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REVIEW PAPER

The use of biofumigation in orchards with apple replant disease – a review

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Abstract

Apple replant disease (ARD) is the consequence of replantation of the same or related species of crops. As a result of the occurrence of ARD, the physicochemical and biological properties of the soil deteriorate. It causes considerable economic losses and may significantly reduce fruit production, especially in regions with high density of orchards. It is difficult to indicate an effective method of preventing the consequences of ARD due to the diversity of causative factors. The development of ARD can be effectively limited by chemical fumigation. However, there are numerous limitations to this method because it is a nuisance to the environment. Not only soil pathogens but also the beneficial microflora may be destroyed, especially by broad-spectrum fumigants. Biofumigation, which involves the use of plants with phytosanitary properties, is an environment-friendly alternative. These plants produce potentially bioactive compounds which, apart from their fungicidal effect, also have nematicidal, insecticidal, antiviral, and cytotoxic properties. This article is a review of the results of research on the effects of biofumigation in orchards with ARD. It shows how the plants used for biofumigation, mainly plants of the Brassicaceae, Asteraceae family, reduced the occurrence of biological causes of ARD (fungi and especially nematodes), and promoted the development of beneficial microorganisms in replanted soil. As a result of biofumigation, both physical and biological properties of the soil are improved, and that leads to improvements of vegetative growth of fruit trees and apple trees in a fruit tree nursery.

Keywords: Apple Replant Disease, biofumigation, *Brassicaceae*, *Tagetes*, soil microbial balance

APPLE REPLANT DISEASE (ARD) SYNDROME

Apple Replant Disease (ARD) is a disturbed physiological and morphological response of plants to the soil whose microbiome has been changed by previous crops of the same or related species (Winkelmann et al. 2019). In consequence of such disturbances, the physicochemical and biological properties of the soil deteriorate. For a long time, ARD has been the subject of numerous scientific studies, using various nomenclature, often interchangeably, e.g. soil fatigue (Wolińska et al. 2018), soil sickness (Cesarano et al. 2017) or replant disease (Nicola et al. 2018). The most common name is Apple Replant Disease (ARD).

ARD, which is the result of planting orchards in place of grubbed ones, has been mentioned for over 200 years. Symptoms of ARD have been found in plantations of ornamental plants (roses), vegetables (asparagus) – Elmer (2018), and medicinal plants (Wu et al. 2015). However, it is the biggest problem in orchards, especially with apple trees. ARD occurs in all major areas with apple orchards around the world (Mazolla, Manici 2012), especially in intensive orchards, where replanting is frequent due to the aging of trees, market requirements concerning species and cultivars, as well as changes in fruit consumers' preferences.

This article is a review of research on the use of biological methods to improve the physicochemical and biological properties of replanted soil in orchards, with a special focus on biofumigation.

CAUSATIVE FACTORS OF ARD

The causes of ARD have been investigated since the 1980s. Despite numerous publications on this subject, researchers have not assumed a clear position on the main causes of ARD. It is assumed that the disease can be caused by both abiotic and biotic factors. The former include insufficient soil moisture, low content of nutrients, low soil pH, and disturbances in soil structure. According to Spath et al. (2015), the influence of abiotic factors on the occurrence of ARD is relatively small, whereas Sobiczewski et al. (2018) treat abiotic factors as the main causes of ARD.

Most authors of studies on methods for restoring fertility to replanted soil discuss the elimination of biological factors, usually specific species of nematodes, fungi, and bacteria. According to Winkelmann et al. (2019), ARD disturbs the soil microbial balance, limits the development of beneficial microorganisms (Long et al. 2019) and increases the activity of harmful microflora. According to Manici et al. (2013), the change in the species structure of soil microorganisms is the main cause of ARD.

