrene, skin spot is a disease which develops during storage. The symptoms of skin spot are typical (Anon., 1971b), though in cases of doubt laboratory methods are needed for identification. It is not clear to what extent the fungus can persist in the soil, although microsclerotia have been found (Hirst et al., 1965), but it is generally accepted that transmission from the seed tubers is largely, if not entirely, responsible for tuber infection (Boyd, 1972). As with dry rot and gangrene assessment of skin spot at an early time in the storage period is difficult, as the disease develops later on during storage (Boyd, 1972).

Silver scurf (*Helminthosporium solani* Dur. & Mont.) is very common on potato tubers. It causes a slight blemish of the skin, which may render the tubers unattractive (Busch, 1958). In severe cases tubers are shrivelled and sprouting vigour inhibited (Mooi, 1968). Silver scurf is tuber-borne. The role of soil inoculum in the epidemiology of the disease is not clear, but if soil contamination, with a crop rotation of three years or more, does exist, it can only be very slight (Mooi, 1968). The symptoms of silver scurf (Burke, 1938) are most obvious towards the end of the storage period. For disease assessment tubers should be washed. As the symptoms may not be well developed laboratory methods could be used to identify the disease. For disease assessment the percentage of affected tubers as well as the proportion of the surface area of the tubers affected by silver scurf, is taken into account.

The symptoms of black scurf and stem canker (*T. cucumeris*, stat. myc. *R. solani*) on the tubers (Anon., 1968) cannot easily be confused with those of other tuber disea-

ses, but in cases of doubt a lens can be used to investigate whether the brown mycelial threads of *R. solani* are present on the tuber surface. Black scurf and stem canker is tuber- as well soil-borne, but according to van Emden et al. (1966) tuber-borne inoculum is an important factor in the epidemiology of the disease. Disease assessment of the tubers is visual and may be carried out with the help of a pictorial key, representing e.g., respectively, light, medium and severe infestation. If necessary, tubers should be cleaned before assessment. The percentage of tubers suffering from the different degrees of infestation determines the quality of the seed lot. Only those lots which are infested to a low degree may be accepted for basic seed. Seed tubers often are treated with fungicides to free them from contamination with *R. solani*. Such a treatment will only result in complete disinfection if no tubers bearing thick sclerotia are present (van Emden et al., 1966). However, even seed lots which are only slightly infested, may contain tubers with thick sclerotia. Disinfection of such seed lots gives disappointing results.

Laboratory methods

Viruses. Despite all care, field inspection gives no guarantee of health. Therefore an extra safeguard, tuber indexing, can be included into the system to check the quality of seed. In countries like Scotland, tuber indexing is not included into the certification scheme, due to the absence of aphids (Hiddema, 1968). Because of the large areas to be checked, samples of 200 or 100 tubers per ha seed are taken. In principle all seed is indexed for the main viruses, viz PLRV, PVA, PVM, PVS, PVX and PVY.

Direct checking on tubers can be performed for the presence of PLRV only with the callose test. From the tubers to be checked longitudinal slices 2–3 mm thick and 3–4 mm wide are cut from the heel end of the potato with a 'double' knife or a mounted razor blade. Slices are stained in a 1% aqueous solution of resorcin blue for 10 min. The sections are then examined under × 25 magnification for the presence of callose which is stained deep blue (de Bokx, 1972). To prepare the stain the following method can be used: 10 g resorcin (metadioxybenzolum) are dissolved in 1000 ml distilled water and 12 ml 25% ammonia added. This mixture is kept in a large open container at room temperature for 10–14 days, by which time the dye is greenish blue and is ready for use.

Opinions differ on the value of the callose test as a routine check. Several investigators consider it very unreliable (Tahon, 1958; Hamann, 1959; Scheibe, 1959; Broadbent & Heathcote, 1960). Arenz (1961), Bérces & Keller (1966) and Sardiña et al. (1958) concluded that the test was satisfactory for diagnosing the virus in tubers from plants infected during the growing season. Bajlova-Yankulova (1961) found that a high percentage of tubers of the varieties Katahdin, Bintje and Eersteling, subsequently found to be free from infection, showed abnormal callose but tubers which did not show abnormal callose were generally free from leafroll virus.

The results obtained with the callose test depend on the skill of the operator and the potato variety. Scheibe (1959) mentioned for instance that in the varieties Eersteling

and Ackersegen 81.8 and 22.7% of the PLRV-infected tubers were detected correctly. The reliability of the test increased as the level of virus infection of the crop also increased. Hamann (1959) concluded therefore that the callose test could be used for certification of certified, but not for that of basic seed.

The test is applied in several Western European countries. Efforts to increase the reliability of the test have had some success (de Bokx, 1967a; Wenzl & Foschum, 1969). It has not proved possible to detect PVY in potato tubers with the callose test (Scheibe 1959; de Bokx, 1967a; Wenzl, 1969).

