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Ten years of biofumigation research in Switzerland

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Summary

Research on biofumigation, which started in Switzerland in 2003, revealed some critical points of this method, hindering its application by growers: Seed availability and winter hardiness of biofumigation mustard cultivars, mustard as host plant of diseases in cruciferous-based vegetable production systems, and space and time needed for the mustard cultivation. The first two points were solved by the commercialisation of adapted cultivars in Switzerland. Greenhouse and pot trials with defatted seed meal products and hay of mustards were conducted by Agroscope to address the two latter points. Solid and liquid defatted seed meal products increased significantly the yield of greenhouse tomato in case of a relatively low, but not in case of a high disease pressure. Mustard hay reduced significantly the number of *Verticillium dahliae* microsclerotia in two greenhouse trials. This effect was most probably not caused by a biofumigation effect and occurred only several months after the hay application.

Key words: Biofumigation, defatted seed meal, mustard hay, silage, soilborne diseases, *Verticillium dahliae*

Introduction

Soilborne diseases are an increasing production constraint for Switzerland's agriculture. The reason is the intensification of the agricultural production systems, caused by decreasing farming surfaces coupled with an increasing population and a demand for higher food quality. Traditional means for controlling soilborne diseases (including nematodes) were mainly the use of well-balanced crop rotations with non-host crops and planting resistant cultivars. Less important was the application of chemical soil disinfestants such as methyl bromide. Actually, only a restricted use of dazomet-containing products is authorised in Switzerland. The application of such products is, similarly to the high energy consuming steam sterilisation method, limited to small areas of very high value crops. Both methods have only a short term effect. They are in low estimation by consumers and retailers representatives, who prefer more ecological production modes.

The biofumigation method based on the cultivation of biocidal mustard plants (Kirkegaard, 2009) offers a new means of control which fits well into the scheme of ecological sound production systems. The traditionally mixed production area pattern of Switzerland, where highly specialised horticultural farms are adjacent to dairy and field crops growing farms, should facilitate its application. Furthermore, mustard species (*Brassica* spp.) are not new crops as they are widely grown as nitrate catch crops during the autumn season.

Biofumigation research in Switzerland started in 2003 with a first field trial at Agroscope. Since then, a number of pot, field and greenhouse trials were conducted, most of them on-station, but also

a few on-farm. Starting 2008, the method was presented to the Swiss producers as fact sheet on the internet and in technical publications. At vegetable and berry grower meetings, talks to introduce this new method were presented.

During on-station and on-farm trial but also based on exchanges with extension officers or growers at meetings or on-farm trials, the following critical points of the biofumigation method were detected:
- Access to the seeds of mustard cultivars specifically selected for biofumigation,
- winter hardiness of the biofumigation cultivars,
- mustard species as host plants for diseases and pests in vegetable production systems rich in cruciferous crops,
- time and space required by the mustard crop in competition with a saleable crop, especially in greenhouse production.

The goal of this publication is to present solutions to the four problems mentioned above. In the two first cases, no research was needed and the solutions will directly be presented in the discussion chapter. For the two latter cases, pot and greenhouse trials were conducted as described in the following chapter.

Materials and Methods

For solving the problems related to the cultivation of mustard on the same site as the main crop i.e. host plant of common diseases and pests, and occupation of time and space, research in two directions was started. One direction is the importation of mustard plants in form of hay, silage or derivate products (defatted seed meal in solid or liquid form) to the production site. The other direction is the replacement of mustard by other plants species. The second approach responds only to the host plant aspect, but growing green manure crops prior to the main crops has other advantages such as an improvement of soil structure or retaining the leaching of nutrients (mainly nitrogen).

Defatted seed meal applications

In 2010, the use of defatted seed meal to control several soilborne pathogens of tomato was tested in a greenhouse trial. It took place in the greenhouse H3 at Agroscope Conthey as its soil is naturally infested with the causal agents of corky root (*Pyrenochaeta lycopersici*), black dot disease (*Colletotrichum coccodes*) and Verticillium wilt (*Verticillium dahliae*). On 9 March, the solid defatted seed meal product Biofence pellets was expanded on soil surface at a rate of 250 g m^{-2}. The second amendment tested at the same rate was the chitin-containing product Agrobiosol. The organic nitrogen content of both products was around 5%, therefore the control treatment was amended with urea at a rate of 25 g m^{-2} to avoid a difference of mineral nitrogen level at the tomato planting date. The amendments were incorporated at 20 cm depth using a spading machine and then the soil was irrigated with 20 mm water. Five weeks later, double lines of tomato seedlings (var. Admiro) were planted; one row with plants grafted on root stock var. Maxifort and the second row with non-grafted plants. One experimental plot measured 5.5 m × 1.6 m, distance between the tomato rows was 0.6 m and between tomato plants in the row 0.5 m. Per treatment (Biofence, Agrobiosol, control) eight experimental plots in a stripe design with four blocks were planted. Fertilizer was applied through a drip irrigation system which was also used for the distribution of the liquid defatted seed meal product Biofence Flowable (Biofence FL). For technical reasons, this product was applied on one half of the greenhouse, which corresponded to two blocks. Before its application, Biofence FL liquid has to be prepared by adding the seed meal to an oil-water emulsion which has to be stirred for 30 minutes. This procedure provokes the transformation of the glucosinolates contained in the seed meal into isothiocyanates which are retained in the water-oil emulsion. Biofence FL was applied three times on May 26, June 23 and July 19 with a rate corresponding to the extract of 1 g seed meal m^{-2}. After the last harvest on September 8, the tomato roots were rated for root rot symptoms. Therefore, the roots in the top soil layer (approximately 30 cm depth) of every second tomato plant were removed and visually rated.