There are about 4,100 species of nematodes parasitising plants around the world. They migrate through the soil in searching of a host plant, invade the roots, and feed on the cytoplasm. Roots damaged in this way are more vulnerable to other soil pathogens. The phytopathogenic nematodes *Pratylenchus* spp. are often mentioned as the main biotic factor of ARD (Mazzola, Manici 2012, Singh et al. 2015, Kanfra et al. 2018). Another group of soil microorganisms mentioned as the biological causes of ARD are fungi of the following genera: *Alternaria* spp., *Rhizoctonia* spp., *Phythium* spp., *Cylindrocarpon* spp., and *Fusarium* spp. (van Schoor et al. 2009, Manici et al. 2013). In the experiment conducted by Cavael et al. (2020), the share of fungi of the *Alternaria* genus in replanted soil amounted to 2% of the total population of soil fungi, which was 10 times more than in agricultural soil. Fungal pathogens attack the root system. According to Yin et al. (2014), *Fusarium proliferatum* is the dominant species in infected roots. Zhao et al. (2022) also found *F. proliferatum* to be an important causative factor of ARD. The role of bacteria in causing ARD has been investigated to a lesser extent than the role of fungi. Researchers usually listed bacteria of the *Bacillus* and *Pseudomonas* genera as the causes of ARD (Mazzola, Manici 2012). However, according to Franke-Whittle et al. (2015), *Pseudomonas* bacteria do not significantly reduce the fertility of replanted soil.

Other causative factors of ARD are phenolic compounds contained in replanted soil. Apple roots contain large amounts of specific polyphenols, such as phlorizin, phloretin, benzoic acid and rutin (Hofmann et al. 2009, Emmet et al. 2014, Yin et al. 2016, Leisso et al. 2017). Phenolic compounds accumulated in the soil in old orchards may strongly inhibit the growth of apple trees.

THE CONSEQUENCES OF ARD IN ORCHARDS

Apple replant disease poses a major challenge for fruit producers. It causes noticeable economic losses and seriously limits the development of fruit production. The profitability of an apple orchard on replanted soils is 50% lower because the yield of fruit is lower and it is harvested later (van Schoor et al. 2009). Fruiting may be delayed by 2-3 years (Mazzola, Manici 2012). The productivity of apple trees, measured with the pppp index, is lower on replanted soils (Cavael 2020). The cross-sectional area of the trunk is commonly used as an indicator of the yield of apple trees in fields.

As mentioned above, ARD deteriorates the physicochemical and biological properties of soil. The enzyme and respiratory activity on replanted soil is reduced (Zydlik et al. 2019, 2020), which indicates lower activity of soil microorganisms responsible for the mineralisation of organic matter. Soil enzymes and soil respiratory activity are reliable indicators of the acti-

vity of soil microflora (Błońska et al. 2017, Meena, Rao 2021). The reduced rate of mineralisation of organic matter in replanted soil results in a smaller amount of nutrients available to plants. This fact was proved in the experiments conducted by Zydlik et al. (2020, 2021a). A smaller amount of available nutrients in replanted soil causes weaker vegetative growth of fruit trees. This effect can be observed in apple rootstocks (Weiß et al. 2017, Zydlik et al. 2023) and apple trees (Manici 2013, Liu et al. 2014, Zhao et al. 2022) – Photo 1.



trees on replanted soil
(third replantation)



trees on non-replanted soil

Photo 1. An eight-year-old apple trees of the Topaz cultivar (photo by Z. Zydlik)

The weaker growth of trees is manifested by smaller growth and a smaller assimilation area of leaves (Emmett et al. 2014, Yim et al. 2016, Sobiczewski et al. 2018). The weaker vegetative growth of apple trees growing on soil with ARD may be affected not only by a lower amount of available nutrients, but also by limited possibilities of their uptake by the root system. Lukas et al. (2018) concluded that apple rootstocks affected by ARD absorb nitrogen in the form of nitrate much worse than rootstocks growing in optimal conditions.

The yield of apples in an orchard affected by ARD is significantly reduced and their quality is worse (Zydlik et al. 2021b). For example, apples may be about 10% smaller (Nikola et al. 2018). The appearance and taste of apples are also worse (Lucas et al. 2018).

The roots are an important organ responsible for the uptake, storage,

and transport of minerals and water to the aerial parts of plants. ARD inhibits these processes because it damages the roots and weakens their function (Photo 2).



Photo 2. The growth of the root system of apple trees on soil with ARD compared to the other variants (photo by Z. Zydlik)

The roots become discoloured and form necrotic tips. The number of root hairs decreases and their growth is limited. These effects can be observed in both fruit trees and nursery material. As early as two weeks after being in the soil with ARD, necrosis appeared on M26 rootstocks and the growth of root hairs weakened (Grunewaldt-Stöcker et al. 2019). The root system usually becomes damaged by the microbiome causing ARD, e.g. nematodes or products of their metabolism in the immediate vicinity of the roots (Lucas et al. 2018).

