Organic seed health. An inventory of issues and a report on case studies

Deliverable number	D 2.5
Dissemination level	Public
Delivery Date	30.11.2020
Status	Final
Lead beneficiary	WR
Authors	**Steven P.C. Groot (WR), Stephanie Klaedtke (ITAB), Monika Messmer (FiBL-CH) and Frederic Rey (ITAB)**

LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090. The information provided reflects the views of the authors. The Research Executive Agency or the SERI are not responsible for any use that may be made of the information provided.

Document Version

Version	Date	Contributor	Summary of Changes
1.0	20.11.2020	Steven P.C. Groot and Stephanie Klaedtke	First draft of deliverable
1.0	26.11.2020	Frederic Rey feedback	Feedback to deliverable
1.1	30.11.2020	Steven P.C. Groot	New version
1.1	20.01.2021	Monika Messmer	Final proofread before uploading

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Executive Summary

Because of the need for more sustainable agriculture, the European commission has set its target for 2030 to have at least one quarter of the farmland in the European Union under organic farming practices. The use of healthy seeds or vegetative propagation material forms the basis of sustainable crop production. The use of organic seeds is an integral and obligatory part of organic farming. An organic seed is defined as a seed of which the mother plant or parent plant has been produced following the principles of organic agriculture. Due to shortage in volume and diversity of organic seeds, a large amount of conventionally produced seeds is still used after official approval by Member States. This derogation for conventional untreated seed in organic agriculture will end in 2036. Both the increase in area of organic agriculture and the use 100% organic seeds call for large efforts in organic seed production and research. This report describes the state of the art and research results on the production of heathy organic seeds, as performed in the frame of the LIVESEED project, with support from the European Horizon 2020 program.

The importance of using high quality organic seeds for crop production, and general challenges in this are described in the introduction, Chapter 1. Unfortunately, organic seed quality is not always optimal. One reason can be pathogens infecting the mother plants, from which some can travel in or on the seeds to the next generation. Seed production in many cases like vegetables or biennial species takes longer than crop harvest used for food or feed production, mother plants are exposed longer to pressure from weeds, pests, diseases, and abiotic stress. Organic seed production is therefore more difficult than conventional and requires more labour, increasing the production costs. Organic seed health is based on a multitude of factors and cannot simply be managed through one-size-fits-all solutions such as curative seed treatments.

Use of seeds produced under organic conditions can also have benefits, as organic soils may have a richer and more diverse microbiome and part of this microbiome enters the seed during development. Although much more research is needed, there are indications that certain microorganisms in this seed microbiome play a role in tolerance of the emerging seedling toward biotic and abiotic stress in the field. Beneficial microorganisms isolated from the seed microbiome can be applied in seed coating as biocontrol agents.

Measures that can be taken to improve organic seed quality are described in more detail in Chapter 2, starting with the effect of seed production conditions. The importance of seed maturity, the seed microbiome, the effect of seed sanitation treatments, the application of biologicals, use of disease resistant varieties and legal regulations are discussed. Shortages still met in the supply of organic seeds also has to do with challenges experienced in producing high quality healthy seeds under organic farming conditions. These challenges are described in Chapter 3, listing the results of an inventory among organic seed stakeholders.

In the frame of the LIVESEED project, case studies have been performed on some of these issues, with the aim of providing background information and tools to tackle them, described in Chapter 4. Most effort went into two studies, one with wheat, in which several approaches have been tested to reduce the risk of infection of seeds with common bunt fungal spores. This case study describes the success of involving farmer seed producers in the development of a successful seed health strategy. In a second case study the role of carrot seed vigour was investigated in relation to tolerance towards a fungal pathogen causing damping-off disease. That study showed that seed vigour is not only important for maximal seedling establishment in the field, but it also improves the tolerance of the seedlings to damping-off disease cause by the pathogen. Other smaller case studies undertaken: a

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literature study and inquiry into management approaches of viral diseases with seed potatoes, survival of the seed microbiome (or applied biologicals) during storage and an analysis of alternative seed packaging material. Most of these smaller case studies are still ongoing and only limited results can be presented in this report prepared several months before the end of LIVESEED.

Conclusions on the studies performed on organic seed quality in LIVESEED are formulated in Chapter 5. Part of organic seeds are supplied by specialised farmers and seed companies, the other part by farm saved seeds. Dissemination of knowledge and technologies already available is one way to support them in producing high-quality seeds, in upgrading through appropriate sorting and treatments, and in maintaining this seed quality during storage. However, even with these improved methods, for some crops it is still a challenge to produce high-quality healthy seeds under organic conditions. The results obtained in case studies and from other activities and research in the LIVESEED project, together with exchange of the sister Projects BRESOV and ECOBREED and further discussions helped to identify research priorities and will lead to recommendations for a new organic seed health strategy. This strategy will be presented at the end of the LIVESEED project as part of the synthesis of the results and a road map to achieve 100% organic seed (Deliverable D6.3). Chapter 6 provides the bibliography with references mentioned in the text. Chapter 7 displays six LIVESEED practice abstracts related to organic seed health.

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1. Introduction

Crop production starts with preparing the soil and sowing of the seed. Farmers' seed choice is mainly based on the cultivar which determines suitability for producing the crop under the environmental conditions available and may include resistance against pests or diseases. But for a successful start of the crop, the physical, physiological, and health quality of the seeds is at least as important. Early plant growth is exponential, which means the earlier the seedling growth, the faster the young plants will develop. Healthy vigorous seeds will emerge rapidly and uniformly, provide fast growing root systems to retrieve nutrients, as well as a fast-growing shoot to capture sunlight and produce energy. This will both give the seedling more tolerance to abiotic and biotic stresses in the field and more capacity to compete with weeds.

In the Green Deal and the Farm to Fork strategy (European Commission, 2020), the European Commission has set the target that in 2030 at least one quarter of the total farmland shall be under organic farming. The use of organic seeds is obligatory and part of an integral organic production system. An organic seed is defined as a seed of which the mother plant (if seed) or the parent plant (if vegetative propagating material) has been produced following the principles of organic agriculture, as laid out by the European Organic Farming Regulations (EC No 834/2007; EU 2018/848). Unfortunately, a considerable share of the seeds used in organic farming is still provided by conventional seed production. The organic seed availability varies greatly from one European country to another. Under certain conditions farmers can get a derogation, because for several crops sufficient quantities are still lacking as organic seeds or even not available for locally adapted cultivars. Moreover, not all seed lots available on the market or farm-saved seeds are of a high quality. Although seed companies and farmers are striving to obtain maximum quality, this is not always possible. Even if the quality is initially high, it may be reduced by non-optimal processing and storage conditions or other deteriorating factors. The European Commission has announced a phasing out of the derogation system for non-organic seed and in 2036 all seeds and vegetative propagation material used for organic farming needs to be produced under organic conditions. This, together with the targeted increase in organic farmland, means that a big task lies ahead for the organic sector to produce considerably higher amounts of organic seeds of a decent quality.

Organic seed production is a demanding activity as it is requiring skills in both organic farming as well as seed production since quality is influenced by many factors. In addition, seed production needs that the crop is on the field till full seed maturation is reached, which is most often longer than crop vegetation period used for food or feed production, exposing the mother plants longer to pressure from weeds, pests, diseases, and abiotic stress.

While organic seed production is a highly specialised activity, it can also represent an attractive income diversification strategy for organic farmers. However, it requires an adequate level of technical skills and infrastructure and entails specific risks, two aspects that should not be underestimated. Organic seed production is more difficult than conventional and requires more labour, increasing the production costs and risks of failure. One of the main challenges is the health status of the mother plant, especially when it concerns pests and diseases. These can reduce the energy from the mother plant available for seed production (reducing seed yield), while some seed borne diseases may also travel through the seeds to the next generation.

The quality standards (germination rate, varietal purity, seed health) which apply to the marketing of organic seed are the same as those for conventional seed: when the quality does not meet the set

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thresholds, seed certification will be denied, leading to a net loss to the seed multiplier unless the seed production contract includes some form of guarantee.

The use of organic seeds for organic crop production is not only obligatory, when available, it is part of a fully coherent value chain (from seed to plate). Although not fully studied, the use of organic seeds may also provide direct benefit to the farmer, as production has been done under organic conditions, potentially adapting the seed to its use in organic fields and harbouring a more diverse microbiome. With an increasing demand for organic seeds, supplying seed companies are getting more experienced in producing seeds under organic management, without chemical crop protection to prevent transmission of diseases with the seeds. Part of this knowledge is also used to make conventional seed production more sustainable. An overview of already established measures to ensure and improve organic seed quality and health is given in Chapter 2. Nevertheless, there are still many challenges to produce high quality organic seeds at reasonable costs. In the frame of the LIVESEED project an inventory has been produced listing current challenges with organic seed quality and health in Europe (Chapter 3). Although some minor crops are missing in this inventory and not all diseases troubling seed production are mentioned, it clearly shows the key issues with organic seed production that need to be addressed.

In the frame of the LIVESEED project case studies have been performed on some of these issues, with the aim of providing background information and tools to tackle them (Chapter 4). Producing high quality, healthy organic seeds requires overarching knowledge of the production process. In the case study on common bunt (Chapter 4.1), the management of this issue was approached by several angles, including seed treatments, legal requirements, the production of appropriate practitioner tools to disseminate available knowledge, and holistic practice-based approaches on farms. This case study demonstrated that organic seed health is based on a multitude of factors and cannot simply be managed through one-size-fits-all solutions such as curative seed treatments.

Health in general, and plant health in particular, is not defined in the same way by all actors of organic production. While some are satisfied with curative measures to sustain plant health, others prefer to base plant health on more self-sustaining ecological and biological interactions, as can be seen in the case of virus management in seed potatoes (Chapter 4.2). Official schemes for seed potato production are highly dependent on serological testing and sanitation through *in vitro* meristem culture to ensure thresholds for tuber-borne viral potato diseases, such as Potato Virus Y. Following the critique of this scheme by some organic seed potato producers, namely that it does not allow the entire production process to happen under organic growing conditions, alternative practices and approaches to potato health were explored. The resilience of seedlings is an important aspect to foster farming systems' sustainability. As mentioned above, high vigour seeds are needed for rapid germination and emergence in the field. From humans we know that physically healthy individuals have more tolerance for pathogens. Surprisingly, this has hardly been studied with regards to seeds and seedlings. In the LIVESEED project this hypothesis has been tested with carrot seeds, comparing their field emergence and tolerance to a pathogen that can cause damping-off disease in the field (Chapter 4.3).

In recent years it has become clear that the health of higher organisms depends for a large part on the microorganisms in their tissues and on the diversity of this microbiome. This holds for plants and likely also for seeds and seedlings. Organic seed production is frequently seen as much more difficult, supported by the higher price farmers must pay for organic seeds. But this does not consider the potential benefit of a richer microbiome of organic seeds due to their production in organic soil with a higher carbon content. Research in a sister European project EU-BRESOV has shown that soils with up to 50% higher carbon content, give a much higher yield in tomato production compared to soils with a low carbon content. Carbon-rich soils contain also a more diverse microbiome and, in theory,

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such a more biodiverse organic soils, should enable to produce seeds with a more diverse microbiome. This hypothesis is tested in the framework of the LIVESEED project.

When producing seeds with a more diverse microbiome is supposed to be beneficial, it is also important to maintain this biodiversity during seed handling and storage. Not only do seeds gradually lose viability during storage, the same holds for the microorganisms forming the seed microbiome. Especially when diverse, they can differ in their rate of viability loss during storage. It is therefore important to optimise seed storage not only for the seed itself, but also for its microbiome. In two case studies research has been initiated to assess the effect of seed storage on the longevity of their microbiome. Seed sanitation treatments, like for instance hot water treatments to irradicate pathogens present in or on the seeds, will also reduce other components of the seed microbiome, especially on those present on outside of the seeds, although there are as yet no data on the extend. When there is no alternative to control the pathogens by a sanitation treatment, it can be useful to supplement the treated seeds with positive microorganisms, so-called biologicals, or biocontrol agents (BCA's), by seed coating. Here challenges lay ahead, especially in the shelf life of the applied BCA's. In one case study it is tested to see if an experimental ageing treatment using high pressure air can provide indications for the shelf life of *Rhizobium* and *Bacillus* bacteria coated on alfalfa seeds. It should be noted that some case studies are still ongoing and that this report provides for those studies only data on the approach and results obtained until preparing this report.

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2. Measures to improve seed quality

2.1. Seed production conditions

Obtaining high quality seeds starts with organising optimal seed production conditions. Since seed production most often takes a longer life span of the mother plants compared to crop production, especially when a second growing season is needed for flowering with biennial plants, the climate conditions are particularly important. Climates which are relatively dry during the period of harvesting reduce risks of diseases and preharvest sprouting. Many diseases can travel on or in the seed to the next plant generation, so-called 'seed borne diseases'. It is therefore important to control disease development frequently. The measures are often similar to crop production. It can be useful to remove diseased plants. Control of weeds in a seed production crop not only avoids competition for nutrients, water, and light, but also reduces risks of contaminating the produced seed lot with weed seeds. Moreover, some weeds can stimulate the spread of pests and diseases. Spreading pathogens during harvesting should also be avoided. Removal of infected plots prior to harvest and thorough cleaning of harvesting equipment are two important measures.

Certain seed borne diseases can cause high amounts of economic damage. Such pathogens require great attention to avoid seeds getting infected. An example is the bacterial disease *Clavibacter michiganensis subsp. michiganensis* (*Cmm*) the causative agent of bacterial wilt and canker of tomato. A worldwide collaboration between seed companies and seed testing organisations has established a so-called 'Good Seed and Plant Practices' chain system (GSPP), with protocols to prevent tomato seed and plant lots from being infected by the pathogen (www.gspp.eu). On the other hand, in personal communications, small-scale, diversified organic seed companies in France and Germany have criticised this GSPP certification system for being inaccessible to small-scale companies, both technically and economically. Also, this system hardly seems compatible with approaches that base plant health on ecosystem and biological interactions (e.g., principles of "Health" and "Ecology" formulated by IFOAM Organics International, 2005). These critiques call for extensive future research on how such interactions can reliably sustain plant health.

2.2. Seed maturity

Seed maturation is another important aspect. For crop production it is often enough to wait till the seeds have reached their maximum dry weight, but that is not the case when the seeds need to be used as propagation material. After the filing of the seed with storage food (proteins, starch, and oil), the seed will gradually shut down its machinery and impose protection mechanisms for survival under dry conditions. During dry storage oxidative damage will accumulate, while repair enzymes cannot be active as they need water for their activity. Protection of DNA, proteins and membranes is therefore important for the survival of the seed. When the seeds are harvested too early, their vigour is low, and they will accumulate more damage during storage. Less mature seeds are also more sensitive to physical sanitation treatments. As damage repair takes time, those seeds germinate more slowly, will be more sensitive to stress or will not provide a healthy seedling at all. One of the case studies performed in the LIVESEED project has shown that a reduction in carrot seed vigour also makes the seeds and seedling more sensitive to attack by the damping-off pathogen *Alternaria radicina* (see 4.3).