For the detection of PVM, PVX and PVY and sometimes PAMV serological methods are generally applied (Wetter, 1965). So far not much has been published about production and use of an antiserum against PVA (Bartels, 1963; Spire, 1970). The agglutination and precipitin tests are mostly used for practical application (van Slogteren, 1972). Various modifications of those tests are known (Vulic & Arenz, 1962; Vulič, 1963; Hamann & Zschüttig, 1965; Vulič & Hunnius, 1967b).

The gel-diffusion test (Purcifull & Shepherd, 1964; van Regenmortel, 1966) is most suitable for isometric viruses and rod-shaped viruses with lengths up to 600 nm. Since most potato viruses are longer, results with this test are not satisfactory, although PLRV is isometric (diam. 32 nm) and should be detectable. However, it has for long proved impossible to produce an antiserum against PLRV (Beemster, 1955), although Japanese research workers have recently claimed to have produced such an antiserum (Murayama et al., 1973). The availability and applicability of a good antiserum against PLRV would be a help in field inspection and tuber indexing but such an antiserum has not yet been used on a practical scale.

Checking serologically for the presence of PVX in potato tubers often leads to negative results (Hoyman, 1951), although Prochal (1953) claimed he could detect PVX in tubers using a serological method.

Staszewicz (verbal communication) claimed that he could detect virus in tubers serologically. Using sap extracted from tubers he could detect 90% of the tubers when infected with PVM or PVS and 50% of the tubers when infected with PVX.

Vulič & Hunnius (1967b) found that, in sap from tubers primarily infected with PVS, no virus could be detected during the growing season, while during storage it could be detected in about 70% of the tubers. However, if tubers with a secondary infection were checked serologically they could detect virus in 95% of the tubers during the growing season, and during storage in 97%. In sap of the sprouts on those tubers virus could always be detected.

Stapp & Bartels (1950, 1952) could also detect PVX reliably in sprouts on tubers grown in the dark. However, Bartels (1957) found that it was not possible to detect PVY reliably in sprouts with a serological method. Checking samples of tubers which were completely infected he could detect PVY serologically in 80% of the tubers from varieties Augusta and Bona, in 70% of the tubers from Ackersegen and Bella and in 60% of the tubers from Heida.

Thus a serological method is in general unreliable for assessment of viruses in potato tubers and sprouts.

Generally serological tests on the sap of foliage are highly reliable, if plants are checked for PVM, PVS, PVX and PVY at the right stage of development. Arenz et al. (1964) and de Bokx (1967b) found that PVS can be detected reliably in plantlets grown in greenhouses, if fully developed leaves from half-way up the stem are used. This is contrary to the findings of Wetter (1957) for field-grown plants. In the greenhouse PVY can be detected reliably only in foliage of plantlets 5 to 7 weeks after planting (Vulič & Arenz, 1963).

Some difficulties may still arise in testing for the presence of PVS and PVY, due to a low virus concentration in the plant, or to a spontaneous flocculation in the serological test.

Methods of improving the common serological tests (agglutination and precipitin test) have been investigated. The addition of bentonite, latex or barium sulphate to the antiserum improved the sensitivity of detection of PVX and other non-potato viruses (Bercks, 1967). However, Maat (1970) checking PVS in the foliage of potato plants found no improvement of sensitivity with the latex agglutination or bentonite-flocculation test. Nor was checking for PVX better with these tests.

The single radial-diffusion technique has now become of interest for testing potato viruses. Antiserum prepared in a special way at the proper dilution is mixed with liquid agar and poured into Petri dishes. The antigen is placed in wells in the gel and diffuses radially into the gel. Around the wells, rings of precipitate form. According to Shepard (1972) the test works with PVM, PVS and PVX. However, in Europe the test is not yet applied routinely.

The use of test plants will be included in certification schemes, if the pathogens, the precence of which has to be checked, cannot be detected with the naked eye, or with serological tests, or if the sensitivity of the relevant serological test is not sufficient.

So far test plants are used only for the detection of viruses. In principle all sap-transmissible viruses can be transmitted to test plants, which are mainly herbaceous hosts. For a general description of test plants see de Bokx (1972).

There are disadvantages to the use of test plants. For instance, the reaction is not always specific: *Gomphrena globosa* will react with local lesions after inoculation with PVX, as well as with some strains of PVM, PAMV, and papaya mosaic virus. Also test plants need much greenhouse space, and the test plant procedure is time-consuming. However, test plants are generally more sensitive than other tests, e.g. serology. As long as the serological tests to detect PVM and PVS have not been improved, a search for test plants to detect PVM and PVS remains necessary. Hiruki (1973) described a local-lesion host for PVM. He found that leaves of French bean in a certain stage of development react with local lesions 3–4 days after inoculation with PVM. However, the problem of greenhouse space still exists, since detached bean leaves cannot be used. The same holds for *Lycopersicum chilense* (Ross, 1968) which reacts with systemic symptoms to inoculation with PVM.