METHODS OF ALLEVIATING THE CONSEQUENCES OF ARD

It is difficult to indicate an effective method of preventing or alleviating the consequences of ARD due to the diversity of causative factors and the interrelations between them. According to Berg et al. (2017), the effect of ARD causative factors largely depends on the cultivation history, environmental conditions (type of soil, weather), and the physiological state of plants. Moreover, the quality and quantity of causative factors may change during one growing season. The most effective solution is to avoid replanting the same species of crops in the same place. However, in practice, this is difficult

or impossible due to the limited number of suitable places for new plantations in orchards and nurseries. All actions aimed at the eradication of ARD symptoms should be targeted at the improvement of the physicochemical properties of the replanted soil and restoration of the species composition of its microflora. According to Deldago-Baqueriro et al. (2016), it is the high diversity of soil microorganisms that determines its multifunctionality and provides protection against pathogens. The physicochemical and biological properties of replanted soil can be improved with agricultural, chemical or biological methods.

Agricultural methods

Proper agricultural practice includes crop rotation and organic fertilisation with manure or compost (Forge et al. 2016, Franke-Whittle et al. 2018). Crop rotation is not often used in perennial orchard plantations. Organic additives, such as biochar, can also be added to the replanted soil. When organic carbon was added to the replanted soil in an apple tree nursery, the enzyme activity of the soil more than doubled. The rate of photosynthesis in the leaves also increased significantly, which improved the vegetative growth of the apple trees (Zydlik et al. 2023) – Photo 3.

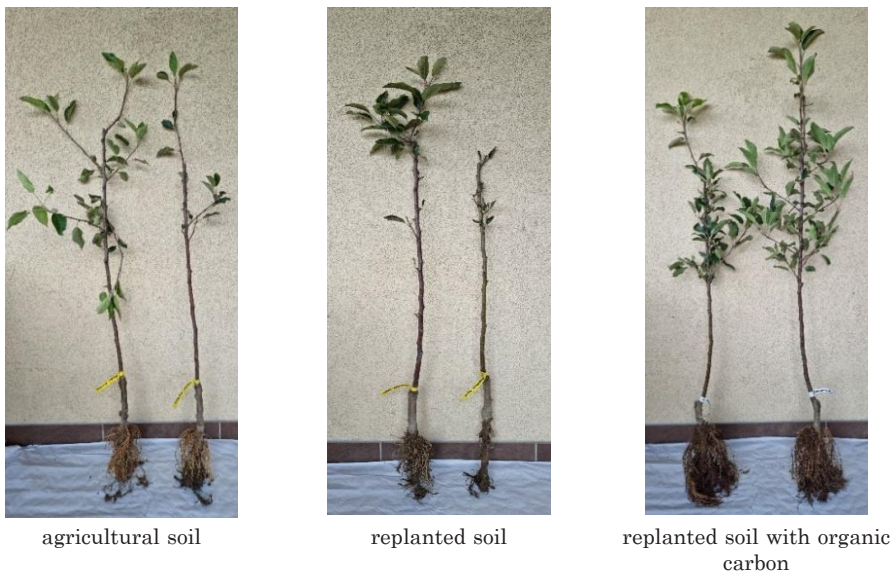


Photo 3. The influence of the soil types on the growth of apple trees (photo by Z. Zydlik)

The productivity of replanted soil can also be improved by adding humic acids, which are components of the soil humus. The spraying with humic acids decreases the salinity of replanted soil, increases the activity of enzymes

(dehydrogenases and proteases) and respiratory activity (Zydlik et al. 2020). Humic acids used in an apple orchard with ARD increase the yield of trees and improve the quality of fruit (Zydlik et al. 2021b).

Agricultural treatments may improve the physicochemical properties of replanted soil, stimulate the development of microflora, as a result of which the soil reaches a state of microbial balance. However, such procedures may be time-consuming or not always sufficient to alleviate the consequences of ARD.

