Bioseedling: a chain approach to the production of healthier seeds and seedlings of Lamb's lettuce *Valerianella locusta*

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Abstract

The project BIOSEEDLING "Robust planting material from seeds to young plants – an implementation oriented chain approach" aims to find improved production procedures for vegetable seedlings of lamb's lettuce. First, the production of lamb's lettuce seeds of a professional seed producer was analyzed and the effect of harvest time and seed size on the germination and disease rate in the seeds was studied. Then, using seeds naturally infected by *Peronospora valerianellae* and *Acidovorax valerianellae*, several seed disinfection methods were compared: aerated steam, hot water, sodium hypochlorite, ethanol, Calcium hydroxide, and compost pellet. After testing methods for the identification of the seed pathogens and the quantification of the infection, an assessment on how the different treatments reduce the pathogens and whether they alter the seeds germination capacity was made. In a third step, substrates suppressive of the soil borne pathogens *Rhizoctonia solanii* and *Pythium ultimum* were developed and several plant protection agents were tested against *Peronospora valerianellae*. Furthermore, the effect of night interruption on the sporulation of lamb's lettuce downy mildew (*Peronospora valerianellae*) using periods of lighting in the red and blue regions was tested. The aim is to combine the best methods resulting from all the experiments cited above in a future experiment and compare them to the standard methods in an on-farm experiment.

Keywords: organic greenhouse horticulture, seed production, seed health, seed treatment, seedling production, seedling vigour

INTRODUCTION

Organic greenhouse horticulture (OGH, certified according to Swiss legislation SR 910.18 or EU Regulation 834/2007) increased from 28 ha in 1997 to 80.2 ha in 2015 in Switzerland (Hartnagel, 1998; Wyssmann et al., 2016). Nowadays the share of OGH production area is 11.6% of the overall organic horticulture area, compared to 7.5% greenhouse area in conventional horticulture (Wyssmann et al., 2016). For this reason, Switzerland belongs to those European countries with the highest importance of the OGH sector (Willer and Kilcher, 2012). Most important crops in OGH are: corn salad (71.5 ha), tomato (23 ha), lettuce (17.5 ha), cucumber (12.1 ha), pepper (9 ha) and rocket (8.2 ha), respectively (Wyssmann et al., 2016). Among these crops, corn salad (lamb's lettuce) and rocket show remarkably high organic shares (18.3 and 15.5%, respectively). For the cropping success of organic greenhouse production, high quality seeds and vigorous seedlings are even more important than in conventional production because options for disease control at a later stage are very limited. Especially downy mildew (e.g., *Peronospora valarianellae* in corn salad) regularly causes severe yield losses. Often the seedlings are already infested with the disease and this seedling-infestation can be traced back to an infestation of the seeds.

In organic seed production only few scientific results are available to outline the possibilities to enhance and to assure the necessary seed health. However, a series of measures for prevention of seed contamination are generally recommended, such as disease

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free production areas, crop sanitation and rotation, cultural practices, disease-free stock seed, preventive treatments, elimination of alternative hosts, irrigation practices, crop rotation, and isolation of seed fields. Köhl et al. (2010) found for open field production of *Brassica* seeds that *Alternaria brassicicola* and *A. brassicae* can infect pods and seeds soon after flowering and that therefore these early infections must be prevented by organic control measures. While for some greenhouse crops like tomato, it is common practice to produce seed in greenhouses, other seed crops are still cultivated in open field due to economic reasons. For the OGH crops of interest, it is important to detect critical control points to avoid disease infestation.

Healthy seeds are a prerequisite for the production of healthy plants and in most cases, seeds need to be treated and freed from pathogens. Several non-chemical seed treatments exist and are gaining more and more importance in both OGH and conventional production. Two physical methods have already proven their efficacy in several plant-pathogen systems; hot water treatment, a long-known technique (Walker, 1948; Bant and Storey, 1952) that has regained attention (e.g., Nega et al., 2003; Schmitt et al., 2009; Koch et al., 2010) and the more recently developed aerated steam method (Forsberg et al., 2002; Heller and Zoller, 2010). The latter has already been implemented in seed treatment practice under the name ThermoSeed™ for the disinfection of large cereal lots (Koch, 2006). Reviews on organic seed treatments have been published (Micheloni et al., 2007; Jahn et al., 2007) and some non-chemical seed treatments were tested on tomato, spinach, bean and pea among others (e.g., Kasselaki et al., 2008, 2011; du Toit and Hernandez-Perez, 2005; Tinivella et al., 2009). These novel methods of seed treatment can improve the seed quality considerably. However, especially with diseases like downy mildew where a small infection rate (<0.1%) may lead to a complete crop loss preventive measures at the other stages of the production are needed.