Mustard hay application

In 2012, the use of defatted seed meal in solid and liquid form and the chitin-containing amendment were tested again in greenhouse H3. As an additional treatment, the use of mustard hay was included in the trial. The mustard hay was produced by growing brown mustard cvs ISCI-99 and Etamine on a field of Agroscope Conthey in early summer 2011. At the full flowering stage, plants were cut and dried with a drying installation of Agroscope (normally used for the research on medicinal plants) to avoid the loss of the fragile leaves of the mustard plants. Dried plants were conserved in jute bags in a dry storage room until their utilisation. The experimental design was a RCBD with for four blocks. The number and dimensions of the experimental plots were the same as in the 2010 experiment. In contrast to the former experiment, Agrobiosol was only tested without the addition of Biofence FL which allowed the test of the mustard hay (equally without Biofence FL). The second difference was the complete randomised distribution of the plots receiving the Biofence FL. On 21 March, soil samples were taken at a depth of 20 cm for the determination of the number of *Verticillium dahliae* microsclerotia in the soil. Two days later, the soil amendments were applied at the same rate as in 2010, the amount of mustard hay was 0.8 kg m^{-2}. The amendments were incorporated at 20 cm depth with a spading machine and 35 mm of water were applied afterwards. On April 16, soil was sampled the second time for the determination of *V. dahliae* in the soil. The planting of the non-grafted tomato (var. Admiro) occurred on April 26, the plants grafted on root stock var. Maxifort were planted the next day. Planting pattern were the same as in the 2010 experiment. Biofence FL was applied at the usual rate six times on 11 May, 6 and 27 June and ,19 July, 9 August and 6 September. After the last harvest on September 28, root rot of every second plant was visually rated. The third soil sampling for the determination of *V. dahliae* in the soil occurred on 3 October.

For the determination of the number of *V. dahliae* in the soil, soil samples were air dried for six weeks, then sieved at 0.5 mm mesh-size and homogenized. For the determination of the number of living *V. dahliae* microsclerotia per g dry soil, five 100 mg-aliquotes were dry-plated (Butterfield & DeVay, 1977) on Sorensen's NP-10 selective medium (Kabir *et al*., 2004). The NP-10 plates were incubated in darkness at 24°C for two weeks, after which soil was removed from the medium surface by adding tap water and scraping gently with a glass slide. The number of *V. dahliae* microsclerotia forming colonies was counted under a dissecting microscope.

Sorghum – Sudangrass hybrids application

In 2013, the effect of another green manure plant than brown mustard on the survival of *V. dahliae* microsclerotia in the soil was investigated in greenhouse H3. The same experimental layout was applied i.e. a RCBD with six treatments and four blocks. A first soil sampling at a depth of 20 cm was taken on 13 May 13 for the determination of the initial *V. dahliae* population in the soil. On June, brown mustard hay (var. Etamine) produced in 2012 (the same way as in 2011), was spread on the soil at a rate of 0.7 kg m^{-2}. A chemical control treatment was included in this trial, therefore, 60 g/m^2 of the product Basamid-Granulat (active ingredient: 98% dazomet) were added to the soil surface. Both amendments were immediately incorporated at 20 cm depth with a spading machine. The following day, brown mustard var. Etamine, sorghum – Sudangrass hybrid (*Sorghum bicolor* × *Sorghum sudanense*) var. BMR 201 and Susu were sown at a seeding rate of 1, 2 and 2 g m^{-2}, respectively. After sowing, all the plots were irrigated with 30 mm water. Throughout the trial, plants were irrigated based on tensiometer readings at 15 cm depth to keep soil moisture above -70 cbar. On August 7, green manure plants were shredded with a flail mowing machine. Brown mustard was in the late flowering stage, sorghum – Sudangrass hybrids were in the late booting stage. Immediately after shredding, the plants were incorporated at 20 cm depth with a spading machine. On 14 August, soil samples at a depth of 20 cm were taken to determine the final *V. dahliae* population.

