# Analysis concerning the rapid spread of apple proliferation phytoplasmas in a breeding orchard of apple seedlings

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**ABSTRACT**: The spread of apple proliferation (AP) phytoplasmas was monitored for 6 years in a breeding orchard in which nearly 16 thousand apple seedlings were planted. Out of this number 1,888 trees were identified as AP infected till the end of the growing season 2004, which corresponded to the proportion of 11.9%. The epidemic started in 1999 when the first 18 trees were diagnosed as contaminated by AP. Since that time the number of AP infected trees in the whole orchard increased exponentially yearly up to 877 specimens in 2004. The decisive factor that influenced this spread of AP in this study was the use of fungicides for pest control in three different parts of this orchard. Some other factors – rootstock, age of trees or top-working were identified as influencing this AP spread in different ways. Their role in this spread is discussed in more detail. Trees of the age 10 years were the most frequently infected on average. Top-working significantly increased numbers of infected trees in the year of the treatment application in comparison with trees from adjoining rows without this treatment. The early removal of all AP infected trees from the orchard did not considerably reduce the rates of AP infection.

**Keywords**: apple proliferation; phytoplasmas; apple; spread; vectors; breeding; insecticides; rootstocks; top-working; monitoring; removal of infected trees

Apple proliferation (AP), caused by a phytoplasma, is a vector-borne and graft-transmissible disease of apple trees diffused in nurseries and orchards all over the world. Economically it is very important because it reduces yields and fruit quality very dramatically. The characteristic symptoms of AP are witches' brooms, enlarged stipules and small fruits with low content of soluble solids. Infected trees are more susceptible to some other diseases, especially to apple mildew. Once infected, the trees cannot be cured in orchard conditions, and furthermore, they become permanent sources of infection for other healthy trees. Sometimes the disease merges into a latent state when its symptoms are much less clear. At that time, however, such diseased trees can still be dangerous sources of the infection (Seidl, Komárková 1976; Seidl 1980; Brzin et al. 2003; Cainelli et al. 2004).

In the Czech Republic, AP was detected in the early sixties of the last century as a common disease mostly in old orchards (Blattný et al. 1963). Using budwood from infected trees it was proved to be the main way of AP dissemination at that time. Transmission of the disease through leafhoppers (mainly by *Philaenus spumarius*) was also proved, but AP spread in this way was considered rather low (Seidl, Komárková 1976; Seidl 1980). After indexing trees in budwood gardens and the use of healthy propagating material, AP practically disappeared for many years from newly planted orchards in the country.

Since 1995, a serious epidemic outbreak of AP has reappeared in some areas of Italy, Slovenia and Germany (Loi et al. 1995; Frisinghelli et al. 2000; Brzin et al. 2003; Jarausch et al. 2004a). This new epidemic is most frequently associated with new more efficient vectors of disease transmission, among which first of all *Cacopsylla costalis* (now *C. picta)* and *C. melanoneura* were designated (Tedeschi et al. 2002; Jarausch 2003; Jarausch et al. 2004a). Another reason for the rapid spread of the disease could be the uprise of new strains of AP (Cainelli et al. 2004; Jarausch et al. 2004b). In recent years, an increased incidence of AP has been observed also in the Czech Republic (Navrátil et al. 1998; Kučerová et al. 2005).

The aim of the present paper was the analysis of AP incidence that was monitored between 1999 and 2004 in a selected apple orchard in Holovousy.

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## MATERIAL AND METHODS

The seedling selection orchard utilised within the apple-breeding program at Holovousy was planted step by step in the years 1986–1997. Climatic conditions of Holovousy are characterised by an average annual temperature of 8.1°C and an average annual rainfall of 650 mm. The orchard located in a plain at an altitude of 280 m above sea level has a rectangular shape and 3 sides adjoin other older apple orchards. It consists of 48 tree rows directed North-South. During the establishment of the orchard, every year usually 1,000 to 2,000 pre-selected apple seedlings grafted on dwarf rootstocks were planted using the primary spacing of 4 × 1 m. A smaller portion of trees was planted at the spacing of 4 × 0.5 m when very dwarf rootstock M 27 was used. Another smaller portion of trees was planted at the spacing of 4 × 2 m in the case of a station trial that was also included in the orchard. All trees were planted as virus free except the lots on M 9 rootstock of Czech origin declared only with the status "virus tested".