To prevent from or to treat against downy mildew infection in corn salad and rocket, no plant protection products are registered and allowed in OGH in Switzerland. As an alternative to fungicides there are substances which can further enhance the level of resistance or tolerance to diseases (resistance elicitors, such as plant based products or biocontrol agents) (Schärer et al., 2006; Tamm et al., 2011). However, their efficacy and suitability for commercial OGH is not sufficiently explored yet and therefore only few products are registered for use in organic production. The potential to prevent diseases by growing robust cultivars is generally well-known by the seed industry and the vegetable growers. However, not all robust cultivars are sufficiently suited for production, e.g., due to low yield or low eating quality (Rascher and Schubert, 2004).

Several research studies have shown that the application of high quality compost can induce the seedlings' internal resistance system and/or can suppress soil-borne diseases. These findings indicate that an optimized substrate composition can contribute to a disease prevention strategy. This is true not only for soil-borne but even for air-borne diseases (Zhang et al., 1996; Hoitink et al., 1997). Plants react relatively slow to organic fertilizers and therefore attention has to be paid to the timing and amount of fertilizer application. Furthermore, certain commercial organic fertilizers can have a phytosanitary side effect (e.g., by increasing chitin decomposition) (Michel and Lazzeri, 2010). It is well-known that the regulation of the climatic conditions in the greenhouse – in particular humidity and temperature – have a large impact on the development of most diseases. The theoretical bases of this issue are known and there is a lot of scientific and commercial activity going on to find optimal climate regulation in greenhouses (Marx and Gärber, 2009). Another possible disease control approach, which is potentially suited for OGH, is the use of light: e.g., interrupting the night period to prevent the sporulation of downy mildew diseases (Koller et al., 2010; López-López et al., 2014). Different intensity, wavelengths and period length of the illumination during the night might differently affect the sporulation of downy mildew. In studies on basil, it has been shown that a sufficiently long dark period is essential to the production of sporocarp and that red and/or blue light can reduce the sporulation of basil downy mildew (*Peronospora belbahrii)* (Cohen et al., 2013).

In this project, every stage of the production chain has been tested with the aim to achieve practical solutions for the overall improvement of the production of lamb's lettuce *Valerianella locusta* and to a smaller extent of perennial rocket salad *Diplotaxis tenuifolia* in the OGH sector.

MATERIAL AND METHODS

Seed production

The production of corn salad seeds *Valerianella locusta* at Sativa Rheinau AG (Rheinau, CH), a Swiss organic seed producer was followed in the spring of 2014. In particular, the effect of harvest time on the germination and disease rate in the seeds of two cultivars 'Etampes' and 'Elan' was studied. In the end of autumn 2013, seeds were sown in rows of 14 cm at a density of 100-300 g a^{-1} (approx. 2000-3500 grains m^{-2}). Seeds were harvested over several dates in May and June 2014 using a machinery of the producer's own construction that consists of a vehicle with a cylinder that rolls over the plants detaching and sucking only mature seeds thanks to a vacuum pump. With this method, seeds are taken out of the field's conditions as soon as they are ready which should reduce the infection rate in seed lots. To test this hypothesis, seed lots from 4 and 8 consecutive harvests of the cultivars 'Etampes' and 'Elan', respectively were tested for their germination rates as well as the percentage of abnormal seeds they contain. Germination tests were performed according to ISTA (International rules for seed testing) and seedling evaluation was carried after the protocol of ISTA handbook of seedling evaluation (3rd edition, 2006; Section 15: Seedling type E – Seedling group A-2-1-1-1). Tests were performed under controlled conditions (20°C day, 15°C night, 12 h daylight). Evaluations were made at day 7, 14, 21 and 28 on 4 repetitions per seed lot. Meteorological conditions (i.e., temperature, relative humidity, and precipitation) of each harvest date were retrieved from www.Agrometeo.ch.

