

EXPRESSION OF DISEASE SYMPTOMS ON DIFFERENT APPLE CULTIVARS INFECTED WITH APPLE PROLIFERATION PHYTOPLASMA

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ABSTRACT

An expression of Apple proliferation (AP) symptoms in the infected young apple trees of 7 cultivars was studied. Two types of symptoms were observed in infected trees; AP specific (changes in size and number of leaf stipules and witches-broom formation) and non-specific symptoms (changes in leaf size, shape and colour, formation of rosettes and stunting of shoots). The percentage of infected trees that expressed one or more AP-specific symptom was 18.9-75.5% in one-year old trees, 39.4-87% in two-year, and 44.8-89.7% in three-year old trees. The expression of non-specific symptoms was similar (43.2-65.3% in one-year, 57.6-85.2% in two-year and 62.1-87.5% in three-year old trees).

Key words: Apple proliferation phytoplasma, apple, disease symptom expression

IZRAŽANJE BOLEZENSKIH ZNAMENJ PRI JABLANAH RAZLIČNIH SORT OKUŽENIH Z APPLE PROLIFERATION FITOPLAZMO

IZVLEČEK

Pri mladih drevesih 7 sort jablan smo preučevali pogostost pojavljanja specifičnih (spremembe velikosti in števila prilistichev, oblikovanje metličavih poganjkov) in nespecifičnih znamenj bolezni (spremembe v velikosti, obliki in barvi listov, oblikovanje listnih rozet in pojav zakrnelih poganjkov) po okužbi s fitoplazmo Apple proliferation (AP) s cepiči ali očesi ob cepljenju. Odstotek okuženih dreves, kjer so se pojavila specifična znamenja je pri enoletnih rastlinah različnih sort znašal 18,9-75,5 %, pri dvoletnih 39,4-87 % in pri triletnih 44,8-89,7 %. Nespecifična znamenja okužbe so se pojavila v približno enakem obsegu; pri 43,2-65,3 % enoletnih, 57,6-85,2 % dvoletnih in 62,1-87,5 % triletnih dreves.

Ključne besede: metličavost jablan, apple proliferation fitoplazma, jablana, izražanje bolezenskih znamenj

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1 INTRODUCTION

Apple proliferation (AP) caused by the AP phytoplasma which was recently assigned as 'Candidatus phytoplasma mali' [24] is one of the most damaging diseases of apple trees in Europe. In recent years studies of potential AP vectors showed that two psillids (*Cacopsylla melanoneura* and *C. costalis* (= *C. picta*) are the main vectors of AP in apple plantations and contribute significantly to the spread of the disease [26]. The main strategies deployed for management of this disease are the control of insect vectors and the production of phytoplasma-free propagation and planting material [14, 22, 26]. Further progress has been made in AP phytoplasma detection methods [3, 5, 15, 7] thus improving the performance of certification schemes. However, the control measures are only partially efficient in the areas where the disease is widespread and insect vectors are present in large populations what was reported for several regions of Europe [9].

Especially in newly established plantations in apple growing areas with high disease occurrence, an early detection of infected trees and their removal is almost equally important as intensive vector control. The authors of several studies of AP phytoplasma pattern of AP symptom expression in apple trees agree that symptom expression is very variable and that many apple cultivars do not show the typical symptoms (changes in size and number of leaf stipules and witches-broom formation) in the first years of tree development [10, 11, 12, 18, 19, 20, 21, 22, 25]. In addition, it has been assumed that many apple trees are latently infected and disease symptoms become visible only as a result of special weather conditions or significant changes in the production practices [2]. Notably, there are no more recent reports on AP disease symptom expression in young apple trees of different cultivars, which could serve for early detection of infected apple trees in new plantations. The main objective of this study was to collect new data on the expression of specific and also non-specific AP symptoms in the young (1-3 years) apple trees, including differences among different cultivars.

