INVESTIGATIONS ON CERTAIN ACTINOMYCETES THAT CAUSE POTATO SCAB.[1])

INTRODUCTION

In addition to *Actinomyces scabies* (THAXT.) GUSS. other species of *Actinomyces* (WOLLENWEBER, 30; MILLARD and BURR, 22) may cause potato Scab. Stress is laid on the great variability amongst the Actinomycetes. On account of this character, and also of the very large number of species belonging to this group, its taxonomy is very incomplete. In the experiments described, no efforts were made to classify the strains isolated; these are indicated by letters and numbers only.

EXPERIMENTAL METHODS

The bacteriological plating out method was used for obtaining pure cultures. Each isolation was plated at least twice, and those mycelia which could be regarded as having developed from single hyphae were used as inocula. Isolations were made either from Scab lesions on potato tubers treated with corrosive sublimate or from mycelia present on tubers just lifted and still moist. These mycelia were washed repeatedly in sterile water and then plated. The last method gave very satisfactory results, especially with re-isolations.

All experiments were made in a greenhouse. The pots and soil were sterilised. A mixture of equal parts of poor sandy soil and leaf-mould was used as potting soil. Tubers themselves were not planted, but only green sprouts that had developed on tubers previously treated with corrosive sublimate.

The Actinomycetes used for inoculation were cultivated on sterilised green rye, or grass; this material allowed uniform distribution of the fungus in the tube and a good mixture afterwards with the soil. Thorough mixing of the inoculum with the soil is a fundamental condition for successful results, and with the method employed no difference in number of mycelia present arises between the forms with and without spores. In most cases the contents of 6 tubes were used for inoculating each pot; a preliminary experiment proved that the contents of even 3 tubes suffice, as no difference was obtained when the contents of either 3 or 6 were employed (fig. 1). However, to avoid the possibility that negative results might be due to too little inoculum, the contents of the larger number of tubes were generally added.

The soil was inoculated before the planting of the sprouts, as this gave rise to a larger number of affected tubers than when it was inoculated during the development of the crop (Table I). All experiments with different strains were made in the same greenhouse, but infection from neighbouring pots was not observed. It may be stated, however, that the tubers of the controls were not always quite free from scab; infection from the atmosphere took place to a certain extent. This occurs in all potato experiments and cannot readily be avoided.

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CONTROL WITH CHEMICALS

The purpose of control measures is either to decrease the number of *Actinomycetes* naturally present in the soil, or to prevent the introduction of others present on affected seed tubers. An experiment was made which proved that *Actinomyces* can remain alive on the potato skin for a period of 8 months, i.e. between digging and planting. Disinfection of affected seed tubers may reduce attack arising from this source. To control the parasite in the soil it may either be killed or the conditions for its growth may be made unfavourable. To this end many chemicals were tried in pots with inoculated soil, already used for other experiments. The only substance which gave satisfactory results was $FeCl_3$, as can be seen in Tables II and III and in fig. 2. The plants suffered a good deal if more than 10 g per pot was used. At the end of the experiment the pH of the soil of some pots was determined, and the results may be seen in Table IV. The difference between treated and untreated soil is not so great as to account for the large decrease of Scab. The pH may have been much lower at the moment of the treatment, which EICHINGER (9) calls „saure Reaktionsstosz". No practical results have been obtained, as the use of $FeCl_3$ in the field would have many disadvantages. None of the other chemicals tried decreased the attack of Scab really satisfactorily, although they did so to varying degrees.

DIFFERENT TYPES OF SCAB.

Deep, *tumulus*, *common* and *superficial* or *russet Scab* are distinguished. WOLLENWEBER (30), MILLARD and BURR (22) and DE BRUYN (4) are of opinion that each type is caused by a different *Actinomyces*, while on the contrary SCHLUMBERGER (26, 1929), BERKNER (2), GOSS (13) and AFANASIEV (1) believe that degree of severity of attack together with the external conditions, are responsible for the differentiation of the various types. Goss makes an exception for russet Scab.