In the course of the seedling selection for disease resistance, no fungicide treatments were used in most parts of the orchards. Similarly, a program of chemical protection against pests was also very limited in most parts of the orchard as natural incidence of aphids, mites and codling moth was rated in search of some resistance of the breeding stock against these pests. There, usually only single trees were treated with chemicals in case the incidence of a pest reached a critical level. In the other parts of the orchard spraying treatments against pests and diseases were conducted according to the recommendations for commercial orchards, mostly based on monitoring of their incidence. Among insecticides, Zolone, Reldan, Calypso, Aztec and Pirimor have been most frequently used in the orchard in recent years.

Original seedlings in the selection orchard were evaluated for selected characteristics in their fruiting stage for 5 or 6 years. Then most of them were discarded from the next evaluation. They were usually utilised for propagation of selected seedlings by their top-working.

All the trees in the selection orchard were monitored from the very beginning for symptoms of AP. During the last three years, when the number of trees with AP symptoms became very high, all the trees were monitored for the disease twice during each growing season – in June and in August. All trees infected by AP were removed from the orchard immediately after they were found.

The differences in the numbers of infected trees by AP between the particular studied factors were tested by analysis of variance or regression analysis.

## RESULTS

In total, 15,924 apple trees were planted in the orchard and monitored for AP infection (Table 1). From this number 1,888 trees were identified as AP infected till the end of the 2004 growing season, which corresponded to the proportion of 11.9%. The epidemic started in 1999, when the first 18 trees were diagnosed as contaminated by AP. Since that time the number of AP infected trees in the whole orchard increased exponentially yearly up to 877 specimens in 2004 (Fig. 1).

The total occurrence of numbers of infected trees throughout the whole orchard varied in particular rows depending on several factors, but the most important of them was the use of insecticides in the program of plant protection of the orchard (Fig. 2).

Table 1. Total numbers of infected trees according to rootstocks

| Rootstock | Number of trees | Infected trees | |
|---|---|---|---|
| | | Number | % |
| J-TE-E | 5,060 | 252 | 5.0 |
| J-TE-F | 1,280 | 101 | 7.9 |
| J-TE-G | 449 | 22 | 4.9 |
| J-TE-H | 489 | 55 | 11.2 |
| M 26 | 307 | 55 | 17.9 |
| M 27 | 1,214 | 41 | 3.4 |
| M 9 | 2,222 | 330 | 14.9 |
| M 9 EMLA | 3,661 | 932 | 25.5 |
| Others | 1,242 | 100 | 8.1 |
| Total | 15,924 | 1,888 | 11.9 |
| LSD ($P$ = 0.05) | | | 4.3 |

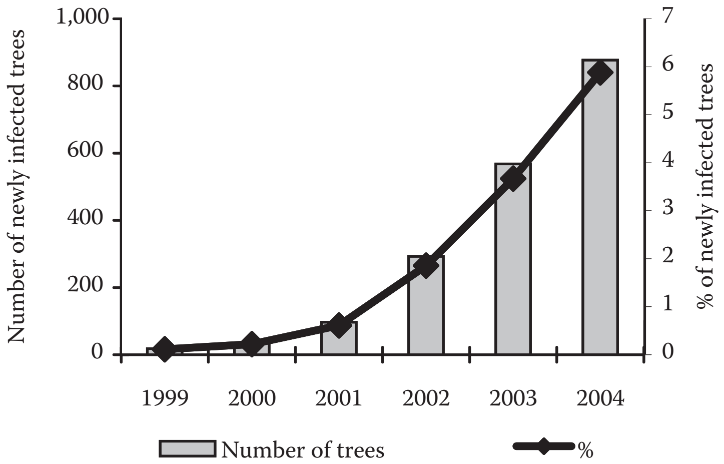


Fig. 1. Rates of infection of trees by apple proliferation in 1999–2004

Numbers of AP infected trees were significantly lower nearly in all rows that were under this protection. The difference between rows with this protection and those without it was on the average of a high-order magnitude.

Another important factor that influenced the rates of the infection was the frequency of infected trees in the row in the previous year (Fig. 3). This relationship (calculated from figures gathered from areas without fungicide use), which was the highest-fitted to a logarithmic equation, proved to be highly significant. According to this relationship the higher the number of trees infected by AP in the current year, the higher the number that can be expected in the following year.

The distance of the tree from the source of infection also played an important role in the spread of AP. It is illustrated by the frequencies of the distance of an infected tree from the next infected tree (Fig. 4). In a majority of cases an infected tree was placed in close neighbourhood to other infected trees. On the other hand, only in 1.4% of cases this distance was longer than 40 m.

The age of the tree was another factor that influenced AP infection rates to some extent in this study. Trees that were 10 years old were the most frequently infected on average (Fig. 5). The youngest trees that were infected here were 5 years old and from this point in time the rate was rapidly increasing. On the other hand, ageing trees older than 10 years were progressively less and less frequently infected.