2 MATERIAL AND METHODS

2.1 Experimental design and conditions

An experimental plantation was established to study the expression of AP disease symptoms in one-, two- and three-year old apple trees. Non-infected (AP-free) and AP infected mother trees were selected as sources of scions and buds for producing of non-infected and infected apple stocks. The term **stock** used in this paper applies to one-, two- or three-year old apple trees obtained by grafting or budding the rootstocks with scions or buds (**grafted stocks, budded stocks, respectively**). All series of non-infected and infected stocks were obtained by the same techniques and were maintained for three years in the experimental plantation under an insect proof screen (4.5 m high construction). Inside screenhouse stocks were planted according to randomised block system. There were 8 blocks, each containing healthy and infected plants of all 7 studied cultivars. Systemic insecticides and fungicides were applied at least six times a year to further prevent any vector transmission and to control fungal diseases. Stocks were fertilized and irrigated according to standard production technology. According to the soil analysis all important micro and macro nutrients were present at optimum level, and the soil pH was at 6.3. In order to avoid interference of chemicals and wounding with AP

symptom expression, no herbicides, hormones or bioregulators were applied and no pruning or tip cutting was performed for branch induction. The applied pesticides exerted no phytotoxic effects which could simulate AP symptoms, as shown by the group of control plants which had not been treated with any pesticide. The presence of AP symptoms was recorded in detail throughout the entire growing season. Laboratory tests for the presence of AP phytoplasma were performed in October each year.

2.2 Mother trees and rootstocks

Experimental mother trees of seven apple cultivars were selected as sources of scions and buds. The apple cultivars studied were: Jonagold, Golden Delicious, Braeburn, Elstar, Gala, Fuji and Idared. For each cultivar, two healthy trees and two AP phytoplasma naturally infected trees were selected and labelled in 1999 at the apple plantation in the Experimental Station of the Faculty of Agriculture Maribor, Slovenia (43° 34' N, 15° 38' E). Altogether, 14 healthy mother trees and 14 infected trees were selected. Laboratory analyses (see 2.4 for details) were performed several times prior to and during the experiment to verify the status of selected experimental trees. Selected mother trees were seven to twelve years old and showed typical AP symptoms in some parts of the crown, while the other parts were free of symptoms. For the production of AP-free stocks, the scions were taken from healthy trees grown in the same orchard. The scions and buds were grafted and budded, respectively, onto one year old virus-free certified EMLA M9 rootstocks (health status also proven by laboratory tests).

2.3 The manner of obtaining scion and bud wood, and propagation techniques

Apple stocks were produced continuously during 2000-2005. The scions and buds from diseased and healthy trees were always collected at the same time and in the same manner. Scions or buds were collected twice a year from shoots. During every propagation period in every experimental year 56 stocks (7 cultivars x 2 trees x 4 scions or buds from different positions per tree) were derived from both AP phytoplasma free and infected mother trees. The scions for dormant grafting (bench grafting) were taken in the first week of February. They were kept in cold storage at 4° C for a week before manual whip-and-tongue grafting to healthy EMLA M9 virus-free rootstocks. Grafted dormant rootstocks with well formed roots were planted in plastic containers with a sterilised substrate (Neuhaus Humin Substrat N8). They were allowed to develop for one month in containers in an insect-proof greenhouse at 15° C, and were transferred to the experimental insect-proof screenhouse nursery in the last week of March. Scions burst in the middle of April and sprout developed into a top of new trees by the fall.

Chip budding was performed in the first week of August each year. For budding, dormant and well rooted EMLA M9 virus-free rootstocks were planted in the experimental screenhouse in the first week of April each year. Shoots with buds for chip budding were taken from mother trees two to three days before budding, and were kept in a cold storage. Buds were inserted and tied with grafting bands. They remained inactive until the following spring. Budded rootstocks were cut back above inserted buds just when the growth started in the spring of the following year. New top developed from the inserted bud.

2.4 Laboratory detection of AP phytoplasma

Stocks were sampled for detection of the AP phytoplasma in the years 2001-2005, each year between the end of September to the end of October. The time for sampling was chosen based on the reports showing that the AP phytoplasma populations in sieve tubes of shoots and leaves reaches the highest level during this time of the year [15, 18]. From each stock, two to three shoots with a minimum of 8 well developed leaves per shoot were taken. If stocks showed any kind of apple proliferation symptoms, the symptomatic shoots were chosen for sampling. If stocks did not show any symptoms, the first terminal shoot and one or two lateral shoots were sampled. Grafted and budded stocks were always tested at the same time. All stocks were tested by ELISA without regard to the AP symptoms expression. All plants that showed symptoms of AP infection and were ELISA negative were additionally tested by PCR. In addition, some plants not showing any symptoms, bud tested ELISA positive, were tested by PCR to confirm the results. Plants which were not confirmed AP positive in the first year were re-tested in the second and third year of development.