The beginning of the development of all types is alike, viz. a small brown spot on the skin of the potato; differentiation occurs afterwards. During this process the stage of another type may be passed; therefore conclusions should be made carefully and are possible only for experiments with pure cultures made under similar conditions. In addition to the fact that severe or light attack is inherent in the particular strain used, the type of Scab eventually produced is due to the particular *Actinomyces* involved.

Experiments with pure cultures in pots again strongly supported the above theory. From a tuber harbouring both deep and superficial (russet) Scabs, the parasite was isolated separately from both types of lesions. Two different *Actinomyces* resulted, and by inoculation both types were reproduced (fig. 3). The results of inoculations with many other isolations, and re-isolations, tend in the same direction.

THE TYPES OF SCAB IN SOME POTATO VARIETIES

Some strains were inoculated on different potato varieties with the result that the types of Scab produced by one and the same strain were not alike. A strain forming russet Scab on Bintje and Industrie was entirely non-pathogenic to 5 other potato varieties. Strain 3*a*, producing deep Scab, did so on 5 potato varieties; on Jubel, however, lesions were very shallow, while on Alpha a type was formed which might be called either russet or common Scab, most of the lesions being of the russet type (fig. 4). Many other strains of *Actinomyces* were tried on the varieties Bintje and Eigenheimer; all russet-forming strains attacked Bintje r– ther severely, while the skins of all Eigenheimer tubers remained sound (fig. 5). Eigenheimer is, however, not resistant to Scab in general, for many deep-forming strains attacked this variety even more severely than Bintje (fig. 6, 8).

Thus, potato varieties are not equally susceptible to attack by different strains of *Actinomyces* and different varieties react differently to the same strain.

PATHOGENICITY

In addition to variability in cultural characters, great variability of the strains was observed with regard to virulence. WOLLENWEBER (30) and AFANASIEV (1) suggested loss of virulence after growth in pure culture for a certain period, although this loss did not occur in all cases. The use of different media may have had some influence on this phenomenon.

In the experiments described here, decrease in virulence, as well as constancy, and even increase, was noted. Table V gives the history of strain 5; decrease of virulence first started after growth for 1½ years in pure culture. Re-isolation produced a renewed strong pathogenic strain, which weakened in virulence again after some time (Table VI).

The behaviour of a large number of strains and their re-isolations was studied during 1937 and 1938 (see Table VII). Strain B_2 was the only one with increased virulence after growth in pure culture (fig. 5). Strains H_{8V} and H_3 remained constant (fig. 6); all other strains decreased in pathogenicity, although to different degrees. With B_7 a total loss of pathogenicity occurred (fig. 7). Strain H_{3a} showed greatly decreased pathogenicity (fig. 8), while H_3, from which it originated, remained constant (fig. 6).

The virulence of all weakened strains was increased through re-isolation, but in different degrees; e.g. Her_a remained a weak parasite.

It might be supposed that certain substances absent in artificial media were the cause of the change. Some weakened strains were therefore cultivated on sterile pieces of cut living potato and carrot. Growth was good, but virulence did not return. As re-isolation gave increased pathogenicity, the necessary substances may be present only in the living growing cells of the potato tuber.

LOSS OF PATHOGENICITY IN THE FIELD

Lately, cases have been mentioned in the literature suggesting change of pathogenicity of Actinomycetes in the field, e.g. LUTMAN, LIVINGSTON and SCHMIDT (19). This theory is in agreement with the results of the experiments described here. They also strengthen the idea of the existence of a strong correlation between virulence and presence of the host. This is confirmed by the more severe attacks of Scab following continuous potato cropping mentioned by MARTIN (20), DIPPENAAR (6), BÖNING and WALLNER (3), SCHLUMBERGER (26, 1930 and 1936) and RODE (23), as well as by the change of pathogenicity found by crop rotation as recorded by CAIRNS, GREEVES and MUSKETT (5) and GOSS (11). In the last case other factors may also play a part.

The change of type of Scab mentioned by LUTMAN c.s. and by GOSS, viz. russet Scab taking the place of deep Scab, may be explained either by loss of virulence, especially of the strain causing deep Scab, or by change of conditions promoting growth of russet-forming strains.

More attention should be paid to the influence of rotation, not only of different crops, but also of different potato varieties, as susceptibility to different strains being not alike in all varieties, even the presence of certain potato varieties may, for some *Actinomyces*, be equivalent to absence of host.