The rootstock is probably a further factor that had a certain influence on the AP infection rate in the study (Table 1). The smallest numbers of seedlings that were infected were found when the rootstock M 27 used. A positive influence in this respect was also exerted by J-TE-G and J-TE-E. On the other hand, seedlings on M 9 EMLA and on M 26 were the most sensitive to the infection.

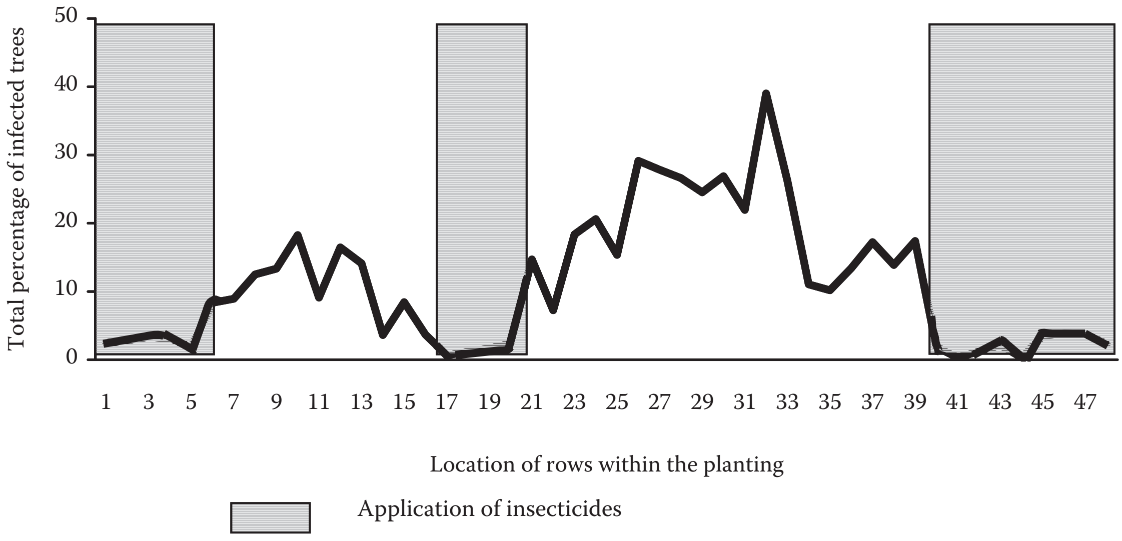


Fig. 2. Occurrence of infected trees in single rows throughout the orchard

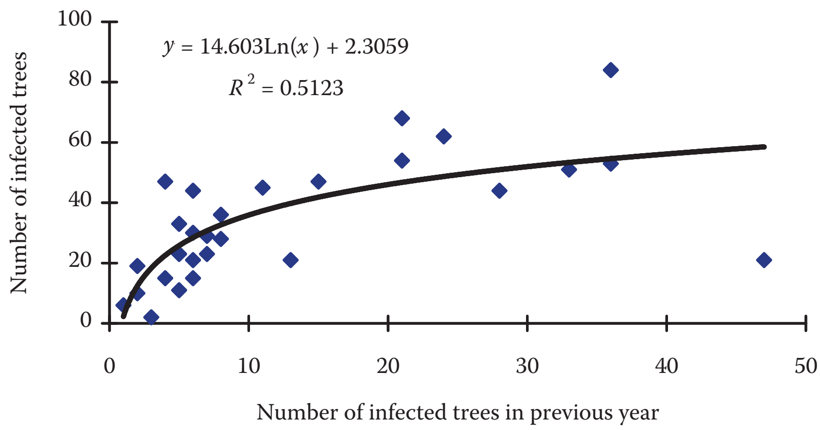


Fig. 3. Relationship between numbers of infected trees in current and previous year within single tree-rows

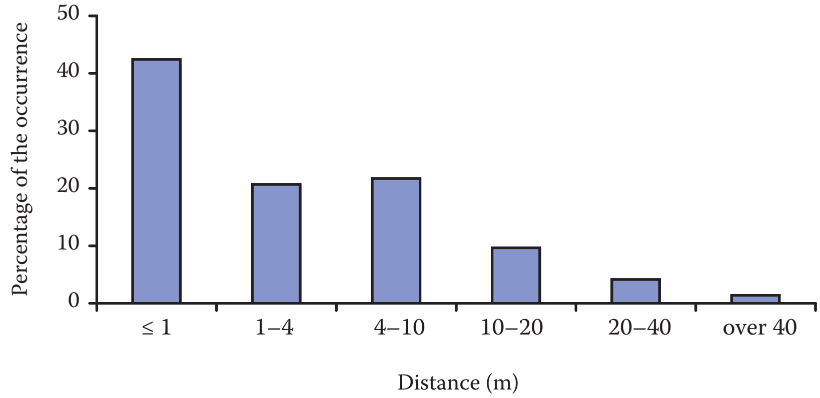


Fig. 4. Frequency of the distance of the infected tree from the next infected tree