The laboratory testing was performed as described by Brzin et al. [5]. 0.5 g of fresh leaf midrib tissue was cut into small pieces and homogenized in 5 ml of ice cold ELISA extraction buffer (20 mM Tris, 137 mM NaCl, 2 % polyvinylpyrrolidone-24, 0.05 % Tween 20, 2.68 mM KCl: pH 7.4) in homogenization bags (U-form, Bioreba Switzerland) using a Homex homogenizer (Bioreba). The ELISA extract was used in ELISA and PCR testing. 1 ml of the ELISA extract was centrifuged at 10.000 g for 10 min at 4°C and DNA was extracted from the resulting pellet for further PCR by method described by Ahrens and Seemüller [1]. For specific detection of AP phytoplasma by ELISA, monoclonal antibodies [15] and their enzyme conjugates were applied as recommended by the manufacturer (Bioreba AG, Switzerland). The absorbance at 405 nm (A_{405}) after 20, 60 and 120 min was measured using a Dynatech MR5000 plate reader. A sample was considered positive if its mean A_{405} value exceeded twice the mean value of the healthy controls.

PCR of 35 cycles was performed in 40 µl reaction volume using the AP group specific pair of primers fO1/rO1 [15] of DNA as template. Negative samples were further tested in the nested PCR assays, where amplification products obtained after 35 cycles in 40 µl reaction volume with the universal primer pair P1/P7 [23] were diluted 1:100 in water and re-amplified with AP group specific primers fO1/rO1 as described above. All sets of reactions included DNA samples from healthy plants and controls lacking template DNA as well as positive controls. Samples (15 µl) of PCR product were resolved by 1 % agarose gel electrophoresis and visualized by staining with ethidium bromide (2×10^{-4} mgml⁻¹) and UV illumination.

3 RESULTS

Plants were examined several times a year for the following morphological and colour changes on AP infected apple plants [10, 11, 12]: the average leaf size, leaf colour (chlorosis and discoloration, browning, reddening, yellowing), the size of leaf stipules and petioles, the number of leaf stipules, the shape of leaf lamina (curling, rolling, malformations), jaggedness and shape of leaf margins, the shape and the colour of leaf veins, the formation of the rosettes on terminal or lateral shoots, witches-broom formation, stunting or dwarfing of shoots and whole stocks. All types of symptoms were observed (Table 1). The present study showed that

the AP phytoplasma-free plants (confirmed by laboratory tests) expressed some non-specific AP-like symptoms in a very low frequency (see the last part of Tables 1). These symptoms were mostly: reddening of leaves, shoot chlorosis, apex rosette formation, leaf curling, and leaf malformations. Based on these results we divided the symptoms in two groups: the AP-specific and non-specific. Witches-broom formation, enlarged stipules and increased number of stipules were considered as AP specific symptoms which do not appear on healthy plants, all other presented symptoms were considered as non-specific symptoms because they have been recorded sporadically also in AP phytoplasma-free plants.

3.1 Expression of AP symptoms in different apple cultivars

Different cultivars showed considerable variability in the time of occurrence, type and intensity of AP symptoms. The differences among cultivars are presented in percentages of infected stocks (confirmed by laboratory tests) expressing a particular AP - specific or non-specific symptom. Witches' broom formation (most specific AP symptom) was frequent in one-year old infected stocks of cvs. Elstar (59.3%), Gala (53.2%), Jonagold (68.3%) and Golden delicious (61.2%). Only a smaller portion of infected Fuji and Braeburn stocks (both 13.5%) showed AP symptoms in the form of witches' broom formation in the first year, but the percentage increased during the second and third year of development. These two cultivars appear to be difficult for early visual detection of AP in the first year. In one-year old Idared stocks typical brooms were recorded in only 24.3% of stocks. 52.8% of the two-year old Idared stocks formed atypical brooms which were different in structure from brooms in other cultivars. Infected stocks of cv. Idared often formed apex rosettes (83.3%) instead of brooms. Rosette formation was often accompanied by stunting and dwarfing of the whole plants or single shoots in that cultivar (55.6, 80.6%). Notably, stunting and dwarfing of whole plants or single shoots were a rarely noticed symptom in other cultivars in the present study. Enlarged stipules, which are also a common and specific symptom of AP, most often appeared in cultivars Elstar (in 61.1% of one-year, and in 77.8% of the two year old stock), Gala (in 36.2% of one-year, and 69% of two year old stock), Golden Delicious (in 40.8% of one-year, and 68.1% of the two year old stock) and Jonagold (in 40% of one-year, and 57.1% of the two year old stock), and was considerably less frequent in Idared, Fuji and Braeburn stocks.