SUMMARY

1. Of various chemicals tried in pots $FeCl_3$ was the only one which decreased Scab.
2. The different types of Scab on the same potato variety are caused by different strains of *Actinomyces*.
3. One strain of *Actinomyces* may cause different types of Scab on different potato varieties; the type is specific for each variety.
4. One strain of *Actinomyces* may be virulent for certain potato varieties and non-pathogenic to others, the latter, however, may behave quite differently with other strains.
5. The virulence of many strains decreased in pure culture; of some it remained constant, with one it increased.
6. The reduced virulence was increased through development on growing, living potato tubers.
7. More attention should be paid to the influence of rotation on the occurrence of Potato Scab.

Laboratorium voor Mycologie
en Aardappelonderzoek

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VERKLARING DER FIGUREN

(*Explanation of figures*)

Fig. 1. Bintje geïnoculeerd met stam 5; links met 3 buizen per pot, rechts met 6 buizen per pot.

Fig. 1. Bintje inoculated with strain 5; left, contents of 3 tubes per pot, right of 6 tubes per pot.

Fig. 2. Bintje, gegroeid in besmetten grond en in denzelfden grond behandeld met $FeCl_3$.

Fig. 2. Bintje grown in infected soil and in the same soil treated with $FeCl_3$

Fig. 3. Bintje, links geïnoculeerd met stam van diepe schurft en rechts met stam van oppervlakkige schurft, beide oorspronkelijk voorkomende op denzelfden knol.

Fig. 3. Bintje, left inoculated with strain causing deep Scab and right with strain causing superficial (russet) Scab, both originally occurring on the same tuber.

Fig. 4. Inoculaties van stam 3*a* op verschillende aardappelvariëteiten.

Fig. 4. Inoculations of strain 3a on different potato varieties.

Fig. 5. Boven: Bintje geinoculeerd met stam B_2 1937; onder: Eigenheimer en Bintje geïnoculeerd met stam B_2 1938. Oppervlakkige schurft, virulentie vooruitgegaan, Eigenheimer immuun.

Fig. 5. Above: Bintje inoculated with strain B_2 1937; *below: Bintje and*

Eigenheimer, inoculated with strain B_2 1938. Superficial (russet) scab, increase of virulence, Eigenheimer immune.

Fig. 6. Boven: Bintje geïnoculeerd met stam H_3 1937; onder: Bintje en Eigenheimer geïnoculeerd met stam H_3 1938. Diepe schurft, virulentie gelijk, Eigenheimer sterker aangetast.

Fig. 6. Above: Bintje inoculated with strain H_3 1937; below: Bintje and Eigenheimer inoculated with strain H_3 1938. Deep scab, the same virulence, Eigenheimer more severely attacked.

Fig. 7. Boven: Bintje geïnoculeerd met stam B_7 1937; onder: Bintje geïnoculeerd met stam B_7 en met her-isolatie B_{7b} 1938. Oppervlakkige schurft, virulentie geheel verloren, versterkte virulentie door her-isolatie.

Fig. 7. Above: Bintje inoculated with strain B_7 1937; below: Bintje inoculated with strain B_7 and with re-isolation B_{7b} 1938. Superficial (russet) Scab, total loss of virulence, increased virulence through re-isolation.

Fig. 8. Boven: Bintje geïnoculeerd met stam H_{3a} 1937; midden: Bintje en Eigenheimer geïnoculeerd met stam H_{3a} 1938; onder: Bintje en Eigenheimer geïnoculeerd met stam H_{3ab} (her-isolatie van H_{3a}). Diepe schurft, virulentie verzwakt, versterkte virulentie door herisolatie, Eigenheimer sterker aangetast.

Fig. 8. Above: Bintje inoculated with strain H_{3a} 1937; middle: Bintje and Eigenheimer inoculated with strain H_{3a} 1938; below: Bintje and Eigenheimer inoculated with strain H_{3ab} (re-isolation of H_{3a}) 1938. Deep Scab, decrease of virulence, increased virulence through re-isolation, attack on Eigenheimer more severe.

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