Because detection of PVY with serological tests is not always reliable, the test plant 'A6' is used in many Western European certification schemes (de Bokx, 1964; Keller & Berces, 1966). The test is used to supplement field inspection as well as to help in

tuber indexing for the detection of PVA and PVY. However, differentiation of those viruses with 'A6' is not readily possible (Bartels, 1970). 'A6' is highly reliable as a means to detect strains of PVA and PVY in seed-potato crops, as long as it is susceptible to the various strains of the two viruses. Strains may however occur which do not induce local lesions on 'A6' (de Bokx et al., 1974).

Although several local-lesion hosts for PVS are known (Vulič & Hunnius, 1967a; de Bokx, 1970) no suitable test plants for PVS are available yet.

PVX can be transmitted directly from tubers and sprouts to hosts like tobacco and *Gomphrena globosa*. However, no data are available on the efficiency of this type of transmission.

The results obtained after direct checking of tubers on 'A6' for the presence of PVA and PVY are not consistent. Nienhaus (1960), Arenz & Hunnius (1961) and Keller & Bérces (1966) obtained reliable results; but de Bokx (1964) found that checking early lifted seed for the presence of PVA and PVY with 'A6' was highly unreliable. In dormant tubers virus could hardly be detected, whereas in tubers of which dormancy was broken, artificially or naturally, virus could be detected to some degree.

Using sprouts on tubers to detect PVY in tubers (Keller & Bérces, 1966) will yield quicker results than checking the plantlets grown from those tubers. Nevertheless this has been abandoned as a means of routine testing because growing sprouts on tubers needs more attention than growing plantlets.

Although PVA and PVY can be detected reliably in sprouts, even in sprouts of tubers the dormancy of which has been broken artificially (de Bokx, 1964), and, according to Arenz & Hunnius (1961), can be detected reliably in cut tubers, the 'A6' test, as yet is routinely used only for detection of PVA and PVY in foliage.

Since direct testing of tubers and sprouts has yielded unreliable results, tubers to be diagnosed are grown in greenhouses or in the open. The foliage of the resultant plantlets is submitted to visual observation or to various diagnostic methods.

From each tuber a top eye is taken and, if necessary, dormancy broken artificially. The pieces of tuber containing an eye are planted in pots, containing potting soil, or in the open. The latter will be done in areas with moderate temperatures and where possibly late blight will not cause much loss (Florida test). A variation of this test has been described by Quemener (1970).

In the main seed-producing European countries tuber indexing is carried out in greenhouses during autumn and winter. Four to six weeks after planting, plantlets can be checked for the presence of virus symptoms.

The visibility of the symptoms is greatly influenced by climatic and soil conditions. This is especially true for the symptoms induced by PLRV. Often, probably due to lack of light (Bode, 1957) symptoms do not appear at all on plantlets in greenhouses during the winter in regions with a moderate climate. The plantlets become etiolated or, if soil conditions are excellent, grow vigorously. In both cases PLRV symptoms will not appear.

Experiments have been made on the prevention of etiolation of the plants by spraying a growth inhibitor (N- dimethylamino succinamic acid) in various doses. However,

even plantlets with a secondary infection of PLRV did not show leafroll after such treatments (Bakker, Sinnema & de Bokx, unpublished). A poor potting soil is recommended for growing potato plantlets when plants have to be checked for the presence of PLRV (Köhler, 1935). Well-fertilized soil is necessary when plantlets have to be grown for a serological checking on viruses (Bartels, 1959).

Apparently factors other than soil conditions also affect the appearance of leafroll. No exact data are available on this subject. Probably duration of illumination, light intensity and temperature effect the development of leafroll symptoms. Bérces et al., (1972) found that only 9% of infected tubers planted in greenhouses in August showed symptoms, whereas all infected tubers showed leafroll when planted in September. However, leafroll symptoms appeared on plantlets grown in poor soil during the winter when the temperature in the greenhouse was kept at 15 °C (Rozendaal, unpublished).

According to Hamann (1966), who observed symptom expression in 32 potato varieties after infection with PVY^N, varieties will generally show similar symptoms in the greenhouse as well as in the open. The symptoms occurring on plants grown in greenhouses, however, are always somewhat weaker.

Masking of symptoms on plants grown in greenhouses sometimes happens not only in the case of PLRV infection, but also after infection with mosaic-causing viruses.

This method of checking seed for the presence of viruses is sufficiently reliable for certified, but not for basic seed (Borchardt, 1962). To check basic seed additional tests (serology and test plant methods) are necessary.