Increased number of stipules (here considered as an AP - specific symptom) mostly appeared in Elstar stocks (in 53.7% of one-year, and 74.1% of the two-year old stock) and very rarely in cultivars Fuji, Gala, Jonagold and Golden Delicious. This symptom did not appear in any of the infected Braeburn and Idared stocks. Early reddening of leaves and spring chlorosis (here considered as a non-specific symptom) appeared in all cultivars in a similar frequency, and were occasionally also recorded in non-infected stocks. Therefore, these symptoms are less reliable in young apple trees, but, they should not be ignored completely, especially in cv. Fuji and Braeburn where those non-specific symptoms appear the first and the brooms appear later. Our results showed that they appeared 5 to 15 times more frequently in infected than in non-infected stocks (see the results at the end of the Table 1). Notably, in infected Fuji stocks, spring chlorosis was frequently recorded. Since no other cultivar expressed such consistency in chlorosis, this symptom could be regarded as specific only for the cv. Fuji. This and other data presented in Table 1 support our hypothesis that young AP infected trees of different apple cultivars show differences in type and frequency of AP - specific as well as in non-specific symptoms. Moreover, some specific and non-specific symptoms could be clearly

associated with a particular cultivar of apple stocks (e.g., specific chlorosis of leaves in Fuji, specific rosette formation in Idared and an increase in number of stipules in Elstar).

3.2 The onset of AP symptom expression

The percentage of infected trees (confirmed by laboratory tests) that showed one or more visually detectable symptom, increased with the age of stocks, i.e. time after grafting. One-year old infected trees showed specific symptoms ranging between 18.9%-75.5%, two-year old stocks between 39.4-87.0%, and three-year old stocks between 44.8 and 89.7%. Results show that approximately one half of the infected stocks do not show AP specific symptoms in the first year of development (see Table 1, age 1). Specific symptom expression increased in the second year of development, but no further significant increase was observed in the third year (see Table 1, age 3). More than 20% of one-year old and more than 10% of two-year old infected stocks did not show any kind of disease symptoms. The method of the stock propagation (budding or grafting) did not considerably influence the symptom expression. Specific and non-specific symptoms appeared with similar frequency in infected budded and grafted stocks in all three years of stock development (data for budded and grafted stocks are not shown separately). Witches-brooms, reddening, enlarged stipules and apex rosettes were the most frequent symptoms expressed more or less equally in budded and grafted stocks.

4 DISCUSSION

Even in case of best certified planting material, a very small percentage of trees placed on the market could still be infected by the AP phytoplasma, and could present an initial source for a disease spread [6]. This is particularly important in case of newly established plantations in apple growing areas with low disease occurrence, where an early detection of infected trees and their removal is as important as intensive vector control. The visual observation of AP disease in apple trees (e.g. nursery stock) is unreliable and very subjective if there are no visible typical symptoms of AP infection (i.e. witches brooms). Laboratory analyses are much more reliable and standardized for assesment of the AP phytoplasma infection [3], although there is no clear data available about their use on young nursery apple stock. Our hypothesis was that the visual detection could still represent an effective tool of preventing the introduction of AP infected planting material, providing that the producers of nursery stocks are well informed and trained in recognizing symptoms of AP in young apple stocks. In order to increase the quality of visual detection of young infected trees it is essential to describe specific cultivar-related and reliable AP symptoms in detail. The obtained results indicate that between 60 - 65% of AP infected two- to three-year old trees can be expected to show one or more visually detectable AP – specific or non-specific symptom. Such a relatively high rate in AP symptom expression (considering also non-specific symptoms) should provide the fruit grower with enough information to detect the presence of AP infected trees in plantations early enough to prevent a further disease spread. Our study shows that differences among apple cultivars exist and are noticeable by thorough observations.

The present experiment showed that also the AP phytoplasma-free plants may express some non-specific symptoms (in this study reddening of leaves, shoot chlorosis, apex rosette formation, leaf curling, and leaf malformations), but in a very low frequency. Non-specific disease symptoms are usually ignored in nurseries, since nobody relates them with

phytoplasma infection. As our results show, in high percentage of infected plants, non-specific symptoms appear earlier than specific symptoms (in this study full witches-broom formation, enlarged stipules and increased number of stipules).