Seed treatment

Using non-commercial corn salad seed lots provided by Rijk Zwaan (De Lier, NL) and bearing different levels of infection by *Peronospora valerianellae*, *Acidovorax valerianellae* and/or *Phoma valerianellae*, the following seed treatments were tested: aerated steam, hot water, sodium hypochlorite, ethanol, calcium hydroxide, and compost pellet. The first three treatments were performed at commercial facilities while the rest was performed in our lab. The reduction of the pathogen as well as seed germination capacity were assessed post-treatment. This part of the project is described in details in Schärer et al. (2017).

Seedling cultivation

Optimization of seedling growth and seedling quality was investigated using amendments for growing media, physical measures (use of light) and organic plant protection products (e.g., resistance elicitors, biocontrol agents).

1. Growing media.

The basis of the substrates tested consisted of 70% peat, which corresponds to the maximal percentage allowed by BioSuisse guidelines. For the remaining 30% substrate, compost, coir (coco peat) or wood fiber were tested. Three fertilizers known for their disease suppressive properties were selected: crab flour – containing chitin from animal origin, biosol – containing chitin from fermented fungi and Condit – containing fermented whey wastes. Phytopellets and horn flour were used as control fertilizers. Growing media resulting from the combination of peat, peat substitute and fertilizer were used in pot experiments for the following plant-pathogen systems: cress-*Pythium ultimum*, basil-*Rhizoctonia solani* and lamb's lettuce-*Rhizoctonia solani*. Combinations with compost as peat substitute and different fertilizers were tested in a pot experiment using 6 pots of 20 lamb's lettuce seeds (cultivar 'Cirilla') each per combination and *R. solani* concentration (i.e., 0, 5 and 25 g L^{-1} substrate). Plants were kept at 22°C day 20°C night, 12h daylight. Plants per pot were counted at day 7, 14, 21 and 28 when plants were harvested and the green biomass per pot was recorded. Experimental designs for cress and basil are described in Fuchs et al.

(2017).

2. Effect of light on the sporulation of downy mildew.

Lamb's lettuce and rocket salad were inoculated at one month ± 1 week old with a suspension of sporangia of their specific downy mildew using a compressed air spray gun (DeVilbiss). The suspensions were prepared from infected leaves washed in cold Volvic mineral water. Inoculated plants were incubated for 24 h in a humidity saturated chamber at 21 and 12-15°C for rocket and corn salad, respectively. After incubation, plants were put in climate chambers with built-in LED Panels of red (670 nm) or blue light (473 nm) (GroBank & GroLeds, CLF Plant Climatics GmbH, Wertingen, Germany) under different light regimes. Light regimes tested vary in the length of the dark period, whether darkness is interrupted by red or blue light, and duration/repetition of the periods of night interruption. Inoculated and control plants were put under every light treatment. After 14 and/or 21 days post-inoculation, individual plants were scored on disease severity and disease incidence.

3. Phytosanitary products.

Treatments consisting of solutions of elicitor/plant strengthening agents, alone or combined with biopesticides at different concentrations are performed on six pots (9 seedlings pot^{-1}, aged 1 month ± 1 week) each. Two periods of application are tested: 3 days and half-day pre-inoculation with spores of downy mildew. For each treatment, 6 pots were randomly chosen and placed in an automatic spraying cabin. This experiment was performed 3 times, reducing in every consecutive series the number of products tested (based on their efficacy and potential for getting officially allowed). In every series, two controls: negative with water and no inoculation, and positive with water where plants were inoculated 4 d later, and a chemical treatment with β-aminobutyric acid (BABA) were included. Considering the positive control plants as 100% infected, infection of the other plants could be visually evaluated.

RESULTS AND DISCUSSION

Seed production

Seeds of the cultivar 'Elan' collected at early harvest obtained higher germination rates compared to seeds from later harvest (average over the first 3 harvests of 85 vs. 35% for the last harvest). Differences in germination rate can be attributed to either the natural germination ability (e.g., size: seeds of smaller caliber had generally lower germination rates) or to seed health. For 'Elan', the seedling evaluation tests resulted in 28% of abnormal seeds for the first harvest compared to 65% for the 8^{th} and last harvest. This trend was not observed in seeds from the cultivar 'Etampes' that were collected in 4 harvests only. No correlation was found between germination rates and weather. In the frame of a two years only project, it was difficult to get enough data to withdraw conclusions on the best harvesting practices. Further tests (i.e., germination tests and pathogen identification tests) are needed over several production years and cultivars. However, based on this work, it can be recommended to test the seed lots from different harvests prior to pooling them in order to avoid a reduction of the quality of a seed lot in the case of high percentage of bad quality seeds in late harvests.