2.3. The seed microbiome

Seeds contain also microorganisms, the seed microbiome (Berg and Raaijmakers, 2018). These can be inside the seeds, in the embryo or endosperm, often called endophytes, or at the outer layers, the

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seed coat of pericarp tissue, in that case called the epiphytes. In fact, pathogens are also part of this microbiome. The seed microbiome is determined largely by the soil microbiome and its diversity, but the composition also varies between species and even between genetically distinct varieties of a crop. The seed microbiome contains microorganisms that have co-evolved with the plant species and can have a negative (pathogens), positive or neutral effect on seed and seedling health. For some microorganisms in the microbiome, it has been shown that they can stimulate seed germination or seedling tolerance to abiotic stress. For others it has been shown that they can have an antagonistic effect on pathogens. The latter can be through the production of compounds that are toxic to the pathogen, through stimulation of the plant defence, or simply through competition. This seed microbiome has evolved with plants over millions of years and is nowadays thought to be largely a symbiotic relationship. It is assumed that domestication, modern agriculture, breeding, seed production and treatments have had a profound effect on the composition of the seed microbiome (Germida and Siciliano, 2001). While seeds are the main intermediary for the transmission of beneficial bacteria (Bergna et al., 2018), seed production conditions, especially the soil, influence the plant microbiome. Although more research is needed, this underscores the potential benefit of the use of seeds produced under organic conditions. Experiments have shown that the microbiome of wild plants is much more diverse compared to that of crop plants (Wassermann et al., 2019). Tapping on the seed microbiome, including that present in wild relatives, has a high potential to improve crop production and cope with both biotic and abiotic stresses. This will require more research efforts to understand for instance the effect of genetics, seed production, seed treatments and storage on the seed microbiome and its effectivity during seedling establishment. Part of this research has been initiated in the frame of the LIVESEED project but will require further experimentation in future projects.

2.4. Seed sanitation treatments (compounds, physical)

Even when during seed production measures against the spread of diseases have been taken, the presence of certain pathogens on the seeds sometimes cannot be avoided. These pathogens may infect the seedling after germination of the seeds. It will depend on the type of pathogen and on the severity of the infection level if and what measures are needed. When it concerns a so-called quarantine pest or disease, the pathogen needs to be eliminated completely before seeds can be marked and sown on the field (see 2.7). An example of such a quarantine disease is *Clavibacter michiganensis subsp. michiganensis* the causative agent of bacterial wilt and canker of tomato. With some other pathogens, for instance *Fusarium,* causing head blight in wheat, low levels of infection may be accepted when it does not harm seedling establishment too much.

There are several methods available to eliminate or reduce the presence of pathogens in or on organic seeds. Brush-cleaning is used for fungal spores adhering to the surface of wheat seeds, for example. Sometimes the pathogen causes a colour or density change of the seeds and colour-sorting equipment can be used to remove infected seeds. Heat treatments with hot water, steam or hot air are frequently used for seed sanitation. A disadvantage of the sanitation treatments is that besides the pathogen also positive microorganisms from the seed microbiome are removed, thereby creating an open niche for soil pathogens upon planting of the seeds. This can be counteracted by coating the seeds with commercially produced microbes (see 2.5). Also, natural compounds can be used to reduce seed pathogens like essential oils (Spadaro et al., 2017). However, they might also have a detrimental effect on the seed microbiome.

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2.5. Application of biologicals

During evolution plants have evolved in the presence of microorganisms and mutual relationships have been established. One of the best known is that between *Rhizobium* bacteria and plants from the *Leguminosae* clade (legume species). The plant roots form nodules in which the bacteria are protected and can multiply, receiving energy from the host. In return the bacteria fix nitrogen from the air and provide the plant with a nitrogen source it can utilize. Since not all soils already contain the best suitable *Rhizobium* bacteria, it has become for decades an agricultural practice to coat seeds from legume crops as soybean, lupin, beans, clover, and alfalfa with *Rhizobium* or *Bradyrhizobium* bacteria before sowing.

In recent years it has become clear that many other microorganisms can aid the plant in the uptake of nutrients and in the tolerance towards biotic and abiotic stresses. When applied to the plants, be it the leaves, roots or seeds, these microorganisms are often called biocontrol agents or biologicals. The number of biologicals that are identified to aid the germinating seed and emerging seedlings is rapidly expanding. However, one of the challenges with the application of biologicals to seeds is their shelf life. Production of biologicals is often in a liquid culture, while seed applications require dry storage after coating to fit in the logistic chain of seed supply to farmers. Selection of biological strains with a good shelf life under dry conditions, or recipe development to improve their shelf life, requires an adequate shelf-life test, which has been lacking until now. In one LIVESEED case study an experimental ageing treatment using high pressure air is been tested, to provide indications for the shelf life of *Rhizobium* and *Bacillus* bacteria coated on alfalfa seeds.

2.6. Resistance breeding

Resistance can be defined as the "inherent capacity of a plant to prevent or restrict the entry or subsequent activities of a pathogenic agent when the plant is exposed, under suitable environmental conditions, to sufficient inoculum of a pathogen to cause disease" (Bhargava and Srivastava, 2019). Several types of resistance exist and are dependent both on the genetic variability in the host plant and in the potential pathogen. Resistance can be horizontal, or quantitative, when it controls a broad range of races of a given pathogen. Horizontal resistance is usually conferred by a combination of many genes, each with a minor effect (polygenic). On the other hand, vertical, or qualitative, resistance results in a total resistance against one or few races, but not against others. It is usually conferred by one or few major genes, which are easily identified and transferred from one genotype to another. However, relying on vertical, monogenic resistance presents a risk, as widespread and continuous use of a particular cultivar or type of resistance may lead to the development of a new pathogenic race or to a shift in pathogen populations. The continuous adaptation of pathogen populations in order to "break" or circumvent resistance genes they are confronted with has been described as an "arms race". Resistances obtained by stacking or pyramiding several resistance genes are therefore preferable, as more difficult to break and therefore more durable. Disease resistance is different from tolerance, as the latter refers to the ability of a plant to limit the impact of a given disease on its development and yield despite infection. Unlike tolerant plants, plants that are resistant to a given plant pathogen are expected to produce seeds free of the respective pathogen, except for cross-infection coming from other seed lots through equipment.

Over past decades, most plant breeding programs have been oriented towards conventional agriculture, which mainly relies on chemical seed and crop treatments to prevent seed and soil borne plant diseases, fungal diseases, in particular. However, specific organic breeding programs have increasingly engaged in resistance breeding, especially in seed and soil borne diseases, to strengthen genetic disease control and make organic systems less vulnerable to plant diseases.

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Topic 11 of LIVESEED's literature review (Nuijten et al., 2020, p. 70, Deliverable 3.5) discusses approaches and challenges of resistance breeding for organic agriculture, based on three examples (common bunt with bread wheat, anthracnose with sweet lupine, several diseases on potato), leading to 3 main considerations. Firstly, disease resistant cultivars should not be perceived as a one-size-fits-all solution taking precedence over other defence mechanisms and resilience factors in cropping systems. They can be considered along with crop diversity, local adaptation, balanced plant-microbe interactions, plant communication and defence strategies in a comprehensive approach to plant and seed health management. Secondly, resistance breeding should be integrated into fair social and economic models. In this perspective, finding solutions for sharing intellectual property rights and benefits is a prerequisite for the participation of all partners, especially if engaging in participatory plant breeding. Thirdly, organic resistance breeding can be reasoned in the framework of organic principles, such as the principles of "Health", "Ecology", "Fairness" and "Care" formulated by IFOAM-OI (IFOAM Organics International. 2005). In particular, this applies to techniques used to introduce resistance genes into plant cultivars (e.g., respecting the integrity of a plant at genome and cell level). In summary, resistance breeding is a powerful tool to strengthen plant health in organic systems, including seed health, but it cannot be considered as a stand-alone solution to ensure plant health. The breeding and use of resistant cultivars should be considered in relation to several other aspects of sustainable and more diverse cropping systems, including its complementarity with other plant health approaches, the social and economic models in which it is embedded and the principles and values behind organic agriculture.

2.7. Legal requirements for seed health in Europe

In the EU, seed health is subject to two main pieces of legislation, one concerned with plant health in general and another concerned with the marketing of seeds and vegetative material regulated through 12 Seed Directives.

The Plant Health regulation (EU 2016-2031) aims at preventing organisms harmful to plants to enter or spread throughout the EU. This regulation groups all organisms harmful to plants under the term "pest", whether they are bacteria, fungi, viruses, insects or parasitic plants, and distinguishes between two main types of pests: quarantine pests and regulated non-quarantine pests (RNPQ). Quarantine pests are pests that could cause substantial economic, social or environmental damage and are not known to be present in the EU. The objective is therefore to prevent these diseases and pests to enter European grounds and destroy any plants that are infected with such a given pest. Outbreaks of *Xylella fastidiosa* and the subsequent massive destruction of olive groves in Southern Europe is a particularly poignant example of this. Among the quarantine pests, some are considered "priority pests", subjected to even more stringent requirements and control. Others are "protected zone quarantine pests", which means that certain geographic areas within the EU (often islands) should be preserved, although the respective pest is already present in other geographic areas. RNPQ are pests that are present in the EU and can cause substantial economic damage, which justifies a joint European effort to control them. While measures for quarantine pests concern any plant material or product, only seeds and other planting material are concerned with RNPQ. For example, tomato seeds must be free of *Clavibacter michiganensis* spp. *michiganensis* (causing bacterial canker) and potato viruses must be below a given threshold on seed potatoes. Regulated pests and concerned crops are listed in an implementing act (EU 2019/2072) and this list can evolve over time.

In order to control the harmful organisms listed in the Plant Health regulation, people and organisations producing seeds (as well as plants in general) are subject to requirements. This implies that entities producing and distributing seeds must register as "professional operator" at their competent national authority, except if they are exclusively producing seed for non-commercial use

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without distance-contracting (in which seeds are shipped rather than transferred directly). The registered entities can then be authorised to issue Plant Passports for the movement of seeds within the EU. This Passport is an official label asserting that the necessary measures to meet the standard for quarantine pests (i.e., absence of the pests) and RNQP (i.e., thresholds) have been implemented. Imports of seed from and exports to third countries require specific documents issued by competent national authorities, called “phytosanitary certificates”.

Since the Plant Health Regulation (EU) 2016/2031 entered into force in December 2019, both a broader range of seed producers are subjected to the Plant Health regulation (i.e., not only commercial seed companies), and a broader range of crop species (especially vegetables) require a Plant Passport, as compared to the former directive (2000/29/EC). An additional administrative burden may affect small-scale producers of vegetable seeds in particular. Thereby, it may constitute an additional obstacle for crop diversity, especially in Member States were the interpretation and implementation of the requirements are very stringent. An impact assessment of the current regulation planned by the European Commission may elucidate this in future.

EU directives for the marketing of seeds and planting material aim at guaranteeing the quality of seeds and planting material for those who buy and cultivate them. Although plant health is not their main concern, these directives also list some requirements for seed health among their minimum conditions for seed quality. This is particularly relevant for certified seeds, which concerns mainly arable crops (but not exclusively). For example, Directive 2002/56/EC concerning seed potatoes, requires that "on official inspection of the growing plants the number affected by blackleg must not exceed 4 %”. Requirements can be quite vague, as for example in the Directive on the marketing of cereal seed (66/402/EEC): “Diseases that reduce the usefulness of the seed shall be of the lowest possible level”. These requirements for seed marketing can be complemented by more stringent national requirements, as is shown in subsection 4.1.2.1 with the example of the diverse common bunt regulations in EU Member States regarding wheat seeds.

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3. Inventory of seed health and quality issues

3.1 Inventory

As we have elaborated above, organic seed production is a challenging process and has specificities that are important to consider for high-quality seed production while respecting the principles and rules of organic production.

An inventory was produced as part of the LIVESEED project, in order to list the current issues faced by professionals producing organic seeds in Europe. The objective was not to be comprehensive, but rather to identify seed health issues that are currently of greatest concern for organic stakeholders (researchers, technical institutes, breeders, etc...), and how they deal with them. The inventory is based on an inquiry – consisting of both targeted interviews and more informal discussions - with LIVESEED partners and third parties during the LIVESEED project.

The inquiry was conducted by WUR (NL) and ITAB (FR), WUR focussing on vegetables and ITAB on arable crops. Participants in the 1st LIVESEED Annual Meeting (Valencia, April 2018) were asked to state according to their own experience, the main current seed health problematics. This produced the following inventory, listing current 34 challenging issues in the domains of arable, vegetable and forage crops (Table 1, next page). In addition, questions on seed health were included in a survey targeting farmers in 2018 and another targeting seed suppliers in 2018/19, to obtain a broader perspective.

For cereals and forage crops, several insect storage pests are mentioned. Challenges due to seed-borne bacterial diseases are recurrent in vegetable crops, as well as for potato. As a next step, it would be worthwhile to conduct a desk study to identify already existing solutions to disseminate. Then, prioritizing among remaining issues would lay the floor for future research and development project.