The results of our study suggest that the non-specific disease symptoms, which also tend to be less obvious, should not be ignored in visual detection of AP diseased young apple trees. They should be regarded as an indication that AP infected trees are present in a plantation. Although they were found in AP phytoplasma free apple stocks and therefore declared non-specific, it should be pointed out that the non-infected trees grown in favourable conditions rarely express the described non-specific symptoms. They could be contributed to the lack of nutrients, use of chemicals for induction of branching, insect damage, drought stress, virus infection and other (observations of the present authors). Especially apex rosette formation is a symptom which often causes confusion among producers of the stocks. It usually occurs due to the irregular water supply (alternating irrigation), or when tip cutting is performed, and in the case of P, B and Zn shortage [8, 17]; observations of the present authors). Even the witches-broom formation could be associated with nutrient deficiency. In case of boron deficiency, after dieback or stunt of shoot tips, branches may proliferate, causing a witches-broom growth. According to our knowledge there is no comprehensive symptoms observation on different cultivars published.

Specific and non-specific symptoms are the consequence of physiological changes influenced by phytoplasmas. It is still unknown how exactly phytoplasmas interact with phloem tissue of hosts on molecular level [4, 13]. There is a close relation between physiological changes in apple phloem tissue caused by phytoplasmas and mobility of micro- and macro nutrients, carbohydrates, amino-acids and other substances important for life of plants [13]. Kartte and Seemüller [11, 12] state that usually the first detectable anatomical aberration is an abnormal deposition of callose on the sieve areas of the sieve tubes, followed by the collapse of these elements and the companion cells. Depending on the susceptibility of the host, a smaller or larger proportion of the sieve tubes necrotize. The carbohydrate content in the roots of infected trees may decrease significantly, indicating the starvation of roots [12]. In apple trees expressing AP symptoms, a strong reduction or virtually complete depletion of starch was observed in the roots, whereas in woody stem tissue the starch content was similar to that of healthy trees [12].

It seems that the symptoms expressed in apple trees due to the lack of nutrients (Mg, P, K, Zn, B) can closely resemble the symptoms developed due to the infection with AP phytoplasma. Lepka et al. [13] have described a striking visual similarity between the symptoms in phytoplasma-infected and the genetically modified tobacco plants with inhibition of nutrient transport. Transport of organic substances and minerals in phytoplasma-infected plants is severely hindered by damaged vascular tissues. In both cases, i.e. in case of real nutrient lack and in case of phytoplasma infection, nutrients are not available in adequate quantity to the sink tissues, therefore disease symptoms start to develop.

We are aware that our experimental design influenced our results significantly. Scions and buds for propagation were taken from mother trees expressing more or less obvious symptoms (i.e. witches-broom formation, enlarged stipules and increased number of stipules). We had to assure the presence of phytoplasma in produced stocks in order to carry out the trial.

Table 1: Percentage of infected trees as confirmed by laboratory tests, which showed visible AP symptoms, according to symptom type, age and apple cultivar. Data printed in bold represent the most obvious differences between cultivars.

Preglednica 1: Delež laboratorijsko potrjenih okuženih dreves, ki so kazala vizualna znamenja metličavosti jablan v odvisnosti od vrste znamenja, starosti in sorte. Podatki v poudarjenem tisku prikazujejo najbolj očitne razlike med sortami.

