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Investigations on the cause of soil sickness in fruit trees VII. An actinomycete isolated from rootlets of apple seedlings, the probable cause of specific apple replant disease

Untersuchung zur Ursache der Bodenmüdigkeit von Obstgehölzen VII: Ein aus Faserwurzeln von Apfelsämlingen isolierter Aktinomyzet, die wahrscheinliche Ursache der Bodenmüdigkeit von Apfelgehölzen

Abstract

From earlier investigations it has been deduced, that actinomycetes, which colonize and damage the rootlets of apple trees, might be the cause of soil sickness. Attempts to isolate these actinomycetes had been without success for a long time. Foremost, recognizing that plant hormones play a decisive role in the colonization of rootlets by actinomycetes and by introducing such plant hormones into culture media it succeeded to isolate a species of actinomycetes, which appeared not earlier. Apple seedlings showed clear diminution of growth in a soil that was steamed and inoculated with this isolate. Additionally, a colonization of rootlets by actinomycetes could be observed, which corresponded in frequency and appearance to earlier results from investigations with roots in sick soils. The results are considered a further underpinning of the assumption that root pathogenic actinomycetes are the cause of apple replant disease.

Key words: Soil sickness, fruit trees

Zusammenfassung

Aus früheren Untersuchungen ist abgeleitet worden, dass in Aktinomyzeten, die die Faserwurzeln von Apfelgehölzen besiedeln und schädigen, die wahrscheinliche Ursache der Bodenmüdigkeit zu sehen ist. Versuche, diese Aktinomyzeten zu isolieren, blieben lange erfolglos. Erst nach Erkenntnissen, dass pflanzliche Wuchsstoffe eine entscheidende Rolle bei der Besiedlung der Faserwurzeln durch die Aktinomyzeten spielen und dem Einsatz solcher Wuchsstoffe in den Nährböden, ist es gelungen, eine bislang nicht in Erscheinung getretene Aktinomyzeten-Art zu isolieren. In einem gedämpften und mit diesem Isolat beimpften Boden wiesen Apfelsämlinge deutliche Minderungen des Triebzuwachses auf. Ebenso konnte in den Faserwurzeln die Besiedlung durch Aktinomyzeten festgestellt werden. Diese entsprach in der Häufigkeit und im Erscheinungsbild dem, was aus Untersuchungen an Wurzeln aus müden Böden hinlänglich bekannt war. In diesen Ergebnissen wird eine weitere Untermauerung der Vorstellung gesehen, dass wurzelpathogene Aktinomyzeten die Ursache der Bodenmüdigkeit bei Apfel sind.

Stichwörter: Bodenmüdigkeit, Obstgehölze

Introduction

The aim of sections I. to V. was to rule out as many as possible of the existing theories about the cause of soil sickness of apple trees and to possibly favour one of the theories (Otto, 1972a, 1972b, 1972c, 1973) and Winkler and Otto (1972). It was concluded that only bacteria or actinomycetes which are specifically associated with rootlets of apple trees can qualify as the cause of soil sickness. Finally, in section VI., for the first time a colonization of rootlets by actinomycetes in sick soils could be demonstrated, Otto and Winkler (1977). Further research

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consolidated the assumption that it must be a root pathogenic actinomycete that causes the rootlets of apple trees to die off.

At the same time several attempts were undertaken to isolate these actinomycetes from and out of rootlets as well as from the soil. These all were without success. Only later they turned out promising by recognizing interactions between the colonization through actinomycetes and balance of the plant hormones of the trees (Otto et al., 1994a). This is the report of one of these isolation experiments.

Methods and Results

While in previous experiments soil extract agar with different additives had been used, for this isolation experiment a culture medium with 1-naphthyl acetic acid was prepared. Rootlets of apple seedlings from a sick soil were rinsed thoroughly with water and grinded slightly in a mortar together with sterile quartz sand. This macerate as well as whole root parts were spread out on the medium. One of the growing colonies was ignored at first, although it occurred quite often. This was due to its morphological appearance which did not resemble usual actinomycetes but rather a colony type of bacteria (Fig. 1). However, on closer examination it turned out to be an actinomycete. Steamed soil was then inoculated with this isolate, which got number M 2/24 of the phylum corpus. In order to do so, young colonies of the isolate were grinded slightly in sterile quartz sand. This turned out to be difficult due to the consistency of the colonies which could only be removed including great amounts of the medium. The experiment was designed as a first trial and was carried out in a phyto chamber in relatively small jars. It was repeated 10 times. The plants were apple seedlings grown from peeled apple cores in steamed substrate. The results are shown in Table 1.

