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# Study on diverse grafting techniques for their capability in rapid and efficient transmission of apple proliferation disease to different host plants

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## Abstract

As phytoplasmas are not cultivable *in vitro*, they must be studied *in situ* within their host plant. Successful transmission of a phytoplasma from a plant to another is therefore a very important step and adequate techniques should be selected. In this study, we evaluated 4 grafting methods: whip graft, bark graft, budding and chip-budding to transfer phytoplasma strains from apple trees to apple indicator (MM106 variety) or periwinkle and from periwinkle to periwinkle. For apple tree to apple tree transfer, the best results were obtained by whip and bark grafting. Even the transmission of phytoplasma strains from transplants non-grown to the rootstock was sometimes recorded. Transmission of phytoplasma from periwinkle to periwinkle was successfully carried out by chip-budding. The surprising results were obtained by grafting apple material on periwinkle. Phytoplasma infection was transferred to approximately 60% of the samples. In conclusion, grafting apple material on MM106 by whip and bark grafting seems to be the more appropriate method for biological indexing of apple proliferation disease.

**Key words:** '*Candidatus* Phytoplasma mali', periwinkle, apple, biological indexing, grafting, certification.

## Introduction

Phytoplasmas are wall-less plant pathogenic bacteria in the class of *Mollicutes*, a group of organisms phylogenetically related to low G+C content gram positive bacteria. Phytoplasmal plant diseases are spread by sap-sucking insect vectors (Lee *et al.*, 2000). They inhabit the plant phloem and cause diseases in hundreds of plant species worldwide. Apple proliferation (AP), caused by '*Candidatus* Phytoplasma mali', is one of the most important apple diseases affecting both the fruit yield and quality. Phytoplasmas are obligate parasites, and up to now, they were not cultivated in axenic culture; therefore Koch postulates are only sometimes fulfilled by using alternative tools, such as graft or insect transmission (Bertaccini, 2007). On the other hand, their accurate detection is a major prerequisite to control the disease and fulfil certification requirements. So, phytoplasma graft transmission in biological indexing processes remains of outstanding importance in disease detection and in the phytosanitary certification schemes.

Different grafting methods, depending on scion and rootstock characteristics, may be used on woody or herbaceous plants to detect phytoplasma infection. In this study, for the first time, comparisons between 4 grafting techniques on fruit trees and periwinkle plants for their success in phytoplasma inoculation and also in phytoplasma detection by biological indexing are presented.

## Materials and methods

For biological indexing on periwinkle, healthy and AP-infected (5 isolates) periwinkles were prepared by FUSAGx and Institut für Pflanzenschutz (Dossenheim, Germany), respectively. For indexing on apple trees, healthy MM106 apple rootstocks and AP-infected (5 isolates) scion woods were provided by CRA-W (Gembloux, Belgium) and Quarantine Station (Lempdes, France), respectively. MM106 rootstocks are very susceptible to '*Ca.* P. mali' (Jarausch *et al.*, 1996). All inoculation experiments were carried out in July-August in insect-proof greenhouse (14 h light, high relative humidity, 20-25 °C).

Four different grafting methods (whip graft, bark graft, budding and chip-budding) were carried out on MM 106 as recommended (Hertz, 1993; Weinmann, 2002; Anonymous, 2005). In brief, whip (tongue) graft method works best when the stock and scion are of similar diameter, preferably between 8 and 12 mm (Hertz, 1993). Bark grafting may be performed on branches ranging from 12 to 25 mm in diameter (Weinmann, 2002). Budding is a form of grafting in which a single bud with a thin layer of bark is used as the scion rather than a section of stem. Budding is carried out in summer, usually from July 15th to August 15th, when the bark of the stock slips easily and when there are well-grown buds (Hertz, 1993). For chip-budding, in mid-summer, non-flowering shoots of rootstocks are selected with similar diameter to the scions from well-ripened, current season's growth as bud (Anonymous, 2005). In all grafting techniques the union (rootstock-scion) was always bound tightly with tape and, for whip and bark grafting the union and scion were carefully covered with plastic bag for 1-2 weeks.

All grafting techniques were carried out on apple rootstocks by AP-infected apple materials. Due to the fineness and fragility of periwinkle tissues, chip-budding was the only feasible method for grafting on this plant. Previously infected apple trees and periwinkle material were used in this case. Healthy apple and periwinkle materials were used as control for grafting on MM106

or periwinkle indicators.

The symptom emergence was monitored on inoculated plants by time. In periwinkle plants, the symptoms as yellowing and reduced leaf size and/or vigour were surveyed. In apple trees, proliferation, chlorosis, small leaves and enlarged stipules were noticed.

## Results

Typical symptoms of phytoplasma infection were observed during the second month after grafting on more than 90% of periwinkles grafted with AP-infected periwinkle. The surprising transmission of phytoplasma from infected apple material to periwinkle was achieved for approximately 60% of the tested samples, but the latency period before symptom observation was notably longer (4-6 months) than those inoculated by infected periwinkles. To our knowledge, this is the first report of phytoplasma direct transmission from apple to periwinkle without intermediate (insect or dodder). No similar symptoms appeared on periwinkles grafted by healthy materials (apple or periwinkle) after 6 months.

In the case of apple trees as rootstock, all grafting methods were performed, and only AP-infected apple material was used as scion for grafting. First symptoms of phytoplasma infection appeared sooner with whip and bark grafting than with budding and chip-budding. Symptoms were observed on all the plants inoculated (efficiency 100%) by whip and bark grafting. In our tests, 7 transplants did not grow after grafting because they were not covered by plastic bag. Nevertheless, the phytoplasma transmission from them to the rootstock was sometimes recorded (28.6%). The efficiency of budding and chip-budding was recorded as 38 and 54%, respectively, and symptoms were observed much later (next year for more than 50% of the trees) with these grafting methods. In the case of apple trees, whatever the grafting method used for inoculation, no symptoms were recorded on trees inoculated with healthy materials.

## Discussion

Biological indexing is laborious, time-consuming, and skill-demanding (Di Terlizzi, 1998). Even if the molecular and serological diagnostic protocols can replace biological indexing as a fast screening technique in certification programs, biological indexing will often remain mandatory as a second screening technique to fulfil the Koch's postulate. In this context, the present work allowed the identification of the best suited methodology for AP indexing. The best results were obtained with whip and bark grafting on MM106 rootstocks, and we perceive that apple trees are more suitable than periwinkle for biological indexing of apple material.

Finally, within a certification program, symptom observation after biological indexing could be confirmed by phytoplasma detection by means of molecular methods to achieve Koch's postulate.

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