Type of symptom:	Age	FU	BR	GA	EL	JO	GD	ID
Spring chlorosis and discoloration of leaves (NS)	1	32.4	10.8	31.9	5.6	18.3	18.4	10.8
	2	33.3	7.3	21.4	9.3	19.6	21.3	19.4
	3	34.5	8.8	18.8	9.3	20.0	23.1	21.9
Early summer and fall reddening of leaves (NS)	1	35.1	54.1	44.7	46.3	31.7	40.8	24.3
	2	33.3	61.0	54.8	37.0	35.7	44.7	61.1
	3	37.9	64.7	53.1	37.2	37.8	43.6	59.4
Leaf curling, rolling and malformations (NS)	1	21.6	27.0	25.5	27.8	33.3	51.0	37.8
	2	24.2	24.4	23.8	9.3	35.7	36.2	38.9
	3	27.6	23.5	25.0	9.3	35.6	38.5	40.6
Changes on the leaf margins (jaggedness) (NS)	1	16.2	16.2	21.3	31.5	33.3	36.7	21.6
	2	9.1	12.2	19.0	9.3	30.4	27.7	33.3
	3	10.3	11.8	18.8	9.3	28.9	28.2	37.5
Changes of leaf vines (branching, colour, ...) (NS)	1	10.8	13.5	31.9	5.6	55.0	22.4	0.0
	2	3.0	14.6	23.8	0.0	51.8	19.1	0.0
	3	3.4	14.7	21.9	0.0	51.1	20.5	0.0
Apex rosette formation on shoots (NS)	1	21.6	32.4	21.3	31.5	30.0	26.5	48.6
	2	21.2	36.6	38.1	37.0	32.1	17.0	83.3
	3	24.1	38.2	37.5	32.6	28.9	20.5	84.4
Stunting and dwarfing of single shoots (NS)	1	24.3	16.2	12.8	14.8	13.3	18.4	62.2
	2	24.3	12.2	26.2	35.2	14.3	25.5	80.6
	3	27.6	11.8	25.0	34.9	13.3	28.2	81.3
Stunting and dwarfing of whole plants (NS)	1	0.0	0.0	0.0	0.0	3.3	0.0	35.1
	2	0.0	0.0	7.1	7.4	3.6	0.0	55.6
	3	0.0	0.0	6.3	7.0	4.4	0.0	62.5
Formation of witch's brooms (S)	1	13.5	13.5	53.2	59.3	68.3	61.2	24.3
	2	42.4	61.0	81.0	83.3	71.5	85.1	52.8
	3	51.7	64.7	78.1	81.4	73.3	87.2	53.1
Enlarged stipules (S)	1	0.0	16.2	36.2	61.1	40.0	40.8	8.1
	2	12.1	19.5	69.0	77.8	57.1	68.1	16.7
	3	13.8	20.6	68.8	74.4	53.3	74.4	15.6
Enlarged number of leaf stipules (S)	1	2.7	0.0	8.5	53.7	0.0	0.0	0.0
	2	3.0	0.0	11.9	74.1	3.6	2.1	0.0
	3	3.4	0.0	12.5	72.1	2.2	2.6	0.0
Infected trees expressing at least one of the specific symptoms (S)	1	18.9	24.3	63.8	70.4	75.0	75.5	29.7
	2	39.4	46.3	76.2	87.0	78.6	85.1	41.7
	3	44.8	47.1	75.0	81.4	75.6	89.7	46.9
Infected trees expressing at least one of the non-specific symptoms (NS)	1	54.1	62.2	61.7	61.1	46.7	65.3	43.2
	2	57.6	63.4	78.6	85.2	75.0	83.0	83.3
	3	62.1	64.7	78.1	83.7	73.3	84.6	87.5
Healthy trees expressing at least one of the specific symptoms (S)	1	0.0	0.0	0.0	0.4	0.3	0.0	0.0
	2	0.0	0.0	0.05	0.3	0.2	0.0	0.0
	3	0.0	0.0	0.0	0.3	0.2	0.0	0.0
Healthy trees expressing at least one of the non-specific symptoms (NS)	1	8.1	5.4	6.4	7.4	5.0	6.1	5.4
	2	6.1	4.9	4.8	3.7	5.4	4.3	5.6
	3	6.9	5.9	6.3	4.7	6.7	5.1	6.3

(FU – Fuji, BR – Braeburn, GA – Gala, EL – Elstar, JO – Jonagold, GD – Golden Delicious, ID – Idared, S – AP specific symptoms, NS – non-specific symptoms; 1, 2, 3 – 1, 2, 3 years old trees)

5 CONCLUSIONS

Besides intensive vector control an early detection of infected trees and their removal is important in newly established plantations in apple growing areas. The results of our study show that the less obvious (non-specific) AP disease symptoms should be regarded as an indication that AP infected trees are present in plantation. We have shown that non-infected trees grown in favourable conditions rarely express the described non-specific symptoms. The obtained results indicate that, on average, 60%-65% of AP infected two- to three-year old trees can be expected to show one or more visually detectable non-specific or specific symptoms. In order to increase the quality of visual detection of young infected trees it is essential to describe specific cultivar-related and reliable AP symptoms more in detail. Our study shows that important differences among apple cultivars exist and are noticeable by thorough observations. On the basis of our results visual inspection could be improved for AP detection in plantations.

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