Inoculating the soil a uniform distribution of the actinomycete isolate resulted problematic due to the fact that the colonies were hardly separable and contained a relatively high amount of the culture medium. So, after removal of the apple seedlings, a first replant was implemented. As expected, growth reduction of the seedlings as well as the frequency of root colonization by the actinomycete was clearly elevated. Microscopic analysis of root cuts showed damage patterns well known from all previous examinations.

In the same experiment other actinomycete isolates were used, but in none of these cases rootlet colonization by actinomycetes occurred. The results finally led to the conclusion that a further step towards clarifying the cause of soil sickness in apple trees was probably completed. This conclusion was already included in the final report for non-cash allowance of the German Research Foundation (Otto, 1993). However, the author considered it not justifiable to publish these results being a first trial with small containers which was carried out under non-natural conditions. Unfortunately, a repetition of the experiment as well as the reisolation of the same actinomycete from the infected rootlets that would be necessary to fulfill the Koch postulate could not be completed as it was not possible to postpone age-related retirement of the author anymore.

Discussion

Otto and Winkler (1977) reported on the colonization of apple tree rootlets by actinomycetes and considered it to be the cause of apple replant disease. Westcott and Beer (1983, 1985a, b) and Westcott et al. (1986) found the same. Though the problem of soil sickness is being issued and worked on in several institutions and research

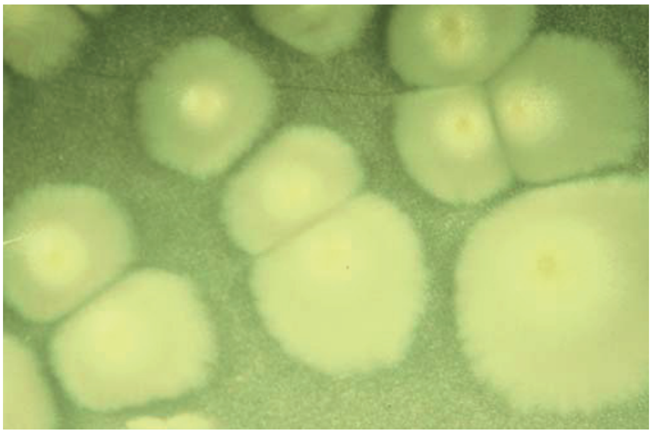

Fig. 1. Colonies of the actinomycete – isolate M2/24.

Table 1. Effect of inoculation of a steamed soil with actinomycete isolate M2/24 on shoot growth of apple seedlings and frequency of rootlet colonization by actinomycetes.
Control: steamed and non-inoculated substrate

	Diminution of shoot growth (%)	Frequency of rootlet colonization by actinomycetes (%)
1. Growing	14,1	19
1. Replant	48,2	55

groups, the author cannot find any clues as to an actual verification of the illustrated ideas on the cause of soil sickness. Therefore, in spite of the still owing final evidence of the pathogenicity of this actinomycete, it may be permitted to discuss the ideas about processes, relations and effects of colonization by actinomycetes which are derived from experimental results so far. This is further justified by the fact that there has been observed clear damage of rootlets in every single case of actinomycete colonization within the epidermal cells and in the cortex of microscopically examined root cuts, see Fig. 2. The root hairs are also damaged (Fig. 3). The stronger actinomycete colonization leads to the more decreasing density of root hairs of rootlets which might be responsible for a lack of efficiency of the fibre root system (OTTO and WINKLER, 1996).

Finally, reactions of root bark cells could be observed in electron microscopic investigations of infested roots which pronounce the pathogenicity of these actinomycetes (SZABÓ et al., 1998).