Chosen chemical substances

Chemicals may also limit the development of pathogenic microbiome in replanted soil very effectively, especially before planting crops. They can be applied into replanted soil by fumigation or sterilisation of the soil. Until recently, methyl bromide was the most common chemical fumigant used to combat soil pathogens (Zhang et al. 2019). However, it was withdrawn because it destroyed stratospheric ozone. Current soil fumigation chemicals mainly include dazomet or metam sodium (both releasing methyl isothiocyanate) and 1,3-dichloropropene/chloropicrin (Nicola et al. 2017, Nyoni et al. 2019). Such broad-spectrum fumigants are used in many countries to combat soil-borne diseases (Li et al. 2017, 2021).

The results of numerous studies have confirmed the effectiveness of chemicals in reducing the effects of ARD. According to Spatch et al. (2015) and Yim et al. (2016), this may indicate that ARD is primarily caused by biotic factors, especially by nematodes. Chemical fumigation of replanted soil limits the development of pathogenic soil microorganisms, e.g. *Fusarium* spp. (Jiang et al. 2022), and plants are affected by the consequences of ARD to a lesser extent (Cheng et al. 2020). When chloropicrin was applied into soil, the populations of *Fusarium* spp. (Li et al. 2017b) and *Phytophthora* spp. (Li et al. 2021) were significantly reduced, which significantly increased the strawberry yield.

The disadvantage of using chemical soil fumigants is their high toxicity. Not only soil pathogens but also the beneficial microflora may be destroyed, especially by broad-spectrum fumigants (Li et al. 2017, Fang et al. 2018). Li et al. (2021) observed that chemical fumigation reduced the population of proteobacteria (responsible for the proper binding and accumulation of nitrogen) and *Acidobacteria*, which are considered a reliable indicator of the degree of plant nutrition. As chemical fumigation is a nuisance to the environment, researchers are searching for non-chemical methods for combating phytopathogenic soil microflora.

Biological methods

In horticultural practice, the effects of ARD can be alleviated biologically by applying biopreparations containing various groups of beneficial micro-

organisms into soil, which are antagonistic to pathogens. These may be arbuscular mycorrhizal fungi competing with phytopathogenic bacteria or *Trichoderma* spp. Zydlik et al. (2021a) observed that the *Trichoderma harzianum* species increased the enzyme and respiratory activities of the soil. Noteworthy is the fact that it is difficult to control soil fungi due to their extensive mycelia and numerous spores.

Plants used for biofumigation

The term *biofumigation* introduced in 1993 is defined as a process of decomposition of plant or animal tissues, leading to the production of volatile biocidal compounds. Biofumigation consists in using phytosanitary plants, which are antagonistic to pathogenic microorganisms in soils with ARD and support the development of beneficial soil microflora. For example, these are plants of the *Brassicaceae* family, with about 350 genera and about 4,000 species. Species of the *Brassica*, *Raphanus*, and *Sinapis* genera are the most common plants of the *Brassicaceae* family used for biofumigation (Hanschen, Winkelmann 2020, Morris et al. 2020). In general, these are usually plants eaten by humans and animals and those used for the production of edible and industrial oils. The most common phytosanitary plants used for biofumigation are: red mustard (*Brassica juncea*), white mustard (*Sinapis alba*), field mustard (*Brassica rapa*), rape (*Brassica napus*), arugula (*Eruca sativa*), and radish (*Raphanus sativus*) – Neubauer et al. (2014), Rios et al. (2016), Ntali and Caboni (2017).

Plants of the *Brassicaceae* family produce secondary metabolites, glucosinolates, which after hydrolysis produce bioactive isothiocyanates: aliphatic allyl isothiocyanate, aromatic isothiocyanates, 2-phenylethyl isothiocyanate, and benzyl isothiocyanate (Gimsing and Kirkegaard 2009). These plants produce over 200 types of glucosinolates, which are present in all parts of the plant. The amounts of these compounds vary considerably depending on the cultivation phase, species, cultivars, and growing season. The highest concentration of glucosinolates was found in the tissues of plants of the *Brassicaceae* family in the summer (Ngala et al. 2015). According to Doheny-Adams et al. (2018), the highest concentration of glucosinolates in the tissues of mustard can be observed at the intensive flowering phase, but later their amount gradually decreases as the plant develops. Other compounds formed during the decomposition of *Brassicaceae* biomass can also be used for biofumigation. These are volatile compounds containing sulphur, e.g. carbon disulphide, dimethyl sulphide, and dimethyl disulphide (Wang et al. 2009).