Bacteria and fungi. Laboratory methods are used for identification of the causal pathogen, but may also give information on the degree of attack.

a. The causal pathogen may be identified by microscopic investigation of propagative organs or of mycelium, either already present on or in the diseased tissue, as e.g. with wart disease (Schick, 1962, p. 1214), or grown on it after storage during five to ten days at room temperature or at 15 °C in a humid environment. In the case of late blight the sporangiophores, bearing sporangia of *P. infestans*, will develop on affected leaves and stems and also on diseased tubers, which have been cut through. On tubers affected by silver scurf, conidiophores, bearing conidia of *H. solani* are produced and on tubers and underground parts of the potato plant, affected by skin spot, the characteristic branched chains of conidia of this fungus appear (Hide et al., 1968).

b. If visual examination is insufficient, isolation and identification of the pathogen on an artificial medium still is the most common way by which the cause of a bacterial or fungal disease can be determined.

Bacterial soft rot and black leg: Graham (1972) describes methods for isolation and identification of *Erwinia carotovora* and *E. c.* var. *atroseptica*. A quick test for the detection of var. *atroseptica* is especially important as this pathogen is the cause of black leg. Pérombelon (1972) describes a semiselective medium for detection of *E.*

carotovora, but this medium cannot distinguish between *E. carotovora* and *E. c.* var. *atroseptica*. Naumann (1972) mentions a selective medium for *E. c.* var. *atroseptica*.

Ringrot: According to Granhall (1961) it is difficult to isolate *C. sepedonicum* from diseased material in which other bacteria are also present. Seldom, if ever, would it be necessary to resort to isolation for identification of the pathogen, as smears from a suspected area of the vascular ring of the potato tuber, subjected to a Gram stain, would give conclusive evidence about its presence (Granhall, 1961). However, according to de Boer & Copeman (1974) coryneform bacteria, which are different from *C. sepedonicum*, occur in healthy potato tissue. Consequently 'false positives', based on Gram staining, may eliminate truly clean stock, whereas contaminated stocks may pass undetected, due to low population levels. De Boer & Copeman conclude that a more selective and reliable diagnostic technique is urgently needed for the detection of *C. sepedonicum*.

Brown rot: Granhall (1961) mentions that *P. solanacearum* is readily isolated from infected plants. A useful diagnostic technique, in addition to other characteristics, is to stain the bacterium with Sudan Black B (Granhall, 1961). It will distinguish between *P. solanacearum* and *E. c.* var. *atroseptica* (Hayward, 1960).

Common scab: Generally there is no difficulty in identifying common scab on account of the symptoms. Also, the need for identification in doubtful cases is less than with black leg or ring rot, as common scab is an ubiquitous disease, which is mainly soil-borne and does not cause much harm during storage. Moreover, it is not possible to distinguish between pathogenic and non-pathogenic isolates on account of cultural characters (Labruyère, 1971).

The usual methods for isolation of pathogenic fungi are also applied for fungal diseases of the potato. For some diseases more specific methods may be used. Sharma & Hodgson (1971) describe such a method for isolation of *P. infestans* from lesions, caused by late blight; Hirst & Salt (1959) for isolation of *O. pustulans* from tubers affected by skin spot; and Burke (1938) for isolation of *H. solani* from lesions, caused by silver scurf. For identification of the pathogens the common taxonomic criteria are used. Van Emden et al. (1966) have described four criteria by which *R. solani*, a fungus which does not produce spores in culture, can be identified. The dangerous *P. exigua* var. *foveata* is distinguished in culture from the rather harmless and ubiquitous var. *exigua* by the production of a yellow pigment and the formation of yellow crystals (Boerema, 1967). This pigment is composed of several anthraquinones, which become yellow in acid conditions and turn red in alkaline conditions (Bick & Rhee, 1966).

c. Identification of the causal pathogen by serological methods is very useful for disease assessment, because they give quick information. However, these methods cannot be applied generally. Until now, little success has been achieved in identification of fungi with serological methods.

Black leg: The presence of *E. carotovora* var. *atroseptica* in potato stems and tubers can usually be determined serologically (Novakova, 1957; Graham, 1963), provided sufficient rotted tissue is available. The method is not completely reliable, because

some strains of *E. carotovora* give an agglutination reaction with serum of the var. *atroseptica*. However, Vruggink (1972) found that antisera against *E. c.* var. *atroseptica* gave a specific precipitation reaction in agar gel, though in agglutination tests the reaction was not specific. Tanii et al. (1973) also stated that agar gel diffusion patterns showed that *E. c.* var. *atroseptica* possessed a specific antigen.

Ring rot: Granhall (1961) describes a serological method for detection of *C. sepedonicum* in potato tissue, used in the USSR for indexing seed stocks. Strobel and Rai (1968) mention a rapid serodiagnostic test for potato ring rot, employing the use of an antiserum, prepared to the crude glycopeptide toxins of *C. michiganense* Jensen.

d. Laboratory methods may be used to stimulate infection, thus stimulating growth of the pathogen and facilitating identification and (or) statement of degree of contamination. Mostly these methods are only used in experiments.