The last factor that affected AP spread in this study was the top-working of the seedlings (Table 2). The rows in which this top-working was used in the orchard from 2001 to 2003 showed significantly increased numbers of infected trees in the year of application of the treatment in comparison with trees from adjoining rows without top-working. These rates, however, were also increased by trees that were not top-worked. The rates of AP infection in these rows, however, were decreased in the next year after this treatment, sometimes even below the infection rates in control trees from adjoining rows.

## DISCUSSION

The most decisive factor that influenced the spread of AP in this study was the use of fungicides for pest control in three different parts of this orchard. These treatments obviously largely reduced the population of insect vectors in particular rows there. The present study is, therefore, focussed on the identification of these vectors. The omission of the plant protection against insects, either in organically grown plantings or in amateur orchards, seems to be very dangerous at the present time regarding the threat of apple tree

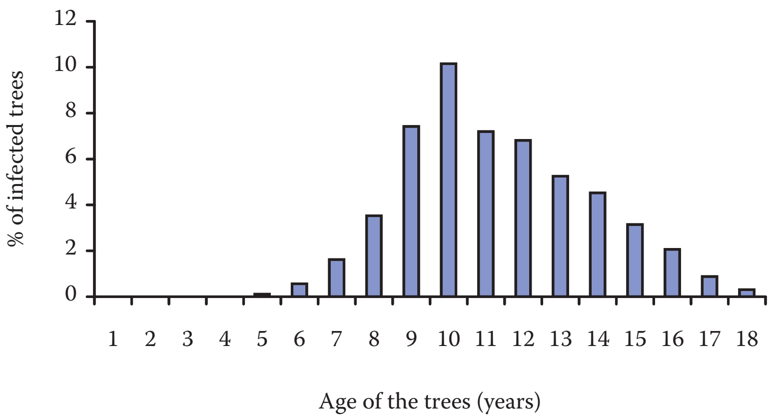


Fig. 5. Influence of the age of trees on their yearly rate of infection

Table 2. Influence of top-working on shares of infected trees

| Year of top-working | Category of trees | % of infected trees by apple proliferation | | | |
|---|---|---|---|---|---|
| | | 2001 | 2002 | 2003 | 2004 |
| 2001 | Top-worked trees | 5.9 | 1.7 | 2.1 | 6.3 |
| | Untop-worked trees | 9.3 | 0.8 | 1.8 | 5.5 |
| | Trees in adjoining rows without topworking | 0.7 | 2.1 | 2.4 | 7.7 |
| 2002 | Top-worked trees | | 15.3 | 4.0 | 6.1 |
| | Untop-worked trees | | 10.1 | 1.5 | 7.2 |
| | Trees in adjoining rows without topworking | | 2.5 | 2.9 | 8.6 |
| 2003 | Top-worked trees | | | 9.8 | 3.5 |
| | Untop-worked trees | | | 11.5 | 2.7 |
| | Trees in adjoining rows without topworking | | | 3.0 | 8.4 |
| LSD ($P$ = 0.05) | | 2.2 | 4.6 | 3.1 | 3.8 |

infestation in the Czech Republic by the disease. Similarly, neglected orchards and roadside alleys, in which apple trees remain without any care (and that are presently very common in the country), should also be included in a state quarantine program as a measure against AP. Plant protection in apple orchards aimed at insect vectors should be considered as the main measure to control AP spread, as it is already recommended in some other countries (Loi et al. 1995; Tedeschi et al. 2002; Jarausch et al. 2004b).

The first AP infected trees in this monitored orchard were found in 1999, when the oldest trees in it were 13 and the youngest ones only 3 years old. Attacking sources were evidently neighbouring orchards of older apple-trees, in which trees with AP were already identified in previous years. During the following 5 years, AP attacked the whole area of the observed orchard, which was overrun step by step through a distance of several hundred meters, in spite of the fact that the most frequent transmission of the disease by means of vectors was by far only to adjoining trees. It is evident from this that with AP disease, potency for its transmission to a larger distance does exist though the frequency of the case is obviously very low.