Silage application

During winter 2012, the replacement of fresh plant material by silage was tested in a preliminary pot trial. As mustard is usually not conserved as silage, grass-clover silage and maize silage were tested in this trial which also comprised a fresh rye, brown mustard hay, dazomet and control treatment. On November 23, fresh soil from greenhouse H3 was mixed with grass-clover silage at rates of 60 (full dose), 30 (half dose) and 15 (quarter dose) g L^{-1} soil; with maize silage at rates of 60 (full dose) and 30 (half dose) g/l soil; with fresh rye at a rate of 60 g L^{-1} soil, with mustard hay at a rate of 7.5 g L^{-1} soil, and with Basamid-Granulat (active ingredient: 98% dazomet) at a rate of 0.6 g L^{-1} soil. Grass-clover silage and maize silage were provided by U. Wyss from Agroscope Posieux, fresh rye plants (tillering stage) were collected from a greenhouse trial, and brown mustard hay was produced in 2012 (same used for the greenhouse trial 2013). All plant material but the grass-clover silage was shredded with a kitchen blender before being thoroughly mixed with the soil in a concrete mixer. Per treatment, two litres of soil were prepared before being added to six 300 ml plastic pots. Water was added to reach field capacity (-30 cbar) and pots were then placed in an incubation room with an average temperature of 15.5°C and no light. Ten days later, soil was removed from the pot for the determination of the number of *V. dahliae* microsclerotia and the soil microbial activity. For latter analysis, a part of the soil was stored at 4°C until measurement of the soil microbial activity with the FDA-method (Schnürer & Rosswall, 1982).

Results

Defatted seed meal application

In 2010, no treatment except grafting, had an effect on root rot (Fig. 1). The average root rot of the non-grafted Admiro tomato plants at the end of the trial was between 10 and 25%, which indicated a relatively low disease pressure by corky root and black dot disease. Grafting on resistant rootstock significantly increased tomato yield by 27% (P<0.001), Biofence FL by 14% (P<0.001) and Biofence pellets by 10% (P=0.027) (Fig. 2).

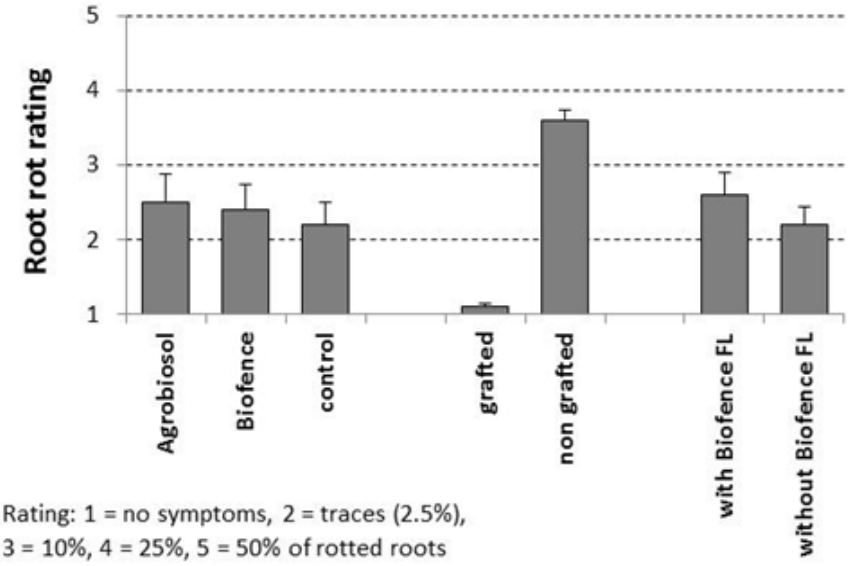

Fig. 1. Rating of the root rot of tomato roots after the final harvest of 2010 greenhouse trial. Roots of non-grafted plants showed typical symptoms of corky root disease (*Pyrenochaeta lycopersici*), roots of grafted plants showed only symptoms of black dot disease (*Colletotrichum coccodes*). Error bars indicate SEM.

Mustard hay application

In 2012, no treatment except grafting had an effect on root rot (Fig. 3). In 2011, another tomato crop and a cucumber crop were grown in the greenhouse H3, which consequently increased the

populations of the already present soil pathogens. Therefore, the average root rot of the non-grafted Admiro tomato plants at the end of the trial had tremendously increased and was rated between 97.5 and 100%. The roots of the rootstock Maxifort still showed only slight, whereas higher symptoms than in 2010, which were again all caused by *C. coccodes*. Grafting was the only treatment with a significant effect on yield (Fig. 4), which was increased by 110% ($P<0.001$). The number of *V. dahliae* microsclerotia was not influenced by any treatment shortly after incorporation (Fig. 5), but mustard hay had a long term effect on the number of *V. dahliae* microsclerotia in soil, which was significantly reduced by 80% ($P=0.01$).