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Table 1. Inventory of current issues for the quality and health of organic seeds, based on inquiries among stakeholders between 2017 and 2020

Crop		Scientific name	Problem	transmitted through seed (or tubers)
Arable Crops	Beetroot	*Beta vulgaris*	Damage by beetroot weevil (*Lixus juncii*) to plants during seed production	no
	Barley Wheat and related Oat	*Hordeum vulgare* *Triticum* spp. *Avena sativa*	Loose smut (*Ustilago* spp.)	yes
	Cereals	*Hordeum vulgare* *Triticum spp.* *Avena sativa* *Secale cereale* …	Reduced seed vigour due to Fusarium Head Blight (*Fusarium* spp.)	some
	Cereals	*Hordeum vulgare* *Triticum spp.* *Avena sativa* *Secale cereale* …	Cereal weevils (*Sitophilus granaries*)	yes (eggs, larvae, pupae in seeds)
	Wheat and related	*Triticum* spp.	Common bunt / stinking smut (*Tilletia caries* and *T. foetida)*	yes
	Maize	*Zea mays*	Fusarium (*Giberella zeae / Fusarium graminearum*), plant disease and resulting mycotoxins	some
	Maize	*Zea mays*	Corn smut (*Ustilago maydis*)	no
	Maize	*Zea mays*	Grain moth (*Sitotroga cerealella*)	yes (larvae in seeds)
	Maize	*Zea mays*	Weevil (*Sitophilus zeamais*)	yes (eggs, larvae, pupae in seeds)
	Potato	*Solanum tuberosum*	Viral diseases (can be propagated by aphids). Mainly PVY, also PVX PNRD, PVS and PVA	yes
	Potato	*Solanum tuberosum*	Late blight (*Phytophthora infestans*)	no
	Potato	*Solanum tuberosum*	Black scurf / stem canker (Rhizoctonia solani)	yes
	Potato	*Solanum tuberosum*	Black leg disease (*Pectobacterium* and *Dickeya* from the Enterobacteriaceae family)	yes
Grain Legumes	Faba bean	*Vicia faba*	Faba bean weevil (*Bruchus rufimanus*)	yes (larvae in seeds)
	Pea	*Pisum sativum*	Pea weevil (*Bruchus pisorum*)	yes (larvae in seeds)
	White lupine (other grain legumes)	*Lupinus* spp.	Anthracnose (*Colletotrichum lupini*)	yes
Forage crops	Alfalfa	*Medicago sativa*	Alfalfa weevil (*Tychius* spp.)	yes
	Clover (red)	*Trifolium* spp.	Seed weevils (*Apion* spp.)	no
	Clover (red)	*Trifolium* spp.	Contamination with seed of Common broomrape (*Orobanche minor*), a parasitic plant	no (but in seed lots)
	Clover (red)	*Trifolium* spp.	Southern anthracnose (*Colletotrichum* spp.)	yes
Vegetable crops and herbs	Basil	*Ocimum basilicum*	Downy mildew (*Peronospora belbahrii*)	yes
	Beans	*Phaseolus vulgaris*	Blights : *Xanthomonas* and *Pseudomonas*	yes
	Beet crops	*Beta spp.*	*Cercospora beticola*	yes
	Brassica (Radish in particular)	*Brassicaceae (Raphanus sativus)*	Seed-borne bacteria: Black rot *Xanthomonas campestris* pv. *campestris, Pseudomonas syryngae* pv. *maculicola*	yes
	Carrot	*Daucus carota*	several diseases as *Xanthomonas campestris* and *Alternaria* spp.	some
	Corn salad	*Valerianella locusta*	downy mildew (*Peronospora valerianellae), Phoma* and *Acidovorax*	yes
	Cress (garden cress)	*Lepidium sativum*	Diverse diseases, as *Alternaria brassicae, Colletotrichum higginsianum, Xanthomonas campestris* and *Phoma lingam*	yes
	Fennel	*Foeniculum vulgare*	Bugs and white mold (*Sclerotinia sclerotiorum*)	yes
	Onion	*Allium cepa*	Loss of mother plants during the two-year production cycle	no
	Oregano	*Origanum majorana*	Parasitic plant (*Cuscuta*) seeds can contaminate crop seeds	yes
	Parsley	*Petroselinum crispum*	*Alternaria* spp.	some
	Parsnip	*Pastinaca sativa*	*Alternaria* spp.	some
	Radish	*Raphanus raphanistrum*	several diseases as blackleg (*Phoma lingam*), white rust (*Albugo candida*), *Alternaria* spp., *Pectobacterium carotovorum,* black rot (*Xanthomonas campestris* pv. *campestris*), *Pseudomonas syryngae* pv. *maculicola*	some
	Spinach	*Spinacia oleracea*	Damping off (*Pythium* spp. or *Rhizoctonia solani*)	no

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3.2 Highlights from the inquiry into issues with seed production

Some general problematics or questionings have been raised during the inquiry, relevant across crops and crop categories. To complete these exchanges, a specific inquiry was conducted in 2020 on arable crops to further detail the inventory.

- First, several actors were concerned about the impact of seed treatments such as vinegar or thermotherapy on seed vigour and seed microbiome. Indeed, as mentioned in the introduction, the seed microbiome biodiversity is a key element of seed health and quality. Seed treatments are effective to protect plant health when a pathogen is detected but, if used systematically, they may suppress seed microbiota and perhaps also endanger the germination rate of the seeds. This is why some actors prefer treating the seeds only if a contamination risk has been identified. These questionings from the stakeholders confirm the need for further research into the role of seed microbiota and seed vigour on seedling health.
- Concerning the interest of seed coatings with biologicals, opinions were mixed among the actors interviewed. Some said that it was an interesting field, and that further research was needed, because seed coating could compensate poor microbiota, both on seeds and in soils. But some other actors mentioned that they were not interested in using such products. Indeed, it was mentioned that those techniques should not replace good agronomical practices which favour a highly diverse microbiota, whereas seed coatings cannot provide such diversity. Moreover, there was a concern that adding ex-situ microorganisms could destabilise the seeds and soil microbiota. Studies are ongoing on the microbiome of different pea cultivars (FiBL-CH) and different maize populations (IPC) to understand the impact of the plant genotype and location on the microbiome.
- Close to the previous comments, actors also mentioned that further work was needed on new seed treatments such as seed coating with biologicals, or essential oils. Indeed, their effectiveness has been shown. However, few practical and large-scale protocols have been developed so that they can be used by farmers and seed companies on large seed batches, and with regular results.
- Remarks were also raised that concern European legislation on seed quality and health. In particular, it was mentioned that pathogen detection thresholds can be different among European countries, which complicates the seed exchanges between countries. An example of this is given below, in part 4.1.2.1 on common bunt.

Issues with two fungal diseases of cereals were described in detail, in particular:

- *Fusarium* is a common and well-known cereal pathogen. But when a seed lot is infected, even with a low incidence on seed appearance (few modifications of seed colour or shape), the pathogen can influence seed vigour, and as a consequence the seed germination rate in the field. The problem is that, when testing only the germination rate under optimal conditions in the laboratory, this seed vigour loss is not always detectible. Indeed, the germination rate can be much lower in the field than in the laboratory germination tests, due to more stressful conditions in the field. This subject will be further described and analysed in part 4.3.1 of this deliverable (case study on carrot).
- In organic as well as in conventional conditions, loose smut on cereals is starting to reappear very occasionally, mainly on barley and oats. This seed transmitted pathogen needs to be studied because no detection techniques exist, nor any organic treatments to control it. One bottleneck to the study of this disease is that it is hard to find infected seed lots to work on. The damage potential of this pathogen can be considerable, but its propagation seems slow. Loose smut also concerns maize production. In this case, farmers manage the risk through mass selection when cultivating populations. The infected spikes are eliminated upon seed production and, according to empirical observations, the pathogen's infection rate decreases over the years. Practitioners have observed that the infection particularly occurs when populations are exchanged and are sown in a new terroir, but it decreases after a few years. Similarly, loose smut has been observed

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on wheat in French farmers' seed networks but is not considered a major problem there because propagation has been observed to be low in diversified wheat populations or mixtures. On the contrary, loose smut on wheat is currently considered a major concern to the RSR network in Italy, due to a lack of detection methods.

Also, with forage, vegetable and herb seed production the major issues are with diseases during seed production and seed borne pathogens. For seed production with corn salad (*Valerianella locusta*), also known under the name lamb's lettuce, mâche, and field salad, crop failure by fungal and bacterial diseases are such a problem that one of the largest organic seed suppliers stopped with the supply of organic seeds for this crop, although there is a strong market for organic corn salad. Therefore, solutions are needed. Onion and carrot seed production takes two years, as they flower after vernalisation only. For both biennial crops there can be considerable losses of parental plants during winter, especially with inbred lines used for hybrid seed production.

For oregano seed production challenges with the presence of parasitic dodder plant (*Cuscuta* spp.) has been mentioned. Besides reducing yield, the dodder seeds may also contaminate the produced oregano seeds. Although not mentioned during the inventory, dodder is known to be a problem for other crops as well.

The inventory indicated issues that are felt to be particularly challenging for seed producing farmers and seed companies. Some of these challenges may be addressed by better dissemination of already available solutions, as shown for the case of bunt in the following section, while other problems need future research to find acceptable solutions.

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4. Preliminary data from seed health case studies

4.1. Managing common bunt in wheat

Common bunt, caused by the fungi *Tilletia caries* and *T. foetida*, is a disease of wheat and related cereals. Starting from just a few spores on the seed, the disease can develop in the crop and considerably reduce grain yield and especially quality. The disease is mainly seed-borne, although it can also persist in soils. Techniques that allow the management of common bunt in organic farming - including sound crop management, observation, seed analyses and seed treatments – are well identified. However, if these are not put into practice, occurrences of common bunt still regularly devastate organic wheat crops.

An inquiry into common bunt management was performed over 4 years in the LIVESEED project, putting emphasis on the exchange of knowledge between European countries and across disciplinary boundaries. It followed three main objectives (Figure 1): Firstly, collecting techniques already available for bunt management and developing appropriate formats to disseminate them (state of play). Secondly, exploring needs and strategies related to bunt management in a variety of contexts. Thirdly, reflecting on coherent seed and plant health approaches, regarding common bunt in particular, and organic systems, in general.

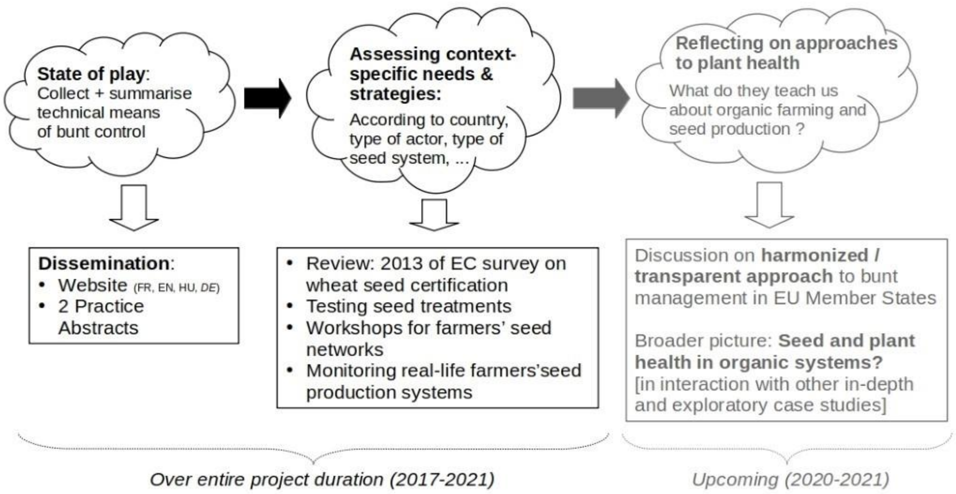

Figure 1. Overview of the objectives and R&D activities on the management of common bunt.

Meetings and workshops among researchers and practitioners facilitated both the exchange of knowledge on existing techniques for bunt management and the emergence of unanswered questions. As described below, the topic was further studied through field trials to test and fine-tune seed treatments, as well as workshops and qualitative interviews to collect empirical experience. Action-research with farmer-breeders based on real-life problematic issues in France (in partnership with the association *Réseau Semences Paysannes*) allowed to take into account farm saved seeds, which pose specific constraints.

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4.1.1 State of Play: Disseminating the knowledge and techniques currently available

The management of common bunt in organic systems requires knowledge of the symptoms to carefully observe wheat crops, knowledge of the disease cycle to integrate preventive measures in the crop management and know-how to apply preventive treatments properly. For example, vinegar treatments are effective to control bunt at low infection levels but may lose effectivity or harm germination capacity of the seed if not applied correctly. Therefore, several forms of communication were developed to disseminate the relevant knowledge and techniques among seed producers.

Website on bunt management: Based on the finding that no or few comprehensive, practice-oriented resources were available on the management of common bunt in organic systems, a website was first developed in French. It is now available in English and Hungarian and will soon be in German to provide actors of organic seed production – farmers, consultants, seed companies – with relevant information. By giving a comprehensive overview of the topic, the website also confers an understanding that organic bunt management cannot rely on seed treatments alone, but that it is based on sound management practices including diversified crop rotations.

Several Practice Abstracts and a video are published or planned to share practical take-home messages and practices on the following topics related to common bunt.

- Managing common bunt in wheat seed lots (Borgen et al., 2019; also available in French) – (Annex 7.1. and a 28 min[1])
- Proper seed storage (Annex 7.3)
- Seed vigour, keep it high (Annex 7.4)
- Seed health in potatoes (Annex 7.5)
- Treating wheat seed with vinegar against common bunt (2020) – (Annex 7.6)
- Treating wheat seed with mustard powder against common bunt (coming up in 2021)

In France, ITAB offered 1h-webinars and more in-depth workshops on bunt management. Taken all together, the described communication tools -website, Practice Abstract, webinars and workshops – form a full-blown communication strategy to share available knowledge on bunt management. Provided with a comprehensive understanding of the issue, users are empowered to make their own decisions and while empowering users to gain autonomy in seed and plant health management.

4.1.2 Assessing context-specific needs and strategies

4.1.2.1 Bunt regulations for certified wheat seeds in EU member states

A survey on standards for common bunt carried out in autumn 2013 upon request by the European Commission's Standing Committee Plant Reproductive Material was summarised and presented at the first annual project meeting in 2018 (Weinhappel, 2018). The survey focused on seed destined for organic farming (which, at the time, could be organic or non-treated conventional seed). This serves as a basis to compare *Tilletia* standards for the regulation of common bunt on certified wheat seed in different Member States.

In the framework of seed certification, two types of standards exist to control for and quantify the presence of bunt. Firstly, standards for field inspection indicate thresholds for the number of plants with bunt symptoms in seed crops. Secondly, standards for seeds indicate thresholds for the number

[1] https://www.youtube.com/watch?v=yAHgVa0I7tU

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of bunt spores on wheat seeds, determined by accredited seed testing methods. The proportion of Member States applying these requirements, respectively, are shown in the Figure 2.

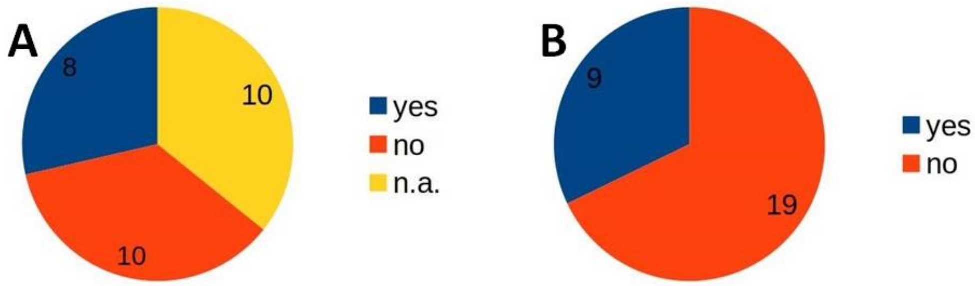

Figure 2. A. Number of EU Member States with bunt requirements for field inspection of wheat seed crops in 2013. (n.a. = no answer). B. Number of EU Member States with bunt requirements for seed testing of wheat seed crops in 2013.