Plants from the *Asteraceae* family (*Asteraceae* Dum) contain various fungicidal components which kill fungi with short reproductive cycles, producing large numbers of spores and easily developing resistance (Perera et al. 2019). These plants produce potentially bioactive compounds which, apart from their fungicidal effect, also have nematicidal, insecticidal, antiviral, and

cytotoxic properties (Karakas et al. 2019). The *Asteraceae* kill nematodes with metabolites released from the roots of mature plants, e.g. thiophene compounds such as α -terthienyl (Hamaguchi et al. 2019). The greatest amount of such compounds is produced by the roots of intensively growing marigolds (*Tagetes* L). The amount of thiophene compounds depends on the species. The highest content was found in *T. tenuifolia*, followed by *T. patula* and *T. erecta* (Marotti et al. 2010).

Allium L. is another genus of bioactive plants which can limit the development of soil diseases and improve plant growth (Arnault et al. 2013) thanks to the substances secreted by the roots, including propyl disulphide and methyl disulphide (Ngala et al. 2015).

The radish (*Raphanus sativus*), also classified as a cruciferous plant, is less frequently used for biofumigation because it has high requirements concerning soil fertility. However, *Raphanus sativus* may combat soil nematodes more effectively than white mustard (*Sinapis alba*).

The methods of using plants of the *Brassicaceae* family may vary depending on the species, the organism being controlled and the soil cultivation method. The most common methods are growing as preceding crop, ploughing green manure, adding fresh or dried plant residues (e.g. meal) to the soil. They are recommended in fruit production before fruit trees or berry bushes are planted. Fresh biomass is particularly recommended because it has high content of glucosinolates. The plant material should be thoroughly crushed and then applied into the soil at a depth of 15-20 cm (Kumar et al. 2018).

As results from reference publications, there have been various studies on using plants from the *Brassicaceae* family to reduce the occurrence of pathogens causing ARD. Experiments have been conducted on tomatoes, cucumbers, peppers, lettuce, onions, napa cabbage, potatoes, sugar beets, wheat, maize, gourds, grasses, pine, and ginger. As regards fruit cultivation, such experiments are usually conducted in apple orchards, fruit tree nurseries, and less often on strawberry plantations.

Effects of biofumigation

Mustard is often used in experiments with fruit plants to limit the occurrence of phytopathogens (fungi and nematodes) in soil. The nematicidal effect is one of the best-investigated effects of biofumigation (Dutta et al. 2019). Hollister et al. (2012) observed that mustard meal effectively inhibited the development of such fungal pathogens as *Bacillus*, *Pseudomonas*, and *Streptomyces*. Mustard seed powder limited the infection of the roots of apple trees growing in replanted soil by *Pythium* spp. (Weerakoon et al. 2012). Barrau et al. (2009) used Ethiopian mustard (*Brassica carinata*) for biofumigation and observed that it reduced the occurrence of the *Phytophthora cactorum* pathogen in a strawberry plantation, which resulted in a higher yield of fruits. Mazzola et al. (2009) and Weerakoon et al. (2012) found that *Brassicaceae* seed meal reduced damage to apple rootstocks by *Pythium*.

It also reduced the population of the phytopathogenic nematode *Pratylenchus penetrans* in the soil. The researchers observed that *B. juncea* was more effective than *B. napus* or *Sinapis alba*.

The marigold (*Tagetes* L.) is an annual herbaceous plant of the *Asteraceae* genus, which has antifungal, bacteriostatic, and insecticidal properties (Padalia, Chanda 2015). Thus far, about 40 species of marigolds have been identified, but nematodes in soil are most effectively destroyed by the French marigold (*Tagetes patula* L.). *Tagetes* plants are the most effective when they are applied before planting fruit trees. A marigold preceding crop significantly increased the vegetative growth of apple trees in an orchard on soil with ARD (Yim et al. 2016, 2017). Du et al. (2017) conducted an experiment with *T. erecta* and observed its fungicidal effect on *F. oxysporum*, which is one of the main causes of ARD. Wang et al. (2022) applied *Tagetes erecta* to replanted soil and observed a decrease in the population of *Fusarium oxysporum* – the fungus which is one of main causative factors of ARD. The number of parasitic nematodes damaging plant roots also decreased significantly. However, Kanfra et al. (2021) observed that *T. patula* was more effective than *T. tenuifolia*.