Seed treatment

Aerated steam and hot water treatment led to the best disinfection with hot water treatment being the only one that eliminated completely both *Acidovorax valerianellae* and *Peronospora valerianellae*. Experimental treatments such as compost pellet need further technical development before their value as a seed treatment can be assessed. These results show that different methods work better against certain pathogens. This highlights the importance of risk analysis in order to perform the seed treatment that targets best the pathogen that will most importantly cause damage to the culture. These results are described in details in Schärer et al. (2017).

Seedling cultivation

1. Growing media.

Pot experiments with the system Lamb's lettuce-*Rhizoctonia* were inconclusive. However, the other experiments showed that compost, especially when combined with a chitin-containing nitrogen fertilizer reduced the effect of *P. ultimum* on cress (Fuchs et al., 2017). Experiments with wood fiber as a peat substitute also showed a suppressive effect on *P. ultimum* (Fuchs et al., 2017). Hence, an experiment using compost, wood fibers and both combined with different levels of *P. ultimum* was performed. The combination of peat with 20% compost and 10% wood fibers lead to the highest cress green biomass in the presence of different levels of *P. ultimum* in the substrate. Further experiments on-farm will show whether this substrate mix fertilized with condit and crab flour improves the organic production of lamb's lettuce seedlings.

2. Effect of light on the sporulation of downy mildew.

In a first experiment, the evaluation at day 14 showed that corn salad plants that were under a light regime with two 3-h periods of red light interrupting the night period had the third of the symptoms of plants under normal day and night regime and plants under a night regime with darkness interruption using blue light. This result is in line with the findings of Cohen et al. (2013) that periods of red light in the dark period suppress the sporulation of downy mildew. However, when this experiment was repeated using a larger number of plants of corn salad, rocket and basil plants as a control for the system, this result could not be repeated. On the contrary, while basil plants' biomass was higher under red light compared to blue light and normal light regime, the severity was higher under both led lights compared to the control. For rocket, while the severity was slightly lower for both red and blue treatments compared to control, there was no significant difference in biomass. Regarding corn salad, there was no difference between green biomass under each of the 3 treatments. The severity and incidence could not be assessed on the latter, probably due to the temperature settings in the climate chambers (higher than previously used for corn salad to accommodate the other two plants). Additional tests in our lab have shown that for *Peronospora valerianellae* to sporulate temperatures not exceeding 15°C are needed. Further research is needed to adapt to first test whether our light system is adequate for such application and second, whether night interruption with either red or blue lights can affect the sporulation of corn salad and rocket downy mildews.

3. Phytosanitary products.

Plants treated with BABA as well as untreated non-inoculated plants showed no infection symptoms. The disease rate was the highest with untreated inoculated plants. In the first series, Kocide (Cu) as well as Prev-am (6% orange skin extract) decreased significantly to completely the infection rate. The elicitors Vacciplant and COS-OGA each used alone decreased the infection slightly. In the last series Prev-am at 0.4 and 0.08% was tested alone, and the lowest concentration was tested in combination to each of the two elicitors. The application of Prev-am 0.4% resulted in 0% infection, comparable to BABA and non-inoculated control plants. Prev-am at the lower concentration of 0.08% in combination with COS-OGA gave similar results. Therefore, the use of this elicitor allows the reduction of the concentration of the bio-fungicide Prev-am and together they can be used as an efficient treatment against the downy mildew of Lamb's lettuce *Peronospora valerianellae*.

CONCLUSIONS

- Gradual harvesting removes mature seeds from field conditions and could improve seed production.
- The efficacy of physical seed disinfection methods was confirmed and new potential methods were tested.
- An improved growing substrate consisting of black peat, compost and wood fibers and fertilized with Condit and crab flour was developed.

- No positive effect of night interruption with red or blue led-light on the sporulation of *P. valerianellae* could be observed. Improvement of the testing method is needed.
- A bio-fungicide combined to an elicitor reduced drastically the infection with *P. valerianellae.*

In a future experiment, all improvements will be implemented in a seedling production which will be quantitatively compared to the standard production. Such an approach will serve as a model for integration of a series of measures for disease prevention in other crop-pathogen interactions, not only in protected organic cropping but also in outdoor cropping. The accumulated benefits of the improvements to every step of the seedlings production can be measured in terms of yield therefore making the changes in some of the actual practices an easy decision to make.

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