Among them, Austria and Finland apply standards both in field inspection and for seed testing. Denmark, Germany and UK don't have legal requirements for seed testing in the framework of seed certification, but agreements in the private sector or recommended limits. Twelve member states do not have any requirements or agreements concerning common bunt for wheat seed production. Regarding seed testing requirements, thresholds can be implemented for non-treated seeds and / or for seeds treated against common bunt. In Table 2, five countries are taken as examples to show that a large diversity of requirements and thresholds prevails among member states.

Table 2. Requirements for seed testing and field inspection in five EU Member States. According to the country, spore thresholds for seed analyses can be expressed in number of spores per gram seed or number of spores per kernel. All thresholds were converted to be expressed in spores / gram seed.

Country	Non-treated seeds	Treated seeds	Field inspection
France	0 sp./g	/	/
Denmark	10 sp./ g *	/	/
Sweden	22 sp./g	1000 sp./g	/
Austria	200 sp./g	6.000 sp./g	/
Germany	400 sp./g **		5 plants / 150m2

*Seed analysis is a pre-requisite to be listed on database of organic seed
**Agreement in the private sector

Among these examples, France and Denmark have very stringent thresholds for untreated seeds, but none for treated seeds. Sweden and Austria have thresholds both for untreated and treated seeds, although Austrian thresholds are higher than Swedish ones. According to a LIVESEED project partner in Austria, AGES, there are ongoing debates at national level whether the current threshold for treated seeds is appropriate for seeds treated with products allowed in organic farming. As a last example, Germany only has an official threshold for field inspection, but actors of organic cereal seed production have found a private agreement to limit the number of spores in seeds.

This diversity of bunt requirements in seed certification gives rise to several questions. On the one hand, regarding untreated seed, the stringent regulations as implemented in France or Denmark

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practically force seed companies to treat their seeds as soon as bunt spores are detected even at very low levels. Treating seeds implies additional labour, and potentially also a loss in storability if part of a treated seed batch is not sold the first year. In comparison, countries such as Austria implement a higher threshold. Engaging in discussions with official authorities across countries to compare experiences with different thresholds on untreated seeds could maybe facilitate the identification of thresholds that would reliably protect users against bunt, while not placing unnecessary burden on seed producers.

On the other hand, countries such as France and Denmark do not have thresholds for treated seeds. However, such thresholds would be necessary as several experiments have shown that treatments with vinegar or copper-based products are much less reliable when treating highly contaminated seed, although they can be effective at medium infection levels (Borgen, 2001; ITAB, 2012). LIVESEED has established a recommended threshold of 1000 spores / gram (Borgen et al., 2019) for treated seeds, but this threshold is based on theoretical calculations aiming at minimizing risks, not on extensive experimentation. In cases where cereal seeds are sold and shipped across the EU, a Member State may import wheat seeds from another Member State with more or less stringent bunt requirements than its own national requirements. In such a situation, it seems necessary to implement increased transparency on bunt thresholds when ex- and importing wheat seeds between EU Member States.

4.1.2.2 Common bunt management in farmers' seed systems: Specific questions and approaches

Farmers' seed networks (Coomes et al., 2015) rely on seeds produced by farmers themselves, sometimes using population varieties selected on farm, to obtain locally adapted wheat populations. In these seed systems it is not always possible to fall back on commercially certified seeds to renew seed lots infected with bunt. Farmers' seed systems thus pose particular challenges in terms of bunt management. At the same time, they provide the opportunity to elucidate how common bunt can be kept at acceptable levels in seed systems that do not have to comply with the bunt thresholds set for seed certification in certain countries.

Workshops

Specific workshops were designed by ITAB in the dual objective of facilitating (i) the exchange of experiences and knowledge on bunt management among farmers producing and selecting their own cereal seeds and (ii) the emergence of research questions from the field.

The workshop consists of three parts. First comes an ice-breaker activity based on the auto-evaluation of knowledge on bunt by participants (Figure 3).

Figure 3. Ice-breaker at a workshop on bunt management in the Rhone-Alpes region, France: "From 0 to 100, how comfortable are you with bunt management?" (ITAB)

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Secondly, knowledge and experience on the topic is shared by participants and recorded in a mind-map including questions or ideas for future research (Figure 4). Thirdly, the facilitator comes back to the information that has been shared to complete or correct it.

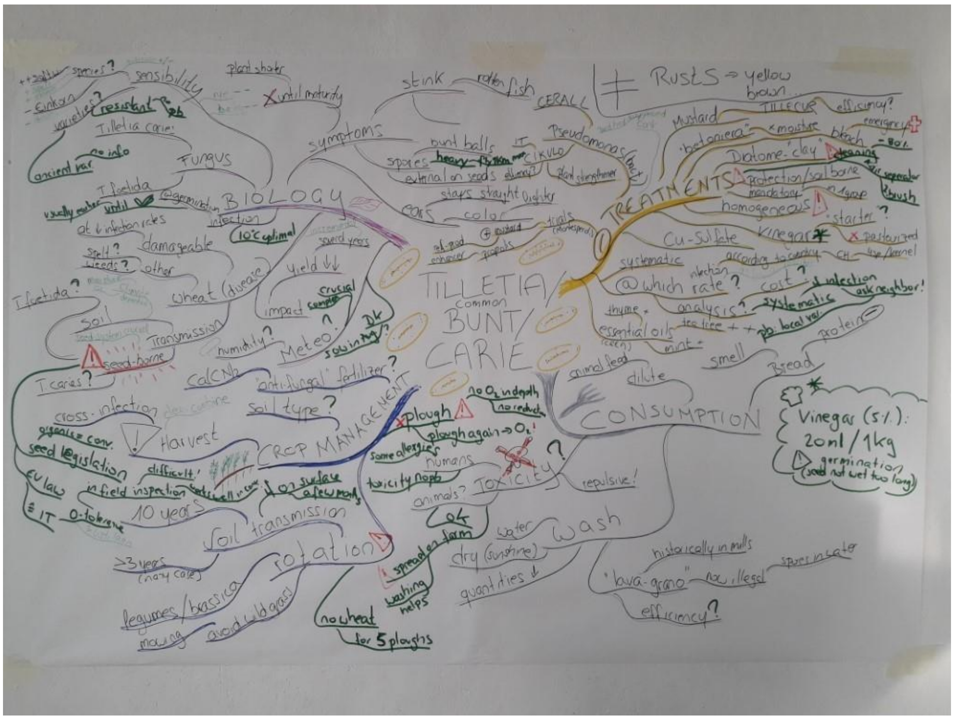

Figure 4. Mind-map constructed at a bunt workshop at the LIVESEED Cross Visit in Italy, June 2019 (ITAB)

Three such workshops were held in crop diversity associations in France and one at the LIVESEED Cross Visit in Italy. Participant numbers ranged from 12 to approximately 50. Experience has shown that at least 1h30 should be given for an in-depth and exhaustive exploration of bunt management.

According to participant feedback, the workshops offered valuable learning opportunities. They are an effective way both to disseminate knowledge on bunt management and to allow for practitioners' experience and questions to feed into the research and development conducted on common bunt at ITAB. Questions concerning the role of seeds and, especially, soil microbiota for reducing the risk of bunt were recurring.

Inquiry into and monitoring common bunt of on-farm bunt management

A qualitative inquiry through both targeted interviews and informal exchange with practitioners in France allowed to identify farms that have relevant experience with bunt management, original approaches to plant health based on ecosystem interactions and /or where bunt has occurred significantly in the past. With ten seed lots on six of these farms a monitoring of bunt infection levels and systems over several years has been started in 2020, with two objectives. Firstly, to take stock of the bunt situation on French farms. Although the farms included in the study are not representative - not in numbers, nor in type of management - this study shall quantify to what extent bunt may be present in farmers' seeds and to what extent symptoms appear. Secondly, on the longer run, the objective is to identify practices and environments that reduce the risk of developing common bunt. The latter objective will only be pursued if further seed lots are added to the study and the study is carried on for several years, beyond the LIVESEED project.

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The rationale and methodology for this study are based on the experiences and observations gained throughout the LIVESEED project. For instance, seed treatment tests described in part 4.1.2.3 (below) have led to the conclusion that it is necessary to test seed treatments at low to intermediate infection levels, as that would be closer to "real-life" conditions. However, this requires larger plots for testing. By monitoring for bunt, over several years and numerous farms, while recording seed treatment practices, enough observations may be collected to get a closer idea about thresholds at which different treatments remain effective. Also, a question that has frequently emerged from the field – in the workshops described above or in more informal interactions with practitioners – is that of the role of seed and soil microbiota for the suppression of common bunt. Hopefully, this study will identify environments and farms where bunt is being suppressed and where a follow-up study could look into the microbiota and their putative interaction with bunt.

In terms of methods, farmers' seed lots are monitored by yearly seed analysis for infection levels with common bunt. The fields sown with those seeds are evaluated for bunted ears, by observing 1 m^2 on 100 random spots all over the field. The total number of bunted ears observed on 100 m^2 is then noted, according to the method often used in field inspection for seed certification. In parallel, the practices (seed treatments, seed cleaning, sowing date and depth, type of machinery used, etc.) of participating farmers are recorded.

This approach aimed at getting an in-depth understanding of bunt dynamics and management in organic systems, in particular organic farmers' seeds, rather than under controlled conditions, is one outcome of the LIVESEED project. Indeed, under the hypothesis that plant and seed health in organic systems is rooted in ecosystem interactions, as put forward by IFOAM Organics International (2005) in its "Organic Principles" elucidating health in those ecosystems seems to be a worthwhile endeavour.

4.1.2.3 Testing and fine-tuning seed treatments

Seed treatments are an important element in bunt management, as seeds are the main vector for the disease. As mentioned above, the seed treatments authorized for use in organic agriculture should be considered with precausion as curative measures, as they have been shown to be less reliable at high contamination rates. The authorised seed treatments include two basic substances (Marchand, 2015): white vinegar (EU, 2019) and mustard seed powder (EC DG SANTE, 2017), as well as the product CERALL®, a biological treatment based on a strain of the bacteria *Pseudomonas chlororaphis*. In some countries, copper-based treatments are still authorised, such as the product COPSEED® in France, but might be forbidden in the near future. To optimize seed treatment applications, both with already authorised and with novel substances, four trials were conducted, as follows.

(i) Investigating the spectrum efficiency of the organic seed treatments CERALL® and white vinegar concerning different infection levels of common bunt on winter wheat

These trials led by AGES in collaboration with Agrologica (Denmark), NARDI-FUNDULEA (Romania) and ÖMKI (Hungary) aimed at testing the effectiveness of vinegar and CERALL treatments at bunt infection levels ranging from 200 to 10.000 spores/g, including a treatment with CERALL following improper storage at too high temperature. Trials based on artificially inoculated seed were sown in 4 locations in 2019. However, the numbers of diseased ears in the field, were too low, even in untreated control plots, to conclude on the effectiveness of the tested treatments (Figure 5). In future, general effectiveness of seed treatments should be tested on highly infected seed lots to obtain reliable results. Thresholds of effectiveness on seed can be tested on low infection levels, but on larger plots (e.g., larger sample size).

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Figure 5. Austrian trial on efficiency of wheat seed treatment with vinegar and CERALL compared to untreated control plots on July 3rd, 2020 (AGES)

(ii) Testing for phytotoxic effect of vinegar on seed germination

The effect of vinegar treatments depends on the concentration of acetic acid, the dose applied and the duration of the treatment. Past research has shown that a dose of 20 ml vinegar per 1 kg of seeds is enough to cover all kernel surfaces without excess (Borgen and Nielsen, 2001; Saidi et al., 2001). The use of white vinegar as seed treatment is limited by law to 10% acetic acid (EU, 2019). However, even within these ranges of doses and concentrations, a reduction of seed germination rates has been observed in practice. Therefore, Ubios (France) investigated the effect of vinegar treatments at different concentrations on seed germination rates. Different vinegar treatments were applied to 20 wheat seed lots with different acetic acid concentrations: no treatment as control, vinegar 4%, 8% and 10%. Germination tests were conducted in sand in climate chambers according to the ISTA protocol. Resulting germination rates decreased progressively with increasing vinegar concentrations from 93% for the untreated to 82% for the highest concentration of acetic acid (10%). Only the germination rate of the lot treated with the highest concentration differed significantly from the untreated control (Figure 6, next page); indicating that vinegar concentrations above 8% may have a phytotoxic effect and impact germination rates, although this probably also depends on how fast the seed is re-dried after treatment. In addition to reducing germination rates, vinegar treatments may also decrease seed vigour, i.e., the speed at which seeds emerge and shelf-life. It would be worthwhile for future research to elucidate the effect of vinegar treatments on wheat seed vigour.

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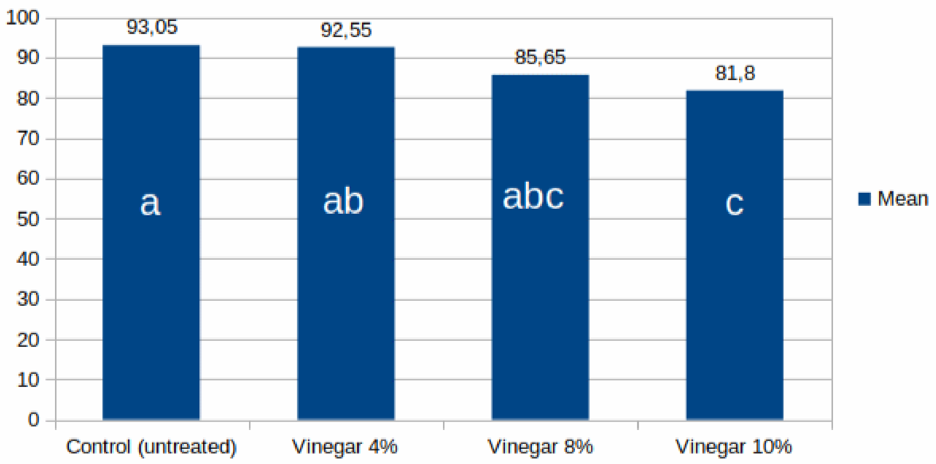

Figure 6. Mean germination rates of wheat according to vinegar treatment concentrations

(iii) Testing farmers' seed treatments with a plant strengthening approach

As emerged in workshops (see part 4.1.2.2), interviews and informal discussions in France, some practitioners aim at moving away from a disinfection approach (i.e., with vinegar) and want to develop farm-based treatments that would strengthen seeds, plants and their microbiota (see Chapter 2.3). To test and compare seed treatments which had been proposed by farmers, ITAB conducted a centralised seed treatment trial in 2018. On wheat seeds, artificially infected with bunt spores (63.800 spores/g), the following treatments were tested: milk whey, sourdough, "Farmer's pro-biotic mix" (mix of 3 pro-biotic ingredients), ground mustard bran, cider vinegar, white vinegar, and copper-sulphate as control (Figure 7). White vinegar and copper sulphate treatments had similar effectiveness of 95% and 96%, respectively.