The highest rate of AP spread expressed itself in 2003 and 2004 in the area of the orchard delineated as the 26$^{th}$ and 33$^{rd}$ rows. This very high occurrence of AP infected trees could be associated with three factors: tree age around 10 years, use of the rootstock M 9 EMLA and extreme susceptibility to AP of the given genotypes (seedlings) planted into this part of the orchard (the last item will be a subject of the next paper). Unfortunately, it is not possible to trace from the gathered data which of the above-mentioned three factors was the most critical, though it is also probable to expect their mutual interference in this case.

The occurrence of AP in this orchard was markedly influenced also by the rootstock. The lowest numbers of invaded trees were recorded on the rootstock M 27, whereas the most frequently infected were trees on M 9 EMLA and M 26. Unfortunately, the trees on the rootstock M 9 EMLA were planted only in that part of the orchard that was the most severely attacked by AP. Despite this, in the case of rootstocks a certain connection was shown between their vigour and the rate of AP infection. The rootstocks that generated smaller trees were infected less often than the more vigorous ones.

The occurrence of AP infected trees in the orchard was considerably influenced also by tree age. In this orchard 10 years old trees had the highest infection rate. Unfortunately, both the youngest and the oldest trees were planted in those parts of the orchard that were treated with insecticides and were, therefore, only very slightly invaded by AP. Nevertheless, the infection rates decreased with the advanced ageing of trees.

Furthermore, the use of top-working had a very interesting influence on the occurrence of AP. Significantly higher rates of AP infected trees were recorded in the tree-rows in the year when this treatment was used. Similarly, increased incidence of AP was reported in the past also after using severe tree pruning (Seidl, Komárková 1976; Seidl 1980). These authors assumed that severe pruning brought trees under stress and so they became more susceptible to AP infection. But here, in tree-rows with this treatment application, those trees that were not top-worked at all also had higher rates of AP infection. This could be generated by an effect of more numerous sources of AP infection (Fig. 4). Another possibility to explain increased infection rates after top-working could be the transition of latently diseased trees through a shock into a state when they show AP symptoms. This version could also explain the fact that the numbers of AP infected tress decreased in the following years after top-working.

The finding that the early removal of infected trees did not reduce much the spread of AP infection in the orchard broke down a definite illusion. This fact could have several causes. Among them it is probably due to the presence of more than coincidence between the moments of a tree's infection, the beginning of its own infectiousness, the time when it produced AP symptoms and the final removal of the tree from the orchard. An infected tree might be a source of infection for other healthy trees for a defined time before it exhibited AP symptoms and was removed from the orchard. These cases might be more prevalent if the infection occurred towards the end of the growing season because these trees could develop AP symptoms in June of the next year and only then they could be removed from the orchard. Likewise, it might also be possible that infected vectors continued to be infectious after the removal of the infected trees. The last possibility could also be that some AP infected trees failed to develop symptoms of AP infection, being only latently diseased but still infected for vectors and thereby able to spread AP further.

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## Rozbor příčin rychlého šíření fytoplazmy proliferace ve výsadbě hybridů jabloní


**ABSTRAKT**: Šíření proliferace jabloní bylo po dobu šesti let monitorováno ve výsadbě hybridů jabloní, do které bylo celkem vysazeno téměř 16 tisíc jabloňových semenáčů. Z nich bylo do doby ukončení vegetace v roce 2004 na základě symptomů celkem identifikováno 1 888 stromů napadených touto chorobou, což odpovídá podílu 11,9 %. Tento epidemický výskyt proliferace začal v roce 1999, kdy bylo s diagnózou výskytu této choroby zaznamenáno 18 stromů. Od této doby se zde počty napadených stromů zvyšovaly exponenciálně až do roku 2004, kdy dosáhly 877 nových výskytů. Rozhodujícím faktorem, který ovlivňoval šíření této choroby, bylo používání fungicidů v rámci programu ochrany rostlin ve třech různých částech této výsadby. Kromě toho zde na šíření proliferace měla vliv řada dalších faktorů, z nichž nejvýznamnější byl vliv použití různých podnoží, stáří stromů a vliv jejich přeroubování. V rámci diskuse je v příspěvku podrobněji rozebírán vliv všech sledovaných faktorů na šíření této fytoplazmy. Nejvíce stromů bylo v průměru napadeno ve věku 10 let. Podíly napadených stromů byly rovněž významně zvýšeny po jejich přeroubování, a to ve srovnání se stromy v sousedních řadách, které přeroubovány nebyly. Naproti tomu včasné odstraňování všech napadených stromů z výsadby nemělo výrazný účinek na omezení šíření této choroby.

**Klíčová slova**: proliferace jabloní; fytoplazma; jabloň; šíření; vektory; šlechtění; insekticidy; podnože; přeroubování; monitoring; odstraňování napadených stromů

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