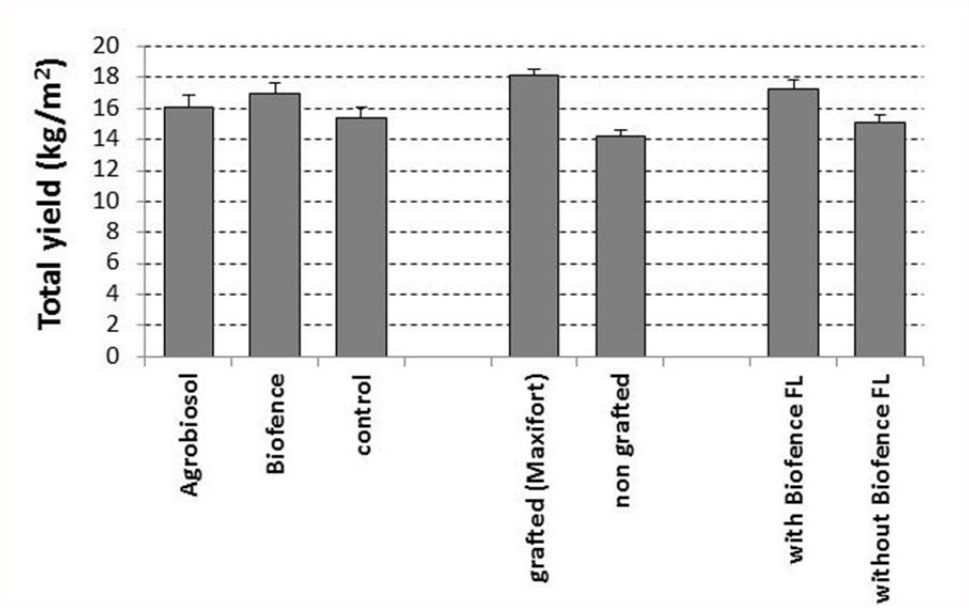

Fig. 2. Total tomato yield in 2010 greenhouse trial. Error bars indicate SEM.

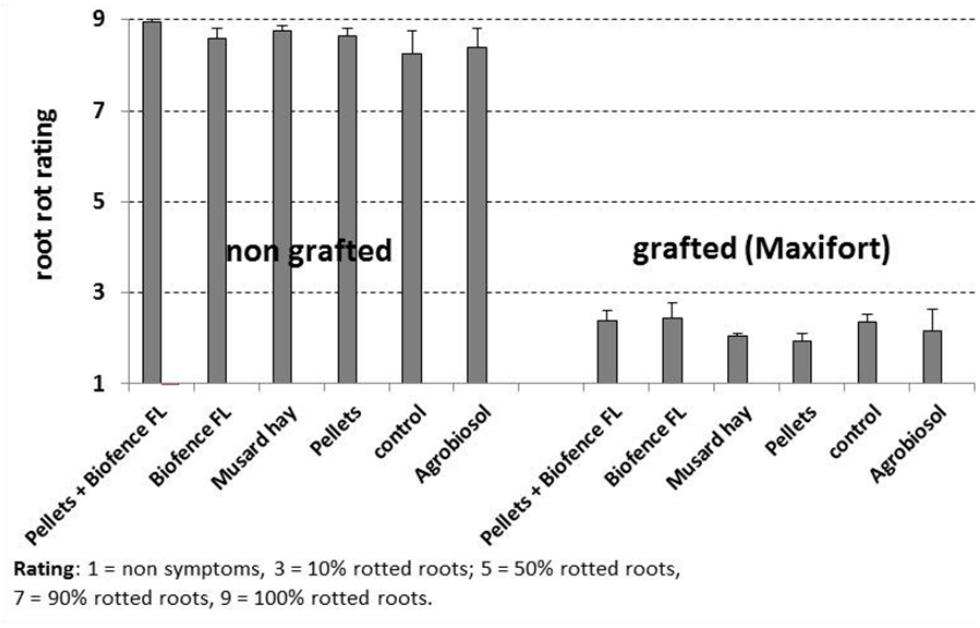

Fig. 3. Rating of the root rot of tomato roots after the final harvest of 2012 greenhouse trial. Roots of non-grafted plants showed typical symptoms of corky root disease (*Pyrenochaeta lycopersici*), roots of grafted plants showed only symptoms of black dot disease (*Colletotrichum coccodes*). Error bars indicate SEM.

Sorghum – Sudangrass hybrids application

In 2013, two treatments had a significant effect on the number of *V. dahliae* microsclerotia in soil. The *sorghum – Sudangrass hybrid* cv. Susu and mustard hay reduced *V. dahliae* by 32% ($P=0.016$) and 65% ($P=0.006$), respectively (Fig. 6).