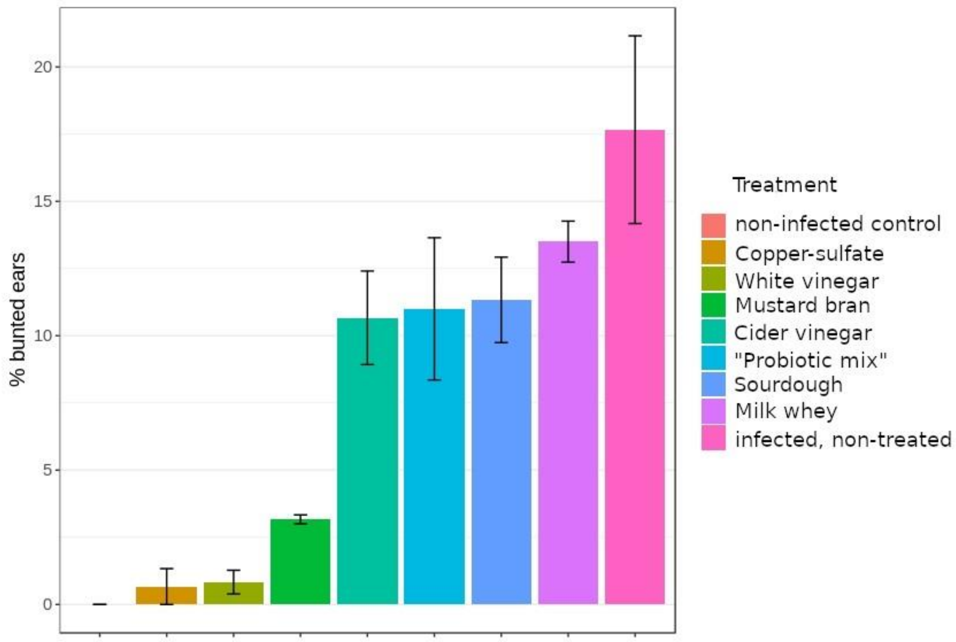

Figure 7. Proportion of wheat ears with bunt observed of artificially infected seed and different farmers' treatments compared to non-treated control (right bar).

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The substances tested in the aim of strengthening plants (milk whey, sourdough, "Farmer's pro-biotic mix", cider vinegar) had an effectiveness ranging from 36 % to 40%, insufficient or not significantly different from the non-treated control. With view to the results obtained, a reflection on the methodology to test these kinds of treatments has been initiated. It may be that these types of seed treatments are more adequately tested at lower infection levels – in other words, these supposed "plant strengtheners" may be efficient against common bunt with low thresholds, when combined with other bunt management practices.

(iv) Quantifying the effect of seed washing on seed contamination rates

Washing has been reported to be an effective means to clean seeds from bunt spores, according to empirical observations (Fontaine et al., 2007), but to our knowledge the effect has never been quantified. In collaboration with the association "Initiatives Paysannes" in the North of France, ITAB therefore aimed to quantify the reduction of infection levels that can be obtained by washing seeds in water. Several seed lots that had naturally been infected with bunt were washed and sent to an accredited laboratory for quantification of bunt spores according to the ISTA working sheet no. 53. Initial infection levels ranged from 1.900 spores to 310.000 spores/g of seed (Table 3). Reduction of infection levels by washing ranged from 54% to 98%, according to seed lot. The reduction obtained may depend on how well the seed had been previously cleaned, for example in an airstream cleaner, to remove bunt balls. Results indicate that seed washing is a simple technique to effectively reduce bunt infection levels, at least in very small seed lots, provided that the seed is quickly dried afterwards (or sown directly). However, this measure should be combined with other treatment when sanitising seed lots. In particular, residual infection levels should be verified by seed analyses to make sure that the seed treatment was effective to reduce spores below threshold.

Table 3. Infection levels with bunt before and after washing of 6 wheat seed lots naturally infected with common bunt. Infection levels after washing in red are still above the recommended threshold for effective seed treatments in organic agriculture.

Seed lot denomination	Infection level (spores / gram seeds)		Reduction
	before washing	after washing	
RedonGuer332	1900	400	79%
Blanc de Flandres	2800	54	98%
Goldritter	9430	560	94%
Goldendrop	30020	484	98%
Prov Normandie	140000	65000	54%
Prov Pommeraye	310000	10000	97%
MEAN			87%

To overcome the need for post treatment drying, tests were performed by Agrologica (Denmark) to "clean" seed by friction in fine sieved dry dirt. The trial shows that it is indeed possible to remove spores in this way without affecting the seed vitality, but a huge amount of spore-free dirt is needed to dilute the spore concentration on the seed surface to an insignificant level, and based on this, the method is not considered practical or economically competitive as compared to other treatments.

Other methods like warm or hot water treatment have also been tested but have not been implemented at farmers' level due to insufficient efficiency and difficult handling to redry the seed, whereas Tillecur® was very effective (Waldow and Jahn, 2007). However, the registration of Tillecur® a plant strengthening agent based on the flour of white mustard on has recently been withdrawn and

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therefore are no longer available for farmers. Thus, further research is needed to develop recipes based on mustard powder that can be produced by farmers.

4.1.3 Conclusions on the management of common bunt and wheat health

The management of common bunt in wheat in organic systems studied in the scope of LIVESEED is an illustrative example showing that seed sanitation is a very important element in ensuring plant health as far as seedborne diseases are concerned, but nevertheless only one element among others. Although seed treatments are available to reduce the risk of bunt (to a certain extent) and are being developed further, they cannot be considered as a stand-alone solution and do not waive from more systemic preventive approaches (diversified crop rotations, observation, thorough seed cleaning etc.) and selection of resistant lines and populations (LIVESEED WP3.4.4). As with the case study on carrot and *Alternaria* described hereafter, managing this seedborne plant disease in organic systems requires not only investigating seed health and sanitation per se, but also extending the view to the system in which the seed is embedded. That requires in-depth knowledge of pathogenic organisms and their biological interactions, as well as adapting strategies to different contexts and needs.

The LIVESEED project has elucidated the bunt issue from several angles, from testing and fine-tuning seed treatments, over inquiring into the wide range of bunt legal requirements concerning bunt and developing appropriate tools (e.g., factsheets, videos, website) for practitioners to disseminate existing knowledge, to exploring holistic, practice-based approaches on farms. In continuation of this work, research priorities would be:

- (i) to better understand, combine and technically fine-tune already known **seed treatments** to decrease side effects on seed physiology and develop guidelines for best practice (applied research and development projects) for farmers. Concerning vinegar treatments, in particular, how does seed disinfection affect seed vigour and the seed microbiome? Are there circumstances under which farm-produced, plant-strengthening substances are effective as preventive treatment, like white mustard powder?
- (ii) to further elucidate **biological interactions** affecting bunt at field level. For instance, do certain previous crops reduce the risk? Do soil microbiota affect the speed at which bunt spores are eliminated in soils? How do interactions between seed treatments and resistance breeding affect bunt development over time? How is the distribution and interaction of common bunt with draft bunt in farmers' field? How fast can races of common bunt evolving to overcome dominant resistance genes?
- (iii) To develop a deeper understanding of the **interactions between bunt and wheat plants**. Beyond monogenic resistance, are there other plant defence mechanisms that come into play (field resistance, tolerance, seed microbiome)? How strongly does seed vigour affect bunt infection under field conditions (the *Alternaria* / carrot case, described below, provides relevant methods for this)?
- (iv) to continue **disseminating available knowledge** to farmers and seed industry, especially in countries with little focus on bunt (demonstration projects).

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4.2. Viruses on seed potato

4.2.1. Introduction

Several potato viruses – known under the abbreviation Potato Leafroll Virus (PLRV), Potato Virus X (PVX) and Potato Virus Y (PVY), to name the 3 most important - are problematic for seed potato production, because they are transmitted by tubers. Secondary infection happens mainly via aphids which can be reduced by insect secure net. Among potato viruses, PVY is the most widespread. In many countries, the production of certified seed potatoes is subjected to very stringent rules to ensure the absence of viruses; some countries even prohibit sowing seed potatoes that are not certified (e.g., Luxembourg, Peru). France, in particular, has a stringent system with low thresholds for viral infection. As is the case in many countries, the French official seed potato production scheme relies on serological testing and sanitising seed potato stocks (every eight to ten generations) through *in vitro* meristem culture.

Although this seed production scheme has proven to effectively control viral diseases, it makes potato seed production under organic conditions during the full process difficult or impossible. Indeed, neither the meristem culture in itself nor the regeneration of plants over several generations can be conducted using only inputs authorised in organic agriculture. For several years « Payzons Ferme », a Bretton cooperative producing organic seed potato, has been questioning the coherence of organic principles on one hand, and official seed potato schemes on the other. In 2001, the cooperative had already contributed to a project aiming to develop a seed potato production process entirely conducted under organic growing conditions based on sanitary selection in vivo, thus without the use of meristem culture (Trehorel, 2001). However, this experimented selection method had been abandoned, mainly for economic reasons and is presently conduced only by few organic breeders (e.g. DottenfelderHof).

In addition to the impossibility to conduct seed potato production entirely under organic growing conditions, the second main critique of the current production scheme is that it renders co-evolutionary processes between potato and its microbiome utterly impossible. Such co-evolutionary processes would be necessary to base plant health on ecosystem interactions, as stated by organic principles (IFOAM Organics International, 2005). These principles are not mandatory rules, but basic principles set by a worldwide federation of associations for organic agriculture. The objective of this LIVESEED exploratory study was to identify topics for future research and development in view of developing a more holistic management of viruses in the production of organic seed potatoes, basing plant health on biological interactions within the ecosystem.

4.2.2. Materials and Methods

A kick-off meeting to the study was organised with « Payzon Ferme » and "Aval Douar Beo" (an association of two organic seed potato cooperatives) in November 2018 for a first approach of the issue from their perspective. Over a period of 3 months, a desk study was then carried out based on bibliography on the management of viruses during the production of seed potatoes, with a particular emphasis on references concerning alternatives to the current production scheme. This allowed identifying 5 types of production strategies to explore, which correspond to different approaches to virus management (Le Grumelec, 2019).

In a second step, about twenty interviews were carried out based on a semi-directive interview guide to explore 4 of the 5 identified strategies. One of them – the strategy employed before the 1970's - was described exclusively through bibliography. These interviews were carried out with actors of seed

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potato production, acting at different levels both within and beyond organic production, with diverse views on potato health. Interviewees included farmers and gardeners, researchers and breeders, as well as representatives of the authorities responsible for the official production scheme. They add up to approximately 20 hours of interview recordings.

4.2.3. Results and Discussion

The following five production systems were identified and described. While the first is the official seed potato production scheme based on in-vitro sanitation, the four others develop alternatives to official production schemes, either because they do not have access to it, or because they deliberately want to circumvent it.

- *French official scheme for certified seed potato production*: The scheme ensures a threshold of 3 % viral infection in field inspection for certified planting material. This is obtained through systematic annual serological testing (ELISA). If thresholds for pre-basic planting material are exceeded or after a maximum of 9 years of multiplication, sanitation through in vitro meristem culture is mandatory (FN3PT, 2020).
- *Before the 1970's*, official seed potato production schemes did not rely on meristem culture, although degeneration of planting material through viral diseases were already a concern. Experiments on environmental conditions allowing for the sanitation of potato were conducted, especially in the former USSR (Mathon, 1953). Some of these works may be worthwhile taking up again. Especially the production at higher temperature should be reconsidered as many viruses are temperature sensitive (Bertschinger et al. 1995).
- Centre of origin of potato: Andean small-scale farmers continue to rely on traditional practices to produce seed potatoes, including the sanitation of potato plants at high altitudes. Under European conditions, Bertschinger et al. (2017) have demonstrated an incomplete transmission of virus from mother to daughter tubers at high altitudes.
- *Actors of crop diversity conservation and management*: Gardeners, some vegetable farmers and associations grow and conserve heirloom or farmers' varieties and usually don't have access to meristem culture. In addition to mass selection for healthy plants, these actors try to sanitise through more handcraft methods, such as planting buds or « tipp cuttings » (e.g., Lorey, 2003). Although these measures are often insufficient and a high number of cultivars have probably been progressively lost due to health problems, these practices may be of interest within an integrated strategy.
- *Botanical seed as propagating material*: Botanical seed are not a vector for the potato viruses of interest here, so using them to propagate potatoes is a solution considered by some. They present the additional advantage of longer shelf-life and easier shipping. The major challenge is that sexual propagation through botanical seed doesn't allow to stabilise cultivars, as vegetative propagation does. Two types of actors are currently investigating botanical seed for potato propagation. On the one hand, gardeners and at least one small-scale vegetable producer are experimenting it, but knowing and stabilising cultivars is not necessary in their context. On the other hand, the Netherlands-based seed company BEJO has released the first seed-propagated potato variety, 'OLIVER F1'. This variety was stabilised by creating dihaploid homozygous plants (whereas potato is usually tetraploid heterozygous).

Drawing on these systems, interactions between pathogens, the environment and potato plants that are relevant for plant health during the production and selection process were highlighted. Accordingly, topics and hypotheses for future research were formulated, as follows:

- *Interactions between plants and their growing environments*: The role of growing environments for potato health in general, and seed potato sanitation in particular, should be elucidated. This includes the effect of altitude and temperature on tuber health, but also the role of soil life.

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- *Interactions between plants and pathogens*: Experimenting and improving mass selection of plant health would be worthwhile to explore co-evolutionary and the capacity of potato plants to adapt. One potato variety named 'Rosa', in particular, was mentioned by producers as being adapted to « living with its viruses ». 'Rosa' and other similar varieties might serve as models to study the range of interactions between potato plants and their viruses.
- *Interactions between plant, pathogens, environment and management practices*: Several interviewees have mentioned the « producer effect » on plant health, i.e. the observation that some producers are particularly good at obtaining healthy plants. This may indicate that these producers have in-depth knowledge and valuable know-how, for example to avoid too strong propagation of aphids transmitting viruses. It would then be relevant to facilitate peer-to-peer knowledge exchange, as well as training (See LIVESEED's Practice Abstract on Seed health in Potatoes[2]).
- *Breeding resistant and tolerant cultivars*: Breeding for genetic resistance or tolerance to viral diseases in potato would create cultivars less dependent on sanitation measures for plant health. Although potential sources for resistance may exist in germplasm collections and resistance genes have been identified (Julius-Kühn-Institut, 2016), introgression of such genes are time consuming. As regards to tolerant cultivars, which could be infected with virus without expressing symptoms, some interviewees feared that they would represent an additional reservoir for spreading the virus.