It can be assumed that the permanent forms of the qualifying actinomycetes can be found ubiquitously in many soils in a virtually more or less numerous number. Certainly, they are also brought into the ground in high numbers with root material from the tree nursery in the case of establishing new apple plantations. When the rootlets die away, the permanent forms of actinomycetes get to the soil and increase in number per ground unit. The formation of permanent forms could be determined in rootlets of apple seedlings (Fig. 4). They show as string-of-pearl-like arrays in the fragmented hyphes of the actinomycetes. This process could also be determined in the substrate mycelium of the colonies of the actinomycete isolate M 2/24 (Fig. 5). Elevated germ numbers of the permanent forms, due to perished rootlets, lead to stronger infection of newly growing roots. In accordance with these ideas, clearance might not be the precondition for occurrence of soil sickness, like SCHANDER (1956) postulated. Rather it should be the case that effects of soil sickness already appear in growing plantations, which

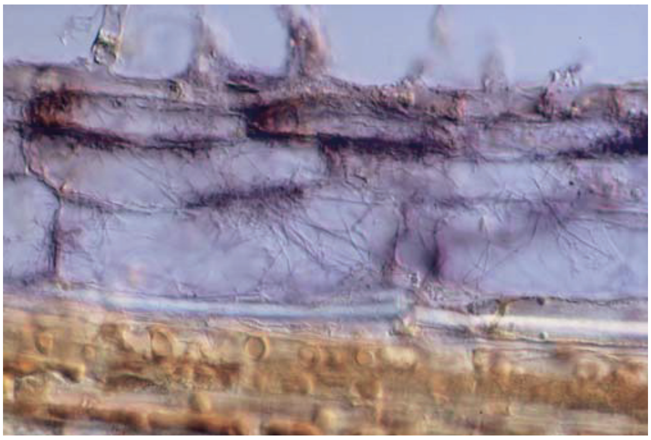

Fig. 2. Invading and beginning of damages of a rootlet of an apple seedling by actinomycetes from a soil with apple replant disease.

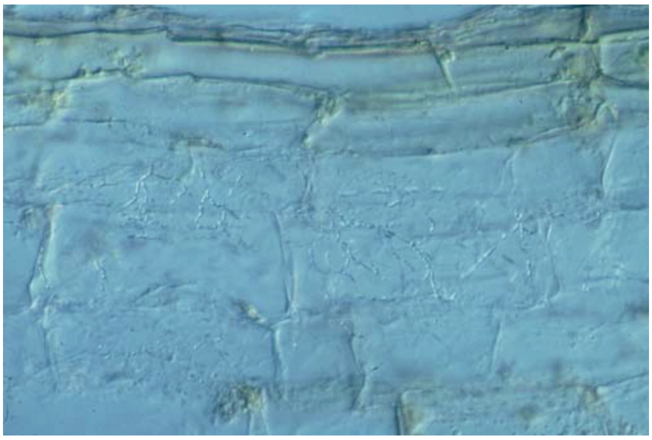

Fig. 4. Permanent forms of actinomycetes in the cortex of a rootlet of an apple seedling from a soil with apple replant disease.

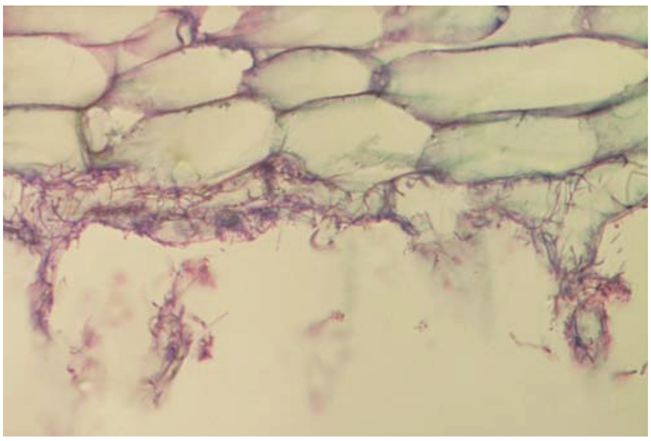

Fig. 3. The beginning of the invading of a rootlet of an apple seedling by actinomycetes from a soil with apple replant disease and damages of the root hairs.