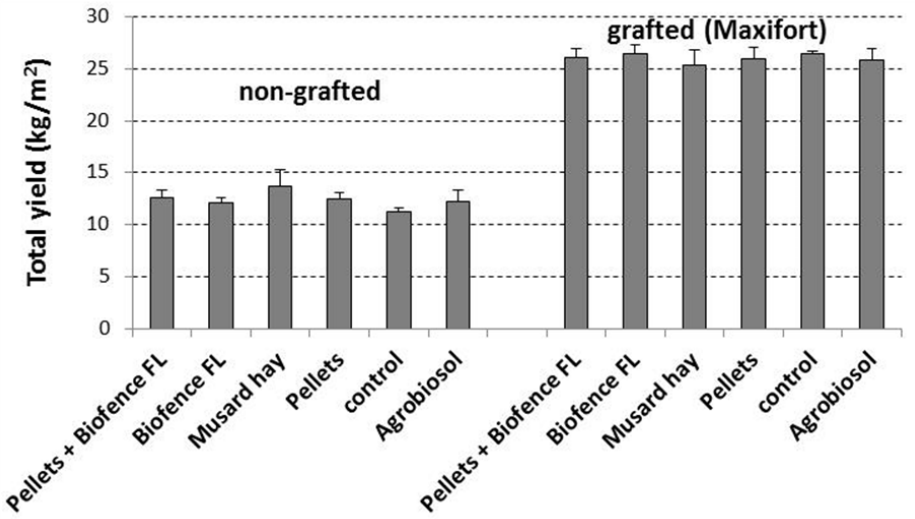

Fig. 4. Total tomato yield in 2012 greenhouse trial. Error bars indicate SEM.

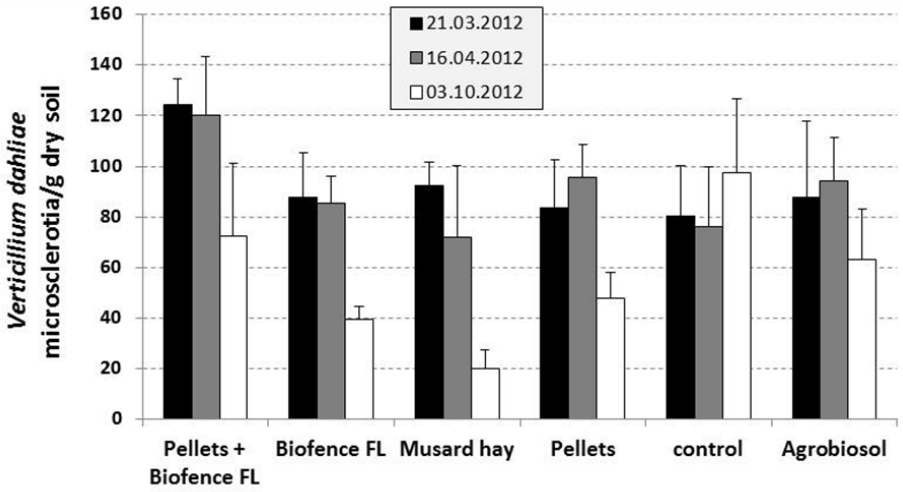

Fig. 5. *Verticillium dahliae* population of 2012 greenhouse trial. Number of *V. dahliae* microsclerotia was measured before trial start (March 21), before planting tomato (April 16) and after final harvest (October 3). Error bars indicate SEM.

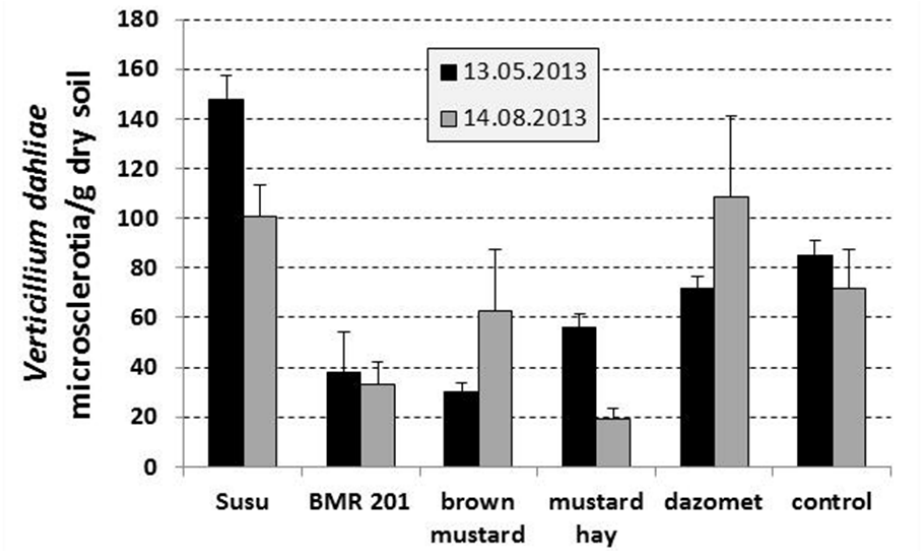

Fig. 6. *Verticillium dahliae* population of 2013 greenhouse trial. Number of *V. dahliae* microsclerotia was measured before trial start (May 13) and after green manures were incorporated (August 14). Susu and BMR 201 are sorghum – Sudangrass hybrids. Error bars indicate SEM.