Soft rot and black leg: Pérombelon (1972) stores samples of tubers under humid and anaerobic conditions, thus causing development of bacterial soft rot. The causal pathogens are isolated and identified by biochemical and pathogenicity tests.

Gangrene is stimulated by wounding the tubers uniformly and storing them for eight to twelve weeks at a low temperature (Khan & Logan, 1968; Hirst et al., 1970, p. 96). Isolations are made from the lesions, which develop during storage. This method is used to determine the degree of contamination, and also to indicate its presence. If contamination is indicated the seed lot may be disqualified for certification (Anon., 1972).

Dry rot is stimulated by cutting tubers through and rubbing soil adhering to the tuber surfaces on the cut surfaces. The number of rots which develop after storage under conditions, favourable for the development of dry rot, is a measure of contamination with *Fusarium* propagules (Nielson & Johnson, 1972).

Physiological stage of development, place of origin

Beemster (1972) found that stems of potato plants reach a stage of development during the growing season after which they cannot be affected with viruses. Experiments carried out in greenhouses showed that this held for PLRV, PVX and PVY. This was also true for PVS (de Bokx & Waterreus, 1967). This phenomenon is called mature plant resistance. However, up to now it has not been possible to develop a method quantitatively to determine mature plant resistance. If this would be possible field inspection will be changed fundamentally.

All research that will help to quantify mature plant resistance must be encouraged.

Temperature of storage has an effect on the yielding capacity of the seed (Krijthe, 1962). However, the user does not have a method to check at which temperature the seed he bought was stored.

According to some authors (Rönnebeck, 1953; Stottmeister, 1958) growing conditions, like place of origin, altitude, type of soil, effect the yielding capacity of the seed. These are physiological effects. However, these are so complex that conclusions cannot

easily be drawn. Among the effects mentioned is that of growing potato crops from seed produced at different altitudes (360–1000 m). The yield from seed ('Arran Pilot' and 'Eva') grown the previous year in the lowlands was higher than the corresponding yield from seed grown in the 'chalet' area (5–15%). Russian papers indicate the same, with yield increases as high as 13–37%.

Low temperatures (17°C) not only effect the production of a potato crop favourably, but also the following generations. Yields were higher than those from seed grown at high temperatures (26°C).

The effect of the physiological age of the seed has been briefly discussed by van der Zaag (1973) and Hunnius (1974). They came to the conclusion that physiological age standard appears to be very important, but less amenable to application in practice than are health standards.

Authenticity and purity of variety

The identification of potato varieties is possible using the properties of foliage, tuber and sprouts grown in light. During a period in summer the growth of the potato plant is so specific that a specialist can determine the variety on the habit of the plant.

The tuber itself does not yield sufficient information for this determination.

A reliable identification method has been developed using the sprouts grown in the light on the tubers to be investigated (Ros, 1972). This usually takes 6–8 weeks and in late summer and autumn even longer. When tubers have been treated with a sprout inhibitor this method cannot be used.

Protein electropherograms of 59 Dutch potato varieties, obtained by paper electrophoresis, turned out to be specific to the varieties (Zwartz, 1966). On this basis a reliable identification method has been developed.

Checking for the presence of nematodes

Seed must have been grown in a field free from cysts of *Heterodera rostochiensis*. The seed tubers must be free from cysts. In some countries potatoes may be grown once in six years in the same soil (Proudfoot & McCallum, 1961).

For sampling sufficient soil per unit area must be examined, at least 200 ml or 250 g. The soil must be derived from sufficient points to provide a representative sample, e.g. at least 50 per unit area of 1 ha.

Conclusions

From the foregoing it is clear that for detection of the major potato viruses diagnostic methods are available. However, the same method cannot be applied for all viruses. And even if the same method can be used for a number of viruses it is not possible to obtain the same reliability for all of them.

Quality assessment of potatoes as far as viruses are concerned, would be simplified

if one diagnostic method could be used for detection of all viruses.

Visual observation, a method used for detection of all viruses, cannot be improved since it depends very much on the weather conditions.

Basically serology would be a method applicable to all viruses, but before this is achieved suitable antisera against PLRV and PVA, and reliable serological tests, must be developed. Testing for the presence of PVY with 'A6' would then be replaced by serology. Using the precipitin test instead of the agglutination test would enhance the reliability of the testing.

Efforts have to be made to produce an antiserum against PVA as this would simplify tuber indexing, since all testing for mosaic viruses (PVA, PVM, PVS, PVX and PVY) could then be carried out serologically. Serological tests can be performed rapidly and moreover they are specific.