4.2.4. Conclusions

The measures for virus control create lock-ins in official seed potato production schemes, both technically and legally. Technically, the established in-vitro systems lead to a low incentive to explore different in-vivo approaches for maintaining virus-free potato planting material for organic production, especially as meristem culture is allowed for production of seed potato in the organic regulation (EU 2018/848). Also, it renders the question of plant-environment-microbiome interactions obsolete because sanitation under aseptic conditions is systematically performed. In France, such sanitation is mandatory at least every eight generations, making it impossible for seed potato producers to experiment with other techniques such as sanitary mass selection, even if they obtain good results.

In an article on the diverse approaches to and definitions of plant health, Döring et al. (2012) conclude that a universal definition of plant health does not exist, and they recommend creating opportunities for a discursive definition, in a process where various and sometimes divergent views on plant health are discussed. In the case of seed potato health, we are far from such a discourse, as the official potato production scheme de facto prescribes its conception of health and how to obtain it.

The present exploratory case study has pointed to a range of topics for future research that may in time lead to alternative techniques and practices for the management of viruses in seed potatoes in organic systems. This would require considerable effort and investment. It is now up to the actors of organic seed potato production to take up the issue, to decide on the stakes and on the approaches to follow.

[2] https://orgprints.org/37870/1/PA28_Seed-health-in-potatoes.pdf

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4.3. Seed vigour and seedling health, a study with carrot and damping-off tolerance

4.3.1. Importance of seed vigour in seedling health

Seed vigour can be described as the ability of a seed lot to provide a high frequency of healthy seedlings. Germination tests in the laboratory are often performed under optimal conditions of temperature, moisture, and light, they provide information on the maximum potential of a seed lot. In the field these conditions are often less optimal and germinating seeds may experience certain stresses. These stresses can be abiotic, like not enough or too much moisture or low temperature, or biotic by the presence of pathogens in the soil (Figure 08). The ability of a seed lot to cope with this biotic and abiotic stress in the field is an important aspect of seed vigour. Seed vigour is tested in laboratory experiments by analysing the tolerance of a seed lot to abiotic stresses.

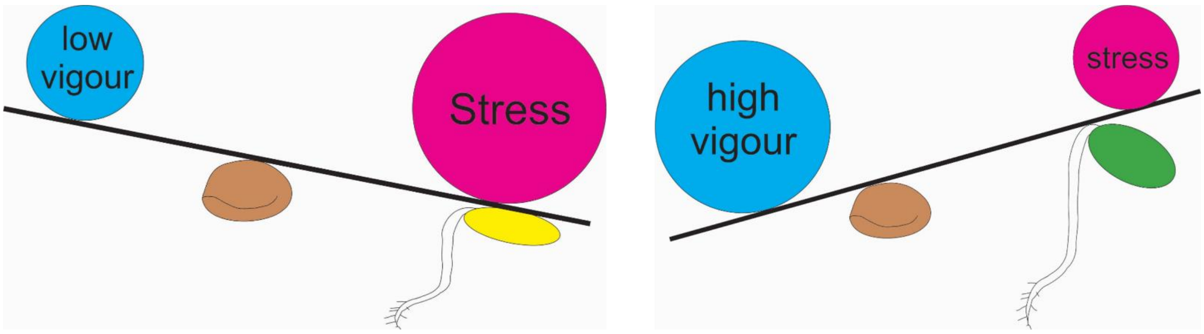

Figure 8. Low vigour seeds have difficulties overcoming biotic and abiotic stress in the field, resulting in poor emergence. High vigour seeds can cope better with stress, resulting in a higher frequency of healthy seedlings in the field.

4.3.2. Laboratory and field experiments with carrot seeds

In the frame of the LIVESEED project it was tested if variation in seed vigour influences tolerance towards pathogens. Carrot (*Daucus carota*) seeds were used as a model. Initial experiments were done with around 20 carrot seed lots that were analysed in the lab for several different seed vigour characteristics under abiotic stress and for emergence in a field contaminated with pathogen, including the *Phytium ultimum*. The field experiments unfortunately failed to provide clear data. One reason was the variation in sowing density of the sowing machine that was used, creating variation in the number of seeds sown per plot, making it impossible to draw conclusions on the frequency of emergence. A second reason was the potential inhomogeneity of the pathogen in the field.

It was decided to start a single seed lot and create sub-samples with different vigour through experimental ageing by storing carrot seeds under high pressure air (Groot et al., 2012, Ann Bot 110 p 1149). The higher oxygen concentration in this system increases the rate of oxidation, which results in a faster ageing of the seeds. The method is called Elevated Partial Pressure of Oxygen (EPPO). The advantage is that this experimental ageing is without increasing humidity or the temperature, which resembles more the aging under dry storage conditions at the seed companies or on farm. These samples were tested in field experiments and in the lab for vigour and tolerance to the *Alternaria radicina* pathogen. This pathogen can cause damping off disease and black rot and can be both seed- and soil-borne. A pathogen tolerance assay had to be developed. Germination tests showed that

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experimental ageing indeed reduced the quality of the seeds. The germination on filter paper was reduced by the ageing and those seeds that germinated did so more slowly. Also, in the soil laboratory test the aged seeds performed worse the longer the duration of the EPPO treatment. This confirmed the decline in seed vigour as result of the experimental ageing treatment.

Field trials with the EPPO aged seed were performed in 2020 at three locations by Bingenheimer Saatgut in Germany, Sativa Rheinau in Switzerland, and Vitalis Biologische Zaden in the Netherlands. The field trials included four ageing treatments (0, 2, 3 and 5 weeks EPPO storage), in six replications in a complete randomised block design (Figure 9).

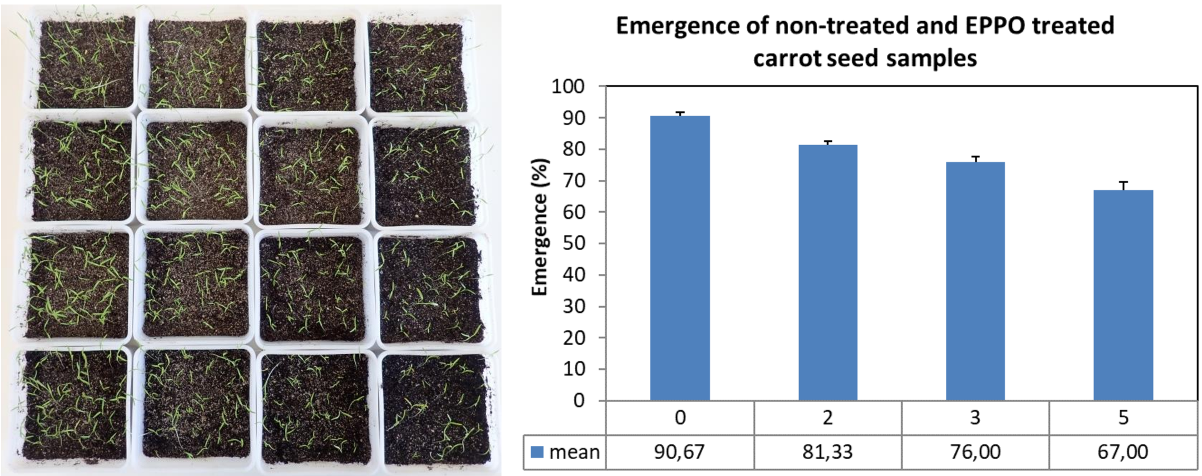

Figure 9. Laboratory soil emergence test for the control (0 weeks) and the EPPO treated (2, 3 and 5 weeks) carrot seed samples, determined by Bingenheimer Saatgut.

100 seeds per replicate were sown by hand (Figure 10).

Figure 10. Hand sowing of hundred seeds per treatment and replicate at the field of Vitalis, the Netherlands.

Emergence in the field experiments differed considerably between the fields. In Switzerland, the emergence was rather low, with 26, 14, 13, and 11% emerging seedlings for the 0, 2, 3 and 5 weeks

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EPPO storage, respectively. In Germany, emergence was 50, 40, 33, and 29%, respectively (Figure 11 A). Frequent counting of the emerging seedlings in Germany allowed us to also analyse the speed of emergence, which was slower for the aged seed lots, another clear sign of lower seed vigour (Figure 11B). In the Netherlands, the field conditions were more optimal as emergence was higher compared to the other field trial, with 82, 64, 57, and 54% for the 0, 2, 3 and 5 weeks EPPO storage, respectively. Vitalis also determined the final yield, both in number of carrots and in weight (Figure 12). The carrots were harvested 54 days after sowing, and the yield was 5.1, 4.7, 3.9 and 3.8 kg per 100 seeds sown for the 0, 2, 3 and 5 weeks EPPO storage, respectively. Although there is some compensation for the lower number of emerged seedlings, it is not enough to reach equal yields, at least not in the eight weeks of cultivation.

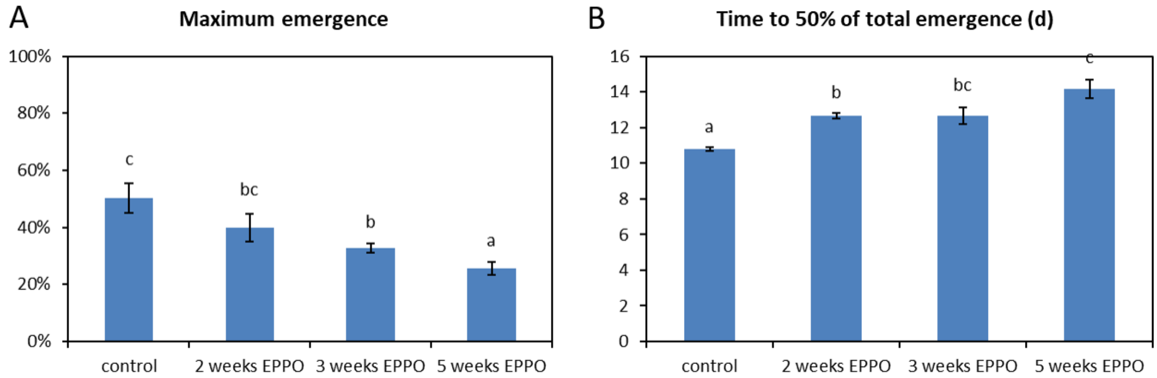

Figure 11. Total emergence frequency (A) and emergence speed (B) in the carrot field trial performed in Germany by Bingenheimer Saatgut.

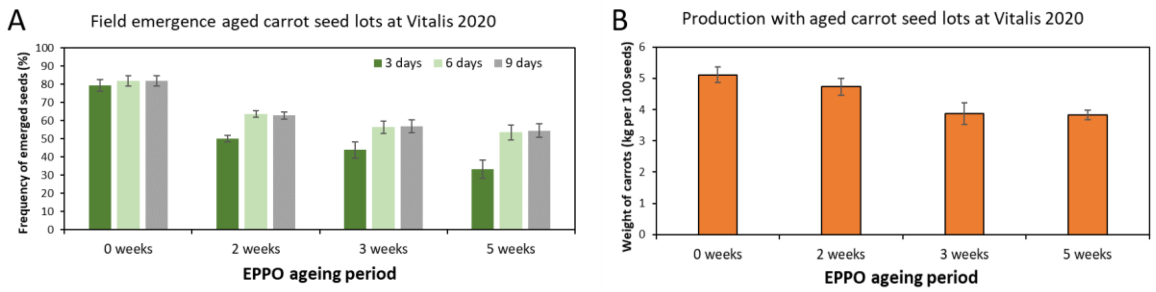

Figure 12. Total emergence frequency (A) and yield (B) in the carrot field trial performed in the Netherlands by Vitalis Biozaden.

Partners from University of Evora (Portugal) are measuring activity of the enzyme alternative oxidase, which they have shown to be linked to seedling vigour in other systems. The results are pending.
In conclusion, a difference in seed vigour for the EPPO aged seed samples was shown both under controlled conditions and in field experiments.

4.3.3. Tolerance of carrot seed towards the fungus *Alternaria radicina*

An assay was developed to test carrot seed lots of different vigour for their tolerance to *A. radicina*. In short, seeds were dispersed in a Petri dish on a stack of three filter papers and wetted with different dilutions of the fungal spore suspension or water as control. The Petri dishes were placed in an incubator at 20 °C. After 5, 7, and 10 days the seedlings were evaluated for infection by the pathogen. In the assay development seeds from a commercial lot were initially compared with lower vigour seeds

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that were mildly reduced in vigour by a controlled deterioration treatment where the seeds were stored for five days at a high humidity and temperature (85%RH and 40°C).