Silage application

In the pot trial, the chemical disinfectant dazomet reduced significantly *V. dahliae* in the soil compared to all other treatments but the full dose of grass-clover silage (Fig. 7). The effect of grass-clover silage seems dosage dependent. Rye had a similar effect as the grass-clover silage. In contrast, maize silage and mustard hay had no impact on the *V. dahliae* soil population. Adding fresh or ensilaged green plants to the soil increased the soil microbial activity (Fig. 8). The maize silage and mustard hay stimulated the soil microbial activity only slightly. The stimulation of the general soil microbial activity, as it is measured by the FDA method, had a detrimental effect on the survival of *V. dahliae* in the soil (Fig. 9).

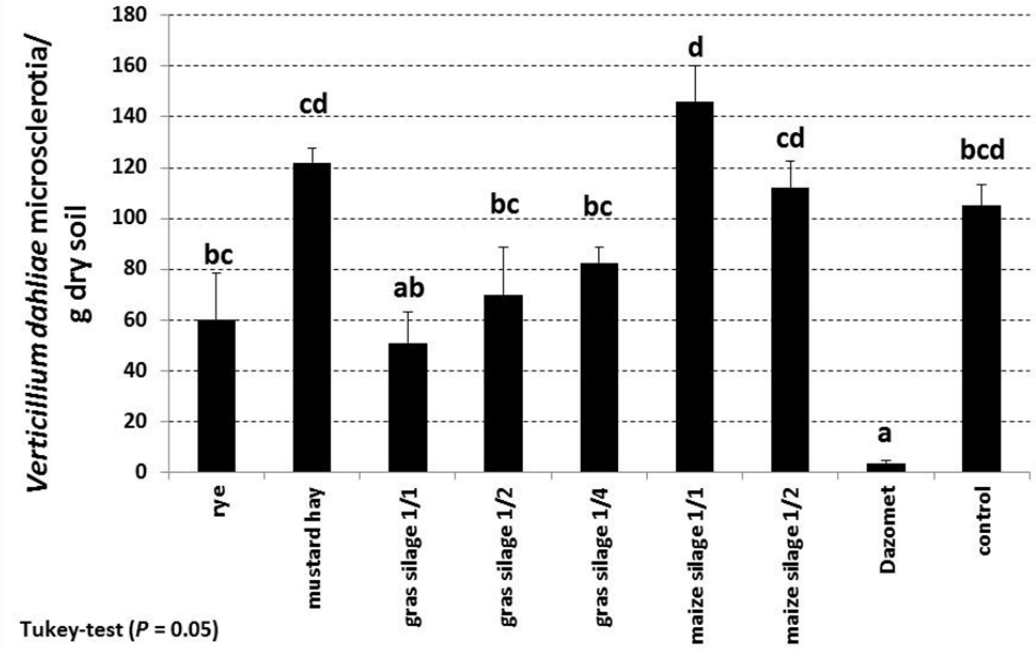

Fig. 7. *Verticillium dahliae* population of 2012 pot trial. The indications 1/1, 1/2 and 1/4 means full, half and quarter dose of silage mixed with same amount of soil. Error bars indicate SEM. Treatments with same letters are not significantly different.