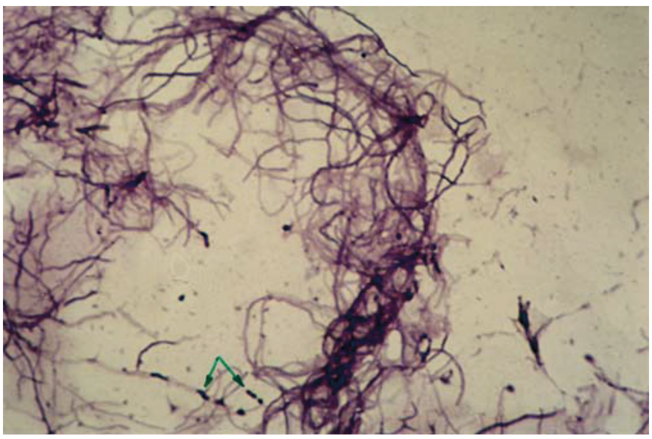

Fig. 5. Permanent forms in the substratemycel of the actinomycete – isolate M2/24.

was already proved by Otto (1966) and Otto and Winkler (1976).

It is well known that soil sickness must not occur on every habitat. It can occur on different habitats with strongly differing effects and it can already show clear effects after the first replant – even after a very short lifetime of the precedent culture – or only after several replants.

All of these occurrences, which are found in many publications on soil sickness, can easily be explained by the accumulation of permanent forms of the actinomycetes in question. In general, actinomycetes develop best under aerobic conditions as well as in substrate with rather neutral pH-value. Consequently, accumulation of the permanent forms will stretch across a longer period of time or several cultivation sequences respectively in soils with a very low pH-value as well as in habitats with regularly occurring high groundwater levels rather than in soils exhibiting better conditions for the development of actinomycetes. This process of accumulation is also strongly determined by the number of plants per unit. An examination of literature on yield progressions in apple tree orchards with different tree numbers per hectare shows that harvesting peaks occurred earlier with the higher the number of trees per hectare (Otto, 1990). Apart from other possible reasons for yield progressions of this kind the accumulation of permanent forms of actinomycetes as a cause should also be taken into account. Plants on virgin soil will not show negative effects in the first years, as the roots can progress to areas of the soil in which germ numbers of the permanent forms are not elevated yet. When the roots progress to root areas of neighbouring trees not reaching any soil without accumulated permanent forms anymore, only then will damage of the rootlet system reduce the efficiency of the root system and consequently the shoot growth and yield capacity. So in this case, not only the number of trees per hectare plays a role, but also the nature of the area that is at disposal for every single tree. Certainly, the short history of the cultivation system of meadow orchards can be explained by that.

Another point of discussion: In many of our experiments it could be observed, that the difference between shoot growth of apple seedlings on sick soils and on control soils reduced slightly in the second half of the growing season. That is, around the time of so called St. John's shoot growth on sick soils was somewhat more noticeable than on the control soils. Further investigation showed that even with very strongly infested rootlets in the first half of the growing season, infestation with actinomycetes reduced only slightly in the second half. In general, a relationship between differences in shoot growth and infestation degrees could be determined (Otto et al., 1993a). That is, the change between damaging and perishing of the rootlets and the growth of new ones is also reflected by the variability of shoot growth. It can be said that root systems are able to recover due to the reduced damage in the second half of the season. This again could explain why there is no considerable loss of trees during apple replantation, even with very strong soil sickness. Therefore it could be assumed that interactions between balance of plant hormones of the trees and changes of infestation degree of the rootlets do exist, which could be validated (Otto et al., 1994a). It could be verified that hormones like 1-naphthyl acetic acid and benzylaminopurine, which are transported from the terminal buds and the youngest primary leaves to the root, lead to higher frequency of actinomycete colonization of the rootlets. In contrast, gibberellic acid which is transported from the root to the sprout had no impact on colonization degree. These results led to the decision to include culture media with plant hormones in the actinomycete isolation experiments discussed above. One of the first results is presented here. It is probable that plant hormones reaching the soil through the rootlets function as a signaling substance for germination activation of the permanent forms of the actinomycetes.