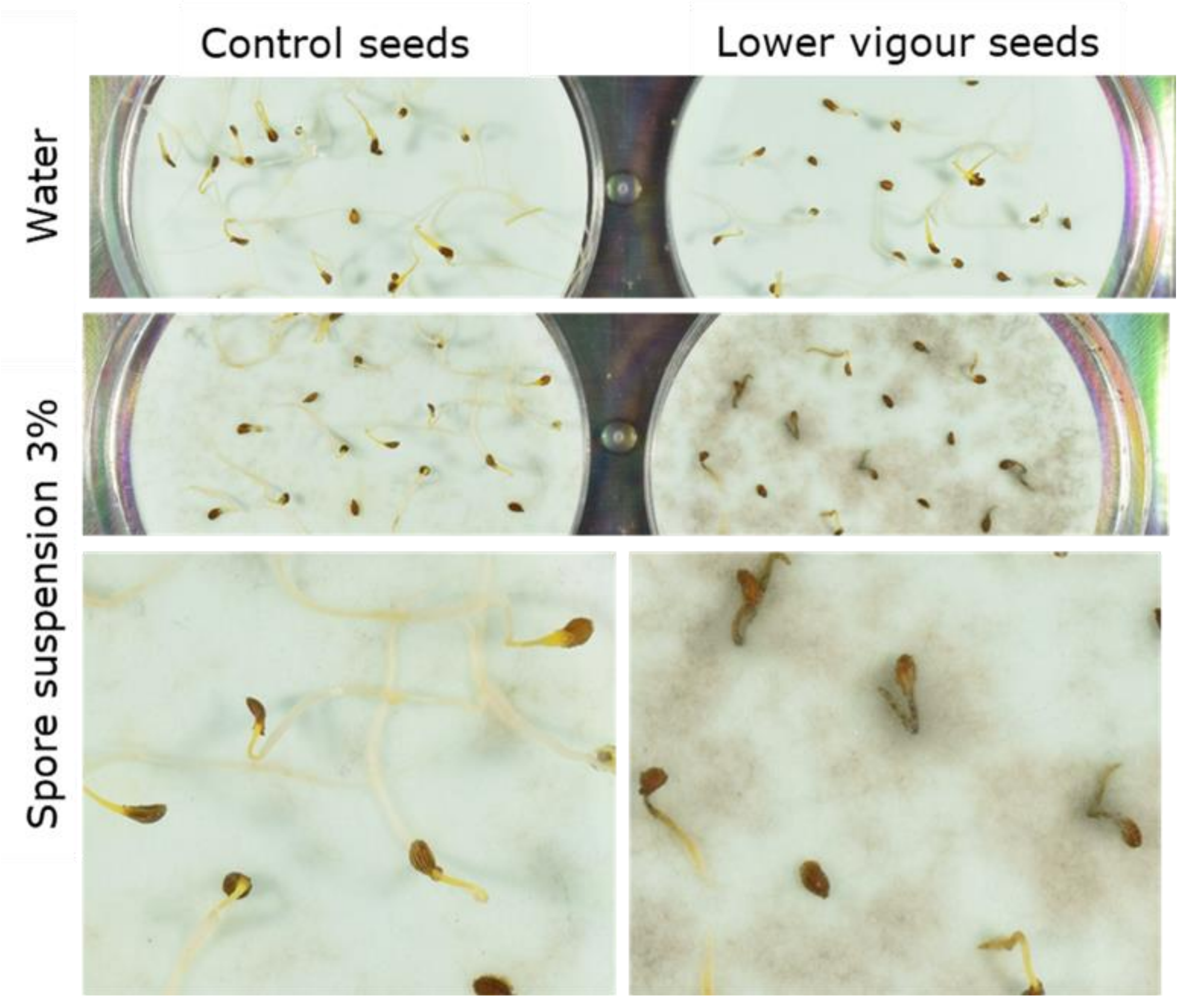

Figure 13. Sensitivity of high (left) and lower (right) vigour seeds to the pathogen Alternaria radicina, causing damping-off under field conditions. Carrot seeds were moistened with water (top) or water with different concentrations of a fungal spore suspension (middle and bottom).

At higher concentrations of *Alternaria radicina* both the high and low vigour seeds were infected. Applying a slight dilution of the spore suspension to the germinating seeds showed that the low vigour seedlings suffered from the pathogen and died, while the high vigour seeds were not or hardly infected (Figure 13). In a follow-up analysis EPPO treated seeds were compared with control. This experiment showed a similar picture, the EPPO aged seed samples with lower vigour germinated more slowly in the assay and were less tolerant to the pathogen, while with the control seed sample more seedlings survived.

Seed deterioration resulted both in a decline in seed vigour and a higher sensitivity of the carrot seeds towards the pathogen. It is tempting to conclude that it is the decline in seed vigour that made the seeds more sensitive. However, it cannot be ruled out yet that the controlled deterioration and the EPPO ageing also resulted in a deterioration of the seed microbiome (see next paragraph), which in a healthy status could have aided the carrot seedlings in their tolerance to the *A. radicina* pathogen. On the other hand, if seeds and their microbiome are considered as a holobiont, then seed vigour should

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also be considered as part of this holobiont. It could then be concluded that the reduction of vigour is responsible for the increased sensitivity towards biotic and abiotic stress.

The experiments show the importance of optimising seed vigour during seed production and maintaining that vigour during further handling and storage. This holds for both commercial seed producers and farm saved seeds. Optimal seed storage is essential as poor storage conditions will result in reduced seed vigour.

4.4. Effect seed production conditions on the seed microbiome

In LIVESEED, studies have been done on the diversity in the microbiome of pea seeds: a study is ongoing to elucidate whether seeds produced under organic soils have a more divers microbiome as compared to seed produced in conventionally cultivated soils. This study also uses carrot as a model plant. Carrot seed production takes two years, as plants produce carrots in the first year after sowing and flower the second year. Carrots were produced from two varieties at three locations in the Netherlands on neighbouring organic and conventional fields. After the winter, the carrots were potted in soil from their production field, brought to flowering in separate compartments and seeds were harvested. At present DNA extraction is performed to test for diversity in the bacterial and fungal microbiome. Results are expected in early 2021.

4.5. Ageing of the seed microbiome during storage

Even if seeds can be produced with a biodiverse microbiome, it is important to maintain that diversity during seed treatments and storage and it can be expected that different components of the microbiome also vary in their tolerance to storage and treatments. For an endophyte (microbiome inside the seed) in grass seeds it has been shown that during storage viability is lost earlier compared to that of the grass seeds.

Two experiments on this aspect were initiated in the frame of the LIVESEED project. Organic produced wheat and alfalfa seeds have been experimentally aged for different durations using the above described EPPO ageing under an elevated partial pressure of oxygen. These samples are presently analysed by the lab of Prof Gabrielle Berg (Graz University of Technology, Austria and scientific advisor for the LIFESEED project) for their microbiome biodiversity over time of storage.

A second experiment is performed with lupin seeds contaminated with the seed born pathogen *Colletotrichum lupini*, also part of the seed microbiome. LIVESEED partner FiBL (CH) had indications that storage of lupin seeds resulted in a weakening of the *C. lupini* when present in the seeds. To test this hypothesis WR performed an EPPO ageing treatment for zero, three and eight weeks and sent the seeds back to FiBL. Due to the corona pandemic evaluation took longer than anticipated. Germination test have been performed, which showed that the three weeks treatment resulted in a slight decline and the eight weeks in a severe decline in germination. A larger greenhouse trial is still ongoing to test the effect on the survival of the pathogen.

4.6. Shelf life of applied biologicals

In the crop protection industry, a shift is going on from chemical crop protection towards the use so-called biocontrol agents (BCA's) or biologicals. As mentioned in Chapter 2, several questions were raised in the inventory about whether application of biologicals can aid in solving organic seed quality

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issues. As described in subsection 2.5, *Rhizobium* bacteria have already been applied to seeds for decades. These bacteria form a symbiotic relation with plants of mainly leguminous species, like alfalfa, by fixing atmospheric nitrogen. The soil where alfalfa seeds are sown does not always contain the right amount of *Rhizobium* bacteria or the right strain that fits this legume plant. For those reasons' seeds are frequently coated with *Rhizobium* bacteria before sowing. After the coating the seeds are dried and stored for some time till the planting season. Other frequently used biologicals are *Bacillus* bacteria, which can aid the seedling for instance in tolerance against pathogens and improve plant vitality through reduction of abiotic stress or improvement of nutrient availability.

The production of biologicals is done in liquid or moist cultures. The seed industry, however, is used to handle, store and sell seeds in dry conditions. Biologicals applied to seeds should therefore, either be dried before or after coating the seeds, or both. Subsequently it is important that the biological should remain alive and vigorous during storage, to be activated after sowing and readily colonise the seedling. For *Bacillus* BCA's, applied as spores, this is not a problem as these spores are very tolerant. However, with many promising new biologicals this is a real challenge. It is therefore important to develop assays that can test desiccation tolerance and shelf life of promising BSA's. Such an assay may also be used as a method to select more tolerant strains or to compare the effect of production recipes on subsequent shelf life. In certain cases (e.g., for Bradyrhizobia in soybean, white lupin) farmers are treating their seed with commercialized biological products directly before sowing according to manufactural recommendations. At present there in no standardised assay for shelf life with BCA's available.

In the LIVESEED project we initiated an experiment to test if the EPPO ageing method, developed for estimating shelf life of seeds under dry conditions, can be used to test survival of BCA's. In 2019 Feldsaaten Freudenberger (FSF) had coated alfalfa seeds with *Rhizobium*, and *Bacillus*. The coated seeds were stored for different periods under EPPO conditions and a control, with limited ageing, a sample was stored at -70 °C. Unfortunately, difficulties were encountered in the plating assays to test the recovery of the two types of the *Rhizobium*, due to overgrowths by the Bacillus bacteria. Although survival for the *Bacillus* was rather low after 40 weeks EPPO, the experiment showed that the *Bacillus* and *Rhizobium* bacteria could both withstand temporary storage at -70 °C. In 2020 a new storage experiment has been started, but the results are not yet known.

4.7. Alternative packaging materials

The seed company Bingenheimer Saatgut AG provides organic seeds of over 480 open pollinated vegetables, herbs, and flower varieties, in different processing forms. This diversity requires a differentiated view with respect to optimal storage conditions to avoid seed deterioration. The seed lots are stored under both short- and long-term conditions. To maintain high seed vigour aging should be limited. Water vapour and oxygen are the main triggers to stimulate seed deterioration by oxidation. Seed companies have the challenge of how to pack und how to store the seeds, with respect to quality and ecological values and strive to use packaging as sustainable as possible and to reduce the use of plastic bags. The principal material they use for packaging is paper, which is permeable for both water vapour and oxygen. For sensitive seeds and long-term storage packaging material with a low oxygen transmission rate is needed. Most seed companies use laminated aluminium foil bags, but these cannot be recycled. In the frame of the LIVESEED project, the company raised the question of an alternative environmentally friendly solution for consumer packages and long-term bulk seed storage. They provide Wageningen Research with several types of bags to test for oxygen permeability. Wageningen Research tested the bags by flushing with nitrogen gas and measuring the oxygen level

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directly after flushing and again after one month. Paper bags with a transparent window and Ziplock bags failed both, as oxygen levels were back to ambient within one month. Heat-sealing the Ziplock bags reduced oxygen permeability. This system might be an alternative for consumer packages that are used within a year, but it is unsuitable for long term storage, as oxygen levels still increased. Tests with large plastic vacuum bags are more promising for long term storage. With these vacuum bags, oxygen levels did not increase during the four months test period. The oxygen and moisture vapour permeability of plastic bags vary with the polymer used. Bingenheimer Saatgut[3] is presently collecting information on the polymers used for the zip lock and vacuum bags. They prefer to use bags from recycled plastics, but it is questionable if these have a reproducible low permeability for water vapour and oxygen, necessary prevention of seed deterioration during long term storage.

[3] (https://polymerdatabase.com/polymer%20physics/Permeability.html)

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5. General conclusions

The need for more sustainable agricultural practices and healthy food is getting more broadly recognised and the European Commission targets to have at least one quarter of the farmland under organic agriculture by 2030. The quality of seeds is important in the establishment of a healthy and well producing crop. The use of high-quality organic seeds is an essential part of the organic food production system. An increase in the area of organic cultivation in the coming years will also increase the demand for organic seeds. Part of organic seeds are supplied by specialised farmers and seed companies, the other part by farm saved seeds. Dissemination of knowledge and technologies already available is one way to support them in producing high-quality seeds, in upgrading through appropriate sorting and treatments, and in maintaining this seed quality during storage. However, even with these improved methods, for some crops it is still a challenge to produce high-quality healthy seeds under organic conditions. An inventory of current issues with organic seed production was produced for arable, vegetable and forage crops and presented in this report. Although the inventory does not cover all crops grown in the European Union, it shows the main obstacles that need to be addressed in order to reach the target of 100% organic seed use and phasing out of derogation for conventional untreated seed by 2036 (EU 2918/848). Seed storage pests (insects) and fungal diseases make up for the vast majority of issues listed with arable crops. Both fungal and bacterial diseases play the most important role in seed production for vegetable crops. Potatoes, prone to several types of diseases challenging both production and seed potato quality, is an issue *per se*. Collecting and disseminating already available solutions to the listed issues would be relevant as next steps, as well as prioritising issues for future research and development.

These obstacles need to be addressed in a holistic and multi-actor approach involving public researchers, the seed industry, and organic farmers. Some of these issues were addressed in case studies during the LIVESEED project. The study to reduce the risk of common bunt with wheat seeds showed that a combination of approaches is most suitable. On the other hand, we have seen that in the case of seed potato production, viral diseases are considered dangerous enough to impose sanitation through meristem culture on all seed potato producers through an official production scheme, at least in France. Alternatives to this production scheme are being experimented only at the margins but merit a closer look as they strive for more coherent processes for growing organic seed potatoes. These case studies highlight the importance of a comprehensive approach to plant and seed health. For instance, when considering the problem of a pathogen that can infect the seedling, total elimination of the pathogen may not be needed when also seed vigour is considered. The carrot *Alternaria* case showed that emphasis on producing high vigour carrot seeds and maintaining that vigour after harvest, can boost the seedling tolerance to that fungal pathogen.

Considering that organic farming strives to strengthen plant health based on robust local (eco-)systems and biological interactions, the microbiota of cropping systems (soil, plants, and seeds) moves into the spotlight as a priority research topic. Studies on the seed microbiome tend to show that the presence of vital beneficial microorganisms in or on the seed can provide a natural defence system for seedlings. Many core questions emerged from these cases, to be addressed as a priority for research in the coming years. What are these beneficial microorganisms? How can seed producers stimulate their presence in or on the seeds? How to maintain them vital during seed treatments and seed storage? Can they be reproduced in fermenters and applied to seeds as biologicals? Or is a natural combination of microorganisms forming the seed microbiome the best defence system for the seedling? Does organic seed production provide a more effective, rich, and diverse microbiome? If the latter can be answered positively, it will also demonstrate a direct advantage to the use of organic seeds. Altogether, these questions and a discussion on it will be the input for a new organic seed health strategy. This strategy will be described in more detail in the deliverable D2.7, expected to be

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delivered near the end of the LIVESEED project. Such a strategy in not only useful for organic farming. Also, conventional seed production systems will need a change in strategy as more chemical crop protection agents will be banned as happened in 2020 with the fungicide Thiram, till that moment widely used for seed coating of conventional seeds. For this reason, also the conventional seed industry is interested in research on the seed microbiome, and the application of biologicals and natural crop protection compounds in seed treatments.

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6. Bibliography

Bhargava, A., and Srivastava, S. 2019. Participatory plant breeding: concept and applications. Springer Singapore. https://doi.org/10.1007/978-981-13-7119-6.

Berg, G., Raaijmakers, J.M., 2018. Saving seed microbiomes. The ISME journal 12, 1167-1170. https://doi.org/https://doi.org/10.1038/s41396-017-0028-2

Bergna, A., Cernava, T., Rändler, M., Grosch, R., Zachow, C., Berg, G. 2018. Tomato Seeds Preferably Transmit Plant Beneficial Endophytes. Phytobiomes Journal 2, 183-193. https://doi.org/10.1094/pbiomes-06-18-0029-r

Bertschinger, L., Bühler, L., Dupuis, B., Duffy, B., Gessler, C., Forbes, G.A., Keller, E.R., Scheidegger, U.C., Struik, P.C. 2017. Incomplete infection of secondarily infected potato plants–an environment dependent underestimated mechanism in plant virology. Frontiers in Plant Science 8, 74. https://doi.org/10.3389/fpls.2017.00074.