Since the tuber is the object which will be planted, development of reliable test methods for direct checking of tubers for the presence of viruses is the goal scientists must aim for.

At the moment it appears that serological testing of tubers must have priority over using test plants. Application of electron microscopy in testing for the presence of potato viruses is of academic value only (Brandes, 1957). If the preparation procedure in electron microscopy could be accelerated, practical application could be considered (de Bokx, 1969).

It may be said that during the last years interest has increased in methods to be used for the assessment of those bacterial and fungal diseases of seed potatoes which cannot easily be identified by symptoms. The development of quick and reliable methods for identification of these diseases, as well as for the detection of contamination with the causal pathogens is now urgently needed. This is not only true in those cases where the elimination of diseases, like ring rot, is being attempted, but also where attempts are being made to reduce bacterial and fungal diseases, as e.g. in those cases where seed stock is developed from stem cuttings.

The development of a quick and completely reliable method for identification of black leg is recommended. Work on a serological method, based on the agar gel diffusion test (Vruggink, 1973; Tanii et al., 1973) indicates the possibility to develop such a method. Another method, based on a selective medium, is described by Naumann (1972). In order to detect contamination of tubers with *E. carotovora* var. *atroseptica*. Pérombelon's (1972) method, or a similar one to stimulate the development of bacterial soft rot, may be used.

Granhall (1961) mentions that Gram staining gives conclusive evidence of the presence of *C. sepedonicum*, but according to de Boer and Copeman (1974) the development of a more selective diagnostic technique for the detection of this pathogen should be recommended. However, these authors do not mention the rapid serodiagnostic test of Strobel & Rai (1968), nor the one used in the USSR (Granhall, 1961). Also it should be ascertained whether the method of Hayward (1960) is a useful diagnostic method for the detection of *P. solanacearum* in seed potatoes (Granhall, 1961).

The method which is used to indicate contamination of seed tubers with *P. exigua* var. *foveata* (Anon., 1972), needs much time before the result is known. It would be important if methods could be developed to decrease this time as much as possible. Also such methods should be more reliable then the one used at present. De Bruin & van Loon (personal communication) found that the method to determine the degree of contamination of seed lots with *Fusarium* propagules, described by Nielson & Johnson (1972) is promising. No assessment is done as yet to determine the degree of contamination of seed lots with pathogens which cause other storage diseases, like bacterial soft rot, gangrene skin spot and silver scurf. However, it would be of great importance to investigate the usefulness of such an assessment, especially if carried out at an early stage during storage, because it may give information on the keeping quality of the seed lots. Several methods, which may be used for the purpose mentioned, are already described in the literature, as e.g. with bacterial soft rot (Pérombelon, 1972), skin spot (Hide et al., 1968) and gangrene (Khan & Logan, 1968; Hirst et al., 1970, p. 96).

Schlussfolgerungen

Methoden der Qualitätsbeurteilung bei Saatkartoffeln

Aus dem bisher Gesagten geht klar hervor, dass für die Erkennung der meisten Kartoffelviren Bestimmungsmethoden vorhanden sind. Dagegen kann nicht für alle Viren die gleiche Methode angewendet werden. Wo die gleiche Methode für eine Anzahl Viren angewendet werden kann, ist es zudem nicht möglich, alle diese Viren mit gleicher Zuverlässigkeit zu beurteilen.

Die Qualitätsbeurteilung von Kartoffeln würde – soweit es die Viren betrifft – vereinfacht, wenn zur Erkennung aller Viren eine einzige Methode zur Diagnose angewendet werden könnte.

Die visuelle Beobachtung – eine Methode, die zur Erkennung aller Viren verwendet wird – kann nicht verbessert werden, da sie sehr stark von den Witterungsbedingungen abhängig ist.

Im Grunde genommen wäre die Serologie eine für alle Viren anwendbare Methode. Es müssten jedoch geeignete Antisera gegen PLRV und PVA und zuverlässige serologische Teste entwickelt werden. Die Prüfung auf das Vorkommen von PVY mit 'A6' müsste dann durch die Serologie ersetzt werden. Die Anwendung des Präzipitations- statt des Agglutinationstest würde die Zuverlässigkeit der Prüfung fördern.

Es müssen Anstrengungen unternommen werden, um ein Antiserum gegen PVA zu erzeugen. Ein Antiserum gegen PVA würde die Knollenbeurteilung vereinfachen, da alle Prüfungen auf Mosaikviren (PVA, PVM, PVS, PVX und PVY) serologisch durchgeführt werden könnten. Serologische Teste können rasch ausgeführt werden; ausserdem sind sie spezifisch.

Da die Knolle das Produkt ist, das ausgepflanzt wird, haben die Forscher die Entwicklung zuverlässiger Prüfungsmethoden, mit denen das Vorkommen von Viren direkt an der Knolle festgestellt werden kann, zum Ziel.