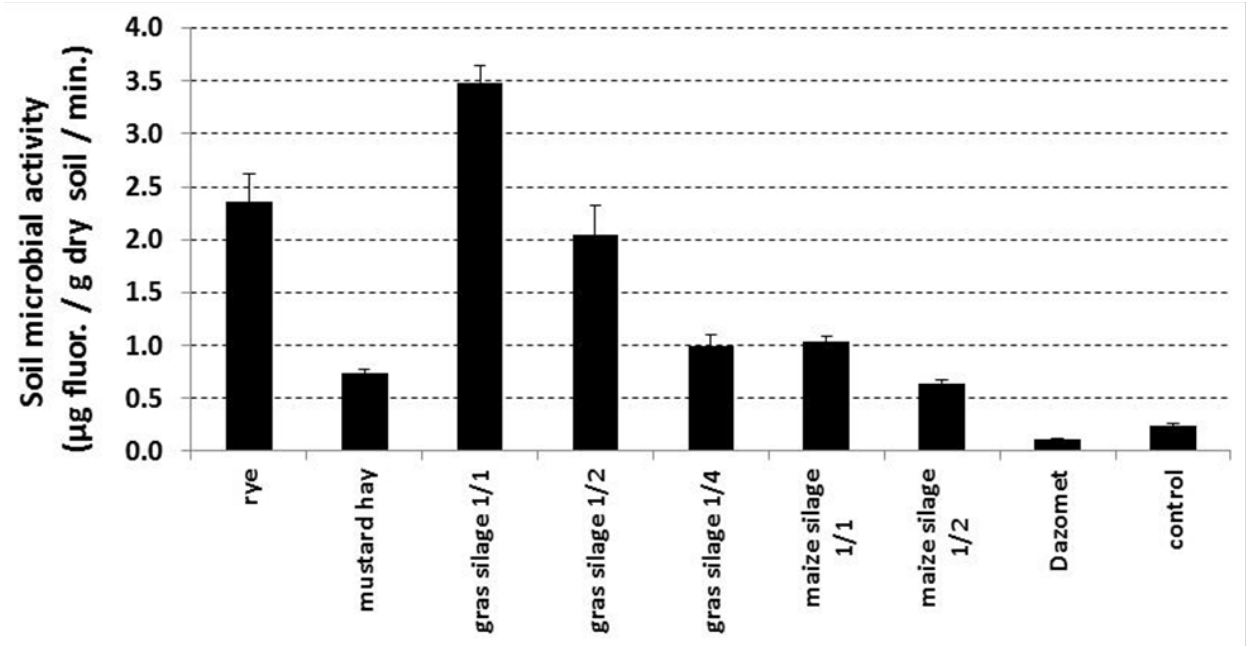

Fig. 8. Soil microbial activity of 2012 pot trial. Higher amount of fluorescein (fluor.) means higher activity. The indications 1/1, 1/2 and 1/4 means full, half and quarter dose of silage mixed with same amount of soil. Error bars indicate SEM.

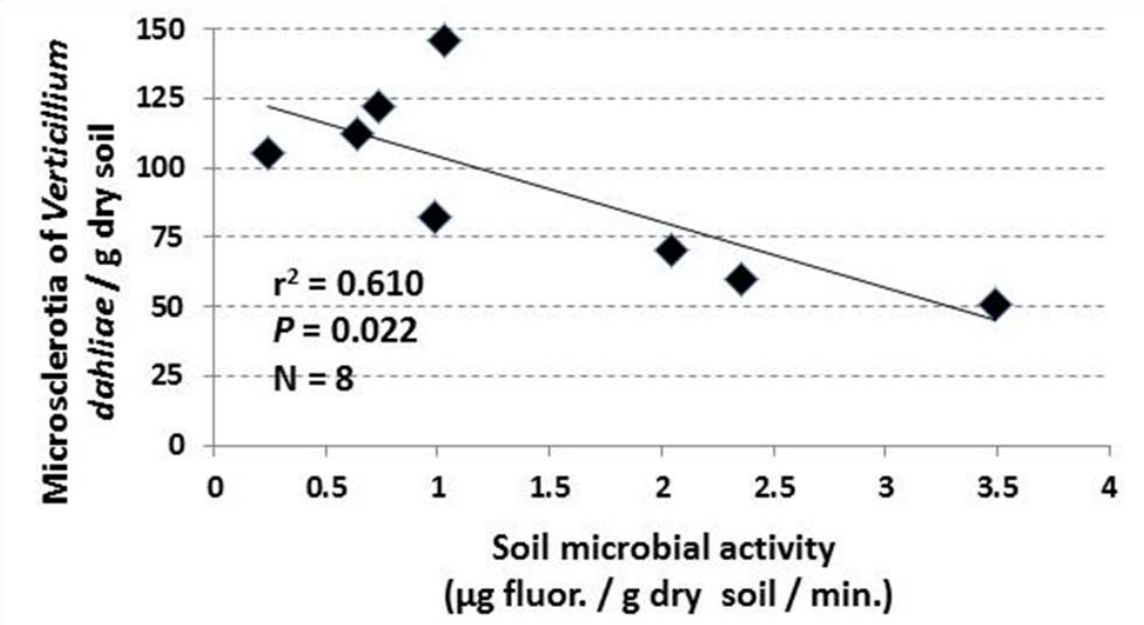

Fig. 9. Correlation between soil microbial activity and *Verticillium dahliae* population of 2012 pot trial. Higher amount of fluorescein (fluor.) means higher activity. The dazomet treatment was excluded from this analysis.