This could explain the strongly marked persistence of soil sickness. It is numbered by decades in literature and clearly confirmed at 30 years by Otto and Winkler (1989). However, in literature there cannot be found any verified indications concerning the change of soil sickness degree during cultivation breaks. It must be assumed then, that the cause of soil sickness remains relatively unscathed in the soil during these large cultivation breaks. It is well known that permanent forms of microorganisms can persist in soils for very long periods of time and are reactivated with an appropriate host. This can also be true for the actinomycetes in question.

So, the question came up if other plants grown in these cultivation breaks would be able to activate the premanent forms, yet without reproducing themselves in the roots. This could be an interesting theoretical approach for the possibility to reduce germ numbers of permanent forms of actinomycetes in the soil. Another possibility would be to transfer signaling substances to the soil through hosts which were treated with plant hormones but are inapt for actinomycetes. If this succeeds, it would be an alternative to soil fumigation by chemical means.

These thoughts led to the question to what extent the actinomycetes found in the rootlets of apple trees can colonize other plants. It was natural to carry out such investigations on other rosaceae species. At the same time this was supposed to be a contribution to the question of specifity of soil sickness. In literature, this specifity generally is considered as a species- or fruit type specifity, due to the fact that normally a replant of stone fruits after apple and otherwise can occur without difficulties. It could be observed that rootlets of pear and rowan tree are also affected by actinomycetes in apple-sick soil (Otto et al., 1993b). However, in the rootlets of stone fruits no actinomycetes could be found. Colonization by actinomycetes could also be observed in some ornamental trees respectively shrubs of the rosaceae family in apple-sick soil as well as in their own replant soil (Otto et al., 1994b). The damage patterns resembled those of apple roots. The frequency of colonization, however, was different. Pear measured only half of the frequency of apple. Rowan tree showed results in between. Intensity of colonization was much lower in pear than in apple roots, too. This coincides with practical experience, in which cultivation of pear after apple is more likely to succeed than otherwise. It can be assumed that different rosaceae species are not equally apt as hosts for actinomycetes, but apple is particularly affected. It is unclear though if tolerance or resistance of apple root stocks against soil sickness can be realized in the near future. Anyway, without a final clarification of the cause of soil sickness a successful search for resistant root stocks remains unrealistic. This is further emphasized by investigations of OTTO and WINKLER (1975), in which no serious difference in the reactions to soil sickness was found between 15 standard apple root stocks, two clones each from three new cross-breeding combinations and combinations of six sorts on four rootstocks. However, it was notable that slowly growing rootstocks tended to show lower diminution of shoot growth, as opposed to frequently expressed opinion. In all trials the seedling rootstocks were affected the most. Again, it can only be assumed if there is a connection between balance of plant hormones of the trees and degree of actinomycete colonization of the rootlets.

Although final evidence of the pathogenicity of the actinomycetes in question still owes, due to the Koch postulate not yet fulfilled, all knowledge, appearance and symptoms of soil sickness of apple can be explained by the results described, while no other theory about the cause of soil sickness can comply with this.

It is the credit of SAVORY (1966), who first used the notion "Specific Apple Replant Disease" (SARD) instead of the common term "soil sickness" to refer precisely to a disease of apple trees. However, looking at the described results the term might no longer be entirely correct. There exists neither species specificity within the rosaceae family nor a specificity of fruit trees. Even the notion "replant" does apply in limited terms only. Of course, soil sickness appears more clearly in replant than in growing plantations. However, clearance preceding cultivation is not the condition for soil sickness.

If we succeed in verifying the found results about the cause of soil sickness of apple trees through the ultimate proof, then the phenomenon of soil sickness would be no less than a common phytopathological process in the area of the rootlets.

The author is aware of the fact that some conclusions derived from these results still must be underpinned by further examination. However, with this publication it is the wish of the author to encourage comprehension of these conclusions and to take them into account in other works of research which aim for the final clarification of the cause of soil sickness.

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