Phytosanitary plants used for biofumigation not only reduce the populations of pathogenic microorganisms responsible for the development of ARD, but they also introduce significant amounts of organic material into soil through the production of large amounts of biomass. This improves the soil structure, increases the amount of nutrients and stimulates the development of beneficial microflora. These may be bacteria from the *Pseudomonas* genus, which are antagonistic to pathogenic fungi in soil (Behera et al. 2014), *Actinobacteria*, which stimulate plant growth, or *Bacillus* (Sobiczewski et al. 2018), which are considered the main biological factor protecting plants from soil-borne diseases. When mustard seed meal was applied to replanted soil and when white mustard and winter wheat were grown as preceding crops before the orchard was established, the content of *Trichoderma* fungi in the soil increased significantly (Sobiczewski et al. 2018). These fungi play a significant role in the decomposition of organic matter and in the reduction of many soil pathogens.

Biofumigation reduces the population of pathogenic microorganisms in replanted soil and thus improves the vegetative growth of fruit trees. This fact was observed by various scientists, including Yim et al. (2017) on marigold and Krzewińska et al. (2008) on mustard. Kanfra et al. (2021) used *Tagetes* for the biofumigation of replanted soil in a fruit tree nursery. As a result, the diameters of the trunks of apple trees grafted on M26 rootstocks were several dozen per cent larger than those in the control variant. The biofumigation of replanted soil with the dry powder of *Tagetes erecta* increased the intensity of photosynthesis in the leaves of apple trees and the respiration rate of their roots, which improved the vegetative growth of the trees (Wang et al. 2022). The activity of root antioxidative enzymes,

which protect plant cells from free radicals, also increased. In consequence, the plants' resistance to pathogens increased.

Good results can also be achieved by combining different methods of using plants for biofumigation. When mustard seed meal was applied to the soil and white mustard and winter wheat were grown as preceding crops before establishing an orchard, the height of apple trees, the area of their leaves and the intensity of photosynthesis increased (Sobiczewski et al. 2018).

Mixtures of plants used for biofumigation also very effectively limit the development of pathogenic microbiome. The mixed cultivation of *Allium fistulosum* and *Brassica juncea* limited the development of *Fusarium proliferatum* for a long time due to the continuous release of bioactive compounds and improved the growth of apple trees growing on soil with ARD (Zhao et al. 2022). The combined use of *B. juncea* and *S. alba* effectively reduced the population of *Pratylenchus penetrans* in the replanted soil, which improved the growth and yield of apple trees (Mazzola et al. 2015, Wang et al. 2019). Yim et al. (2016) also observed better growth of apple trees after using *B. juncea* with *R. sativus* on replanted soil.

SUMMARY

The biofumigation of replanted soil is a promising method of mitigating the consequences of ARD in fruit plantations. This fact has been proved by the results of numerous studies, which showed that this method effectively limited the development and reduced the populations of biological causative factors of replantation disease – mainly fungi and nematodes. Biofumigation enables the restoration of microbiological balance in replanted soil, which improves the health, growth, and yield of fruit trees and berry plants growing on soil with ARD.

Further research is necessary due to the variety of causative factors of ARD, the large number of plant species with phytosanitary properties, and the influence of external factors on the effectiveness of biofumigation. New plant species with the potential to combat pathogenic microorganisms in orchards should be sought. It is also necessary to pay greater attention to the timing of biofumigation, selection of the right amounts of phytosanitary plants and optimal methods of their application in soil, and find the most effective mixtures of these plants. New experiments should be conducted not only on apple trees but also on other species of fruit plants that are sensitive to ARD.

Author contributions

R.W. – writing – original draft preparation, funding acquisition, data curation; P.Z. – writing – original draft preparation, supervision, writing – review and editing.

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Conflict of interest

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