Discussion

Seed accessibility and winter hardiness of mustard cultivars

After the first information spread to the growers via technical publications and talks at grower meetings, several innovative farmers were eager to test the biofumigation method. Not used to contact foreign companies themselves, the growers asked Agroscope to help them getting the seeds of specific biofumigation mustard cultivars. Several seed trading companies in Switzerland were contacted and one of them, OH Seeds, organised the importation of the brown mustard cv. ISCI-99, which was selected for biofumigation purpose (Patalano, 2004). The same company later also organised the import and sale of the defatted seed meal products Biofence pellets and Biofence FL. In Switzerland, green manures are mainly grown in late autumn as catch crop to avoid nitrate leaching. Plant used for this purpose are fast growing, deep rooting crops such as brown, black (*Brassica nigra*) and white mustard (*Sinapis alba*). However, the cultivars used are not frost-resistant to avoid additional work in spring caused by the shredding and incorporation of big amounts of green organic matter. For biofumigation, such behaviour is contra productive, as freezing and thawing of the plants will provoke the loss of the glucosinolates contained in the plants. Therefore, mustard cultivars that a winter hard are needed. Such a brown mustard cultivar was developed in France and is commercialised under the name Etamine. A comparison of ISCI-99 and Etamine at Agroscope Conthey during the winter 2010–2011 clearly showed the superior winter hardiness of the latter cultivar. In contrast, when sown in spring, ISCI-99 is slightly more productive than Etamine.

Effect of defatted seed meal and hay of mustard plants

Defatted seed meal products, in solid or liquid form, provided a significant increase of tomato yield when disease pressure was relatively low, like in the 2010 trial. In 2009, the same treatments were already tested in the same greenhouse. As disease pressure was very low i.e., all ratings on roots of non-grafted plants were bellow rating 2 (traces) by the end of the trial, all treatments with exception of grafting had no effect on yield (data not shown). In the 2012 trial, in contrast, with a very high disease pressure, the defatted seed meal products had no more effect on yield. The absence of a measurable effect on root rot in the 2010 trial might be due to assessment only after

the final harvest. Root rot evolves during the growing period of tomato plants with a strong final increase, which might mask differences that occurred earlier.

The addition of mustard hay was only tested at a very high disease pressure in the 2012 trial and had no effect on root rot or yield. This was different for its effect on the survival of *V. dahliae* microsclerotia. In the 2012 trial, mustard hay had no immediate but a long-term effect which was confirmed by the results of the 2013 greenhouse trial. As the tomato cv. Admiro is resistant to Verticillium wilt (it was chosen for its susceptibility to corky root), this beneficial effect of mustard hay found no expression in a higher yield. The absence of a short term effect, confirmed by the result of the pot trial, indicates that most probably biofumigation is not involved. Based on the 2012 pot trial, the stimulation of the soil microbial activity can also be excluded as control mechanism. The stimulation of specific groups of soil microorganisms by the specific composition of the mustard hay might be the reason, new technologies such as pyro-sequencing could eventually be an approach to elucidate this question.

The absence of an effect of the defatted seed meal products and brown mustard fresh plants on the number of *V. dahliae* microsclerotia is not surprising as even the dazomet treatment had no impact in the 2013 trial. On one hand, *V. dahliae* has been shown to be a most resistant pathogen to methyl-isothiocyanate (Klose *et al*., 2008), the active substance not only of dazomet but also of metam sodium. On the other hand, the amount of allyl-isothiocyanate generated after incorporation of fresh brown mustard is too low to be lethal to *V. dahliae* microsclerotia (Neubauer *et al*., 2014) Even so no brown mustard silage was tested in the 2012 pot trial, the results show that potentially fresh green plants (rye) can be replaced by ensilaged green plants (grass-clover silage). Also important is the correlation between the soil microbial activity and the reduction of *V. dahliae* microsclerotia. An increase of the soil microbial activity was reported to decrease the survival of *Pythium ultimum*, a soil- and substrate-borne pathogen of poinsettia (Boehm & Hoitink, 1992). The three doses of grass-clover silage show clearly that the soil microbial activity can be influenced by the amount of organic matter added to the soil and thereby increase the efficacy of this control method.

Adding green manure plants, in form of fresh plants, as hay or as silage, to a soil has not only an impact on the soil microorganisms, beneficial or pathogenic, but also on other soil inhabitant e.g. earthworms or nematodes. Especially the incorporation mode can strongly influence the soil fauna, mostly in a detrimental way. Another factor is the importation of important amounts of nutrients which have to be taken into account for the fertilisation of the following crop. Some growers of organic greenhouse vegetables in Germany and Switzerland are using silage rich in leguminous species as an organic nitrogen fertiliser.

Swiss growers are more and more interested in biofumigation or the use of other green manure crops to control soilborne diseases. But still their number is relatively small and the general implementation of these methods needs time and resources (Sherman & Gent, 2014). Therefore, biofumigation research will be continued at Agroscope, with ongoing and future trials including brown mustard silage and hay. But an increasing part of the Agroscope activity will be invested in the communication with growers.

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