Im Augenblick hat es den Anschein, dass die serologische Untersuchung der Knollen den Vorrang vor der Verwendung von Testpflanzen haben müsse. Der Anwendung der Elektronenmikroskopie zur Untersuchung auf das Vorkommen von Kartoffelviren kommt nur akademischer Wert zu (Brandes, 1957). Wenn das Vorbereitungsverfahren bei der Elektronenmikroskopie beschleunigt werden könnte, käme eine praktische Anwendung eventuell in Frage (de Bokx, 1969).

In den letzten Jahren hat das Interesse für Methoden zur Beurteilung von Bakterien- und Pilzkrankheiten an Pflanzkartoffeln, die nicht leicht durch Symptome identifiziert werden können, zugenommen. Die Entwicklung von schnellen und zuverlässigen Methoden zur Bestimmung dieser Krankheiten wie auch zur Entdeckung der Ansteckung durch die ursächlichen Erreger ist nun dringend notwendig geworden. Dies ist nicht nur dort der Fall, wo versucht

wurde, Krankheiten zu eliminieren, (z.B. Bakterienringfäule) sondern auch da, wo man versucht, Bakterien- und Pilzkrankheiten zu reduzieren, wie z.B. bei Pflanzgut, das auf der Basis von Stengelstecklingen angezogen wird.

Die Entwicklung einer schnellen und vollständig zuverlässigen Methode zur Feststellung von Schwarzbeinigkeit wird empfohlen. Arbeiten an einer serologischen Methode, die auf dem Agar-Gel-Diffusionstest beruht (Vruggink, 1973; Tanii et al., 1973), weisen auf die Möglichkeit, eine solche Methode zu entwickeln, hin. Eine andere Methode, basierend auf einem selektiven Medium, ist bei Naumann (1972) beschrieben. Um die Ansteckung von Knollen mit *Erwinia carotovora* var. *atroseptica* zu erkennen, kann die Methode von Pérombelon (1972) oder eine ähnliche, die die Entwicklung der Bakterien-Nassfäule anregt, angewendet werden.

Granhall (1961) erwähnt, dass die Gram-Färbung das Vorkommen von *Corynebacterium sepedonicum* überzeugend beweist, aber nach de Boer & Copeman (1974) sollte die Entwicklung einer mehr selektiven und diagnostischen Technik für die Entdeckung dieses Erregers empfohlen werden. Diese Autoren erwähnen jedoch weder den raschen serodiagnostischen Test von Strobel & Rai (1968), noch die in der USSR übliche Prüfung (Granhall, 1961). Ferner sollte klargestellt werden, ob die Methode von Hayward (1960) eine brauchbare diagnostische Methode zur Entdeckung von *Pseudomonas solanacearum* in Pflanzkartoffeln ist (Granhall, 1961).

Die Methode, die angewendet wird, um die Ansteckung von Pflanzknollen mit *Phoma exigua* var. *foveata* (Anon., 1972) festzustellen, benötigt viel Zeit, bis das Ergebnis bekannt ist. Es wäre wichtig, Methoden zu entwickeln, die diese Zeit so viel wie möglich verkürzen. Solche Methoden sollten auch zuverlässiger sein als die bis jetzt angewendete.

De Bruin & van Loon (persönliche Mitteilung) fanden, dass die Methode, den Grad der Ansteckung von Pflanzgutposten mit *Fusarium* zu bestimmen, beschrieben von Nielsen & Johnson (1972), vielversprechend ist. Bis jetzt ist keine Beurteilung durchgeführt worden, um den Grad der Verseuchung von Pflanzgutposten mit Erregern anderer Lagerkrankheiten, z.B. Bakterien-Nassfäule, Phomafäule, Tüpfelfleckigkeit und Silberschorf, festzustellen. Es wäre jedoch von grosser Wichtigkeit, die Nützlichkeit einer solchen Beurteilung zu untersuchen, besonders wenn sie zu Beginn der Lagerperiode durchgeführt würde, weil sie über die Haltbarkeit des Pflanzgutpostens Auskunft geben könnte. Verschiedene Methoden, die zum erwähnten Zweck angewendet werden könnten, sind schon in der Literatur beschrieben worden, z.B. für Bakterien-Nassfäule (Pérombelon, 1972), Tüpfelfleckigkeit (Hide et al., 1968) und Phomafäule (Khan & Logan, 1968; Hirst et al., 1970, p. 96).

Conclusions

Méthodes de fixation de la qualité des plants de pomme de terre

Il apparaît clairement de ce qui précède qu'il existe des méthodes valables de détermination des virus les plus importants. Cependant ces mêmes méthodes ne sont pas applicables à tous les virus. De plus ces méthodes valables pour un certain nombre de virus ne donnent pas la même fiabilité pour chacun d'entr'eux.

La détermination de la qualité des pommes de terre pour ce qui concerne les virus serait simplifiée si l'on pouvait utiliser une seule méthode pour tous les virus.

L'observation visuelle, utilisée pour la détection de tous les virus, ne peut être améliorée parce que trop dépendante des conditions atmosphériques.

Fondamentalement la sérologie serait une méthode applicable à tous les virus. Dès lors, il y aurait lieu de développer des antisera valables contre les PLRV et PVA pour obtenir des tests sérologiques dignes de confiance.

La détection de la présence de PVY à l'aide de 'A6' devrait alors être remplacé par la sérologie. L'usage du test par précipitation plutôt que le test par agglutination devrait accroitre la fiabilité de la détermination.

Des efforts doivent été réalisés pour produire un antiserum contre le PVA. Un antiserum contre le PVA simplifierait l'indexage des tubercules, puisque alors le test pour les virus de mosaïque (PVA, PVM, PVS et PVY) pourraient être réali-

sés sérologiquement. Les tests sérologiques peuvent être réalisés rapidement, au surplus ils sont spécifiques.

Etant donné que le tubercule est l'elément qui sera planté, les scientifiques doivent viser à développer des méthodes de test utilisables pour la détection des virus dans les tubercules.

En ce moment il semble probable que le test sérologique des tubercules doive avoir la priorité sur les tests de plantes. L'application du microscope électronique dans les tests pour la présence du virus de la pomme de terre a seulement une valeur académique (Brandes, 1957). Si le processus de préparation pour la microscopie électronique pouvait être accéléré, on pourrait envisager une application pratique (de Bokx, 1969).

On peut dire qu'au cours des dernières années l'intérêt s'est accru pour les méthodes à utiliser dans la détermination des maladies bactériennes et fongiques des plants de pommes de terre qui ne peuvent être identifiées par les symptômes. Le développement de méthodes rapides et fiables pour l'identification de ces maladies, aussi bien que pour l'étude de la contamination par des agents pathogènes, est devenu une nécessité urgente. Ceci n'est pas seulement vrai dans le but d'éliminer ces maladies, telle la bactériose annulaire, mais aussi pour tenter de réduire les maladies bactériennes et fongiques, par exemple dans le cas de production de plants à partir de boutures de tiges.

Le développement d'une méthode rapide et complètement fiable pour l'identification de la jambe noire est vraiment utile. L'étude d'une méthode sérologique basée sur un test de diffusion sur gel agar (Vruggink, 1973; Tanii et al., 1973) révèle la possibilité de développer pareille méthode. Naumann (1972) décrit une autre méthode basée sur un milieu sélectif. Dans le but de détecter la contamination des tubercules par *Erwinia carotovora* var. *atroseptica*, on peut utiliser la méthode de Pérombelon (Pérombelon, 1972) ou une semblable pour stimuler la pourriture humide bactérienne.

Granhall (1961) signale que la coloration Gram apporte une preuve certaine de la présence de *Corynebacterium sepedonicum*, mais selon de Boer et Copeman (1974) il y aurait lieu de recommander le développement d'une technique de diagnostic plus sélective. En tout cas, ces auteurs ne mentionnent pas le test rapide de sérodiagnostic de Strobel et Rai (1968) ni celle utilisée en URSS (Granhall, 1961). Aussi serait-il utile de savoir si la méthode de Hayward (1960) constitue un diagnostic vraiment utile pour la détection de *Pseudomonas solanacearum* (Granhall, 1961).

La méthode en usage pour établir la contamination des plants de pommes de terre pour *Phoma exigua* var. *foveata* (Anon., 1972) prend beaucoup de temps avant que les résultats ne soient connus. Il serait important de développer des méthodes pour diminuer autant que possible ce délai. De plus de telles méthodes devraient être plus fiables que celle utilisée présentement.

De Bruin et van Loon (communication personnelle) trouvent prometteuse la méthode décrite par Nielsen et Johnson (1972) pour déterminer le degré de contamination de lots de plants par de germes de *Fusarium*.

On ne signale aucune évolution relative à la détermination du degré de contamination de lots de plants par les pathogènes responsables d'autres maladies de conservation, telles que la pourriture humide bactérienne, la gangrène, l'oosporiose et la gale argentée. Cependant, il serait de la plus grande importance de rechercher l'utilité de telles déterminations, plus particulièrement quand elles soient exécutées au premier temps de conservation, parce que cela peut donner des informations sur le maintien de la qualité des lots de plants. Plusieurs méthodes, qui peuvent être utilisées dans ce but, sont déjà décrites dans la littérature; c'est le cas par ex. pour la pourriture humide bactérienne (Pérombelon, 1972), l'oosporiose (Hide et al., 1968) et la gangrène (Khan & Logan, 1968; Hirst et al., 1970, p. 96).

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