Bertschinger, L., Keller, E.R., Gessler, C. (1995) Characterization of the virus X temperature interaction in secondarily infected potato plants using EPIVIT. Phytopathology 85(7) 815-819

Borgen, A. 2005. Removal of bunt spores from wheat seed lots by brush cleaning. ICARDA Seed Info 29.

Borgen, A., Klaedtke S., and Thueringer A. 2019. Managing common bunt in wheat seed lots. https://www.liveseed.eu/tools-for-practitioners/practice-abstracts/ (accessed 9 November 2020).

Borgen, A., and Nielsen B.J. 2001. Effect of seed treatment with acetic acid for control of seed borne diseases. In: British Crop Protection Council, editor, Seed Treatment Challenges and Opportunities. Sympsium Proceedings No. 76. Wishaw, North Warxickshire, UK. p. 135–140

Coomes, O.T., McGuire, S.J., Garine, E., Caillon, S., McKey, D., Demeulenaere, E., Jarvis, D., Aistara, G., Barnaud, A., Clouvel, P., Emperaire, L., Louafi, S., Martin, P., Massol, F., Pautasso, M., Violon, C., Wencélius, J., 2015. Farmer seed networks make a limited contribution to agriculture? Four common misconceptions. Food Policy 56, 41-50. https://doi.org/https://doi.org/10.1016/j.foodpol.2015.07.008.

Döring, T.F., Pautasso M., Finckh M.R., and Wolfe M.S. 2012. Concepts of plant health - reviewing and challenging the foundations of plant protection. Plant Pathology 61(1): 1–15. https://doi.org/10.1111/j.1365-3059.2011.02501.x.

EC DG SANTE. 2017. Final Review report for the basic substance mustard seeds powder Finalised in the Standing Committee on Plants, Animals, Food and Feed at its meeting on 6 October 2017 in view of the approval of mustard seeds powder as basic substance in accordance with Regulation (EC) No 1107/2009.

European Commission. 2020. Farm to Fork Strategy. For a fair, healthy and environmentally-friendly food system. https://ec.europa.eu/food/sites/food/files/safety/docs/f2f_action-plan_2020_strategy-info_en.pdf.

FN3PT. 2020. La filière française, plant de pomme de terre français. http://www.plantdepommedeterre.org/index/la-filiere-francaise (accessed 24 November 2020).

Fontaine, L. 2007. Cahier Technique “Carie du blé” - Agir avant qu’il ne soit trop tard. https://orgprints.org/29384/ (accessed 23 November 2020).

Germida, J., Siciliano, S., 2001. Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biology and Fertility of Soils 33, 410-415. https://doi.org/10.1007/s003740100343.

IFOAM Organics International. 2005. Principles of Organic Agriculture. https://www.ifoam.bio/sites/default/files/poa_english_web.pdf (accessed 28 November 2019).

LIVESEED is funded by the European Union’s Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

ITAB. 2012. Carie du blé - agir avant qu'il ne soit trop tard. Actes Journée Technique Jeudi 9 favrier 2012, Paris. http://itab.asso.fr/downloads/actes%20suite/carie-actes2012.pdf (accessed 23 November 2020).

Julius-Kühn-Institut. 2016. Schlussbericht für das Verbundprojekt "KMU-innovativ-10: Schaffung von Grundlagen für die Verbesserung der komplexen Krankheitsresistenz von Kartoffeln (ECOPOT-RESI) (Teilprojekt D)".

Le Grumelec, S. 2019. Gestion des virus sur plants de pomme de terre en Agriculture Biologique. Ecole Supérieure d'Agricultures, Angers, France.

Lorey, H. 2003. Was tun gegen Abbauerscheinungen bei Kartoffeln? Samensurium 14.

Marchand, P.A. 2015. Basic substances: an opportunity for approval of low-concern substances under EU pesticide regulation: Basic substances of low concern under EU pesticide regulation. Pest Management Science 71(9): 1197–1200. https://doi.org/10.1002/ps.3997.

Mathon, C.C. 1953. La pomme de terre, la dégénérescence vaincue et l'amélioration des variétés.

Nuijten, E., Lazzaro, M., Costanzo, A., Chable, V., Annicchiarico, P., Rodriguez Burruezo, A., Klaedtke, S., Ceccarelli, S., Kölling, A., Messmer, M., 2020. Liveseed deliverable 3.5 : Innovative organic breeding concepts: challenges and examples. https://www.liveseed.eu/wp-content/uploads/2020/10/LIVESEED_D3.5_Report-on-novel-breeding-concepts-and-strategies-for-low-input-agriculture.pdf (accessed on November 10th, 2020).

Saidi, B., Azmeh, F., Mamluk, O., Sikora, R., 2001. Effect of seed treatment with organic acids on the control of common bunt (*Tilletia tritici* and *T. laevis*) in wheat. Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen) 66, 213-221.

Spadaro, D., Herforth-Rahmé, J. and van der Wolf, J., 2017. Organic seed treatments of vegetables to prevent seedborne diseases. Acta Hortic. 1164, 23-32. DOI: 10.17660/ActaHortic.2017.1164.3 https://doi.org/10.17660/ActaHortic.2017.1164.3

Trehorel, F. 2001. La production de plant de pomme de terre biologique : aspects phytosanitaires et organisationnels.

Waldow, F., M. Jahn, 2007. Investigations in the regulation of common bunt (*Tilletia Tritici*) of winter wheat with regard to threshold values, cultivar susceptibility and non-chemical protection measures. Journal of Plant Diseases and Protection, 114 (6), 269–275.

Wassermann, B., Cernava, T., Müller, H., Berg, C., Berg, G., 2019. Seeds of native alpine plants host unique microbial communities embedded in cross-kingdom networks. Microbiome 7. https://doi.org/10.1186/s40168-019-0723-5

Weinhappel, M. 2018. Survey on EU Member States standards for *Tilletia caries*, *Tilletia foetida* and *Tilletia controversa*. Based on a survey of the Standing Committee Plant Reproductive Material.

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7. Published practice abstracts related to seed health

7.1. Managing common bunt in wheat seed lots

PRACTICE ABSTRACT No. 2

Target audience: farmers, farm advisors, seed networks

Agrologica

Plant breeding and research

itab
l'Institut de l'agriculture et de l'alimentation biologiques

Managing common bunt in wheat seed lots

Figure 1: *Bunt balls, a mass of spores replace the kernels. (Photo: S. Klaedtke (ITAB))*

Problems

In wheat and related cereals, common bunt can cause considerable damage in yield and grain quality. The disease is caused by seed-borne fungi, which can persist in soils as well.

Practical recommendations

Seed analyses: A seed analysis, as performed by state-accredited labs for example, will confirm and quantify the infection of a seed lot with common bunt.

Thorough seed cleaning: Thoroughly cleaning an infected seed lot with an air stream or similar gravity cleaning equipment can remove most of the intact bunt balls and some of the free spores. As a second step, brush-cleaning is very efficient to reduce the number of free spores in the seed lot.

Seed treatments: Seed treatments are essential to prevent and control common bunt. Several seed treatments are authorized for organic farming, namely white vinegar, mustard powder, products based on antagonist microorganisms (e.g. Cerall (R)) and products based on copper (e.g. Copseed), depending on the country.

When harvesting...
If an infection with common bunt is suspected, harvest healthy wheat fields first and infected fields last. Then clean the harvester by harvesting crops which are not susceptible to common bunt, e.g. oats or any non-cereal crop (e.g. pea, soybean).

The decision diagram on the next page summarizes all the necessary information when managing an infested seed lot.

Further information

1. Matanguihan J.B., Murphy K.M.,2011. Control of Common Bunt in Organic Wheat. The American Phytopathological Society, Plant Disease Vol. 95 No. 2: 92-103. Available at: https://doi.org/10.1094/PDIS-09-10-0620

2. On brush cleaning in particular: Borgen, Anders (2005) Removal of bunt spores from wheat seed lots by brush cleaning. Seed Info, ICARDA, 29, pp. 13-15. Available at: http://orgprints.org/3202/

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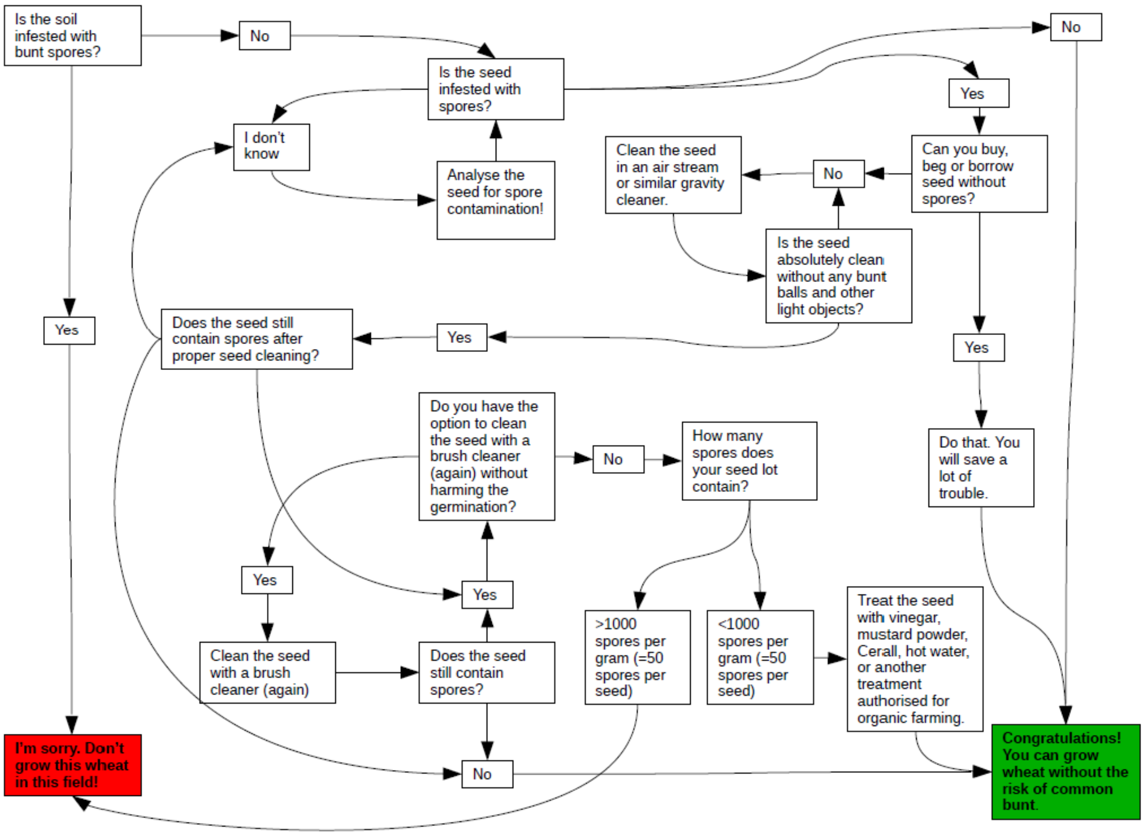
7.2. Necessary information when managing an infested seed lot

PRACTICE ABSTRACT No. 2

Target audience: farmers, farm advisors, seed networks

The following decision diagram summarizes all the necessary information when managing an infested seed lot.



Authors: Anders Borgen (Agrologica), Stephanie Klaedtke (ITAB) and Angela Thueringer (AGES)
Contact: stephanie.klaedtke@itab.asso.fr
Publisher: ÖMKi Hungarian Research Institute of Organic Agriculture
Date: October 2019
LIVESEED: Boosting organic seed and plant breeding across Europe. LIVESEED is based on the concept that cultivars adapted to organic systems are key for realising the full potential of organic agriculture in Europe. Research project 2017-2021.
Social Media: Facebook [LIVESEED] & Twitter [@LIVESEEDeu]

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7.3. Proper seed storage

PRACTICE ABSTRACT No. 25

Target audience: farmers

Proper seed storage

Figure 1*. Seed quality test with fresh and aged barley seeds*

Problems

Seed quality is very important for the start of a crop. Accumulation of damage during storage can result in abnormal seedlings or even failure of emergence (Fig 1). To avoid too much ageing, seeds need to be stored in the most optimal way.

Solutions

What causes seed ageing?

Seed ageing is caused by oxidation of the cell membranes, mitochondria, DNA, RNA and proteins in the seeds. This oxidation is stimulated by four factors: seed moisture level, temperature, oxygen and time. The main factors stimulating this ageing are moisture and oxygen.

How to reduce ageing

Keep sealed commercial seed packages closed until use, to avoid moisture uptake from the air. Never store an open package in a cold place like a refrigerator, were the humidity is high and the seeds will absorb moisture. If not all seeds are used, store the remainder in a dry environment. For this we developed an easy system with a 'seed drying and storage box' (Fig 2).

Figure 2*. Box for seed drying and storage*

The principle is an airtight transparent box. In the box is a bag with silica gel and a relative humidity (RH) meter. The optimal RH is between 20 and 40%. Home produced seeds can also be dried in the box. If the RH surpasses the 40%, the silica gel needs to be regenerated in an oven at 100 °C. The dried silica gel can be cooled down in a closed clean jam jar or alike. It is possible to store the airtight box with seeds in a cooler place, to reduce ageing further. For larger amount of seeds the box could be replaced by a large vacuum bag, as available for storage of clothes.

Practical recommendations

- To reduce seed quality loss you need to store seeds under dry and cool conditions.
- The seed drying box is a tool to keep the seeds dry and can be home made from readily available material.

Further information

Read more on seed storage: http://library.wur.nl/WebQuery/wurpubs/534005

Authors: Steven P.C. Groot (Wageningen University & Research)
Contact: **steven.groot@wur.nl**
Publisher: ÖMKi Hungarian Research Institute of Organic Agriculture
Date: March2020
LIVESEED: Boosting organic seed and plant breeding across Europe. LIVESEED is based on the concept that cultivars adapted to organic systems are key for realising the full potential of organic agriculture in Europe. Research project 2017-2021.
Social Media: Facebook [LIVESEED] & Twitter [@LIVESEEDeu]

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7.4. Seed vigour, keep it high!

PRACTICE ABSTRACT No. 30

Target audience: farmers, seed producers, seed networks

Seed vigour, keep it high!

Problems

Seeds are living organisms and sensitive to stress during storage or treatments, which can result in reduction of seed vigour. Vigour can be seen as the tolerance of seeds to emerge under non-optimal conditions. Low vigour seeds give upon sowing in the field no or weaker seedlings.

Solutions

What causes seed vigour loss?

When seeds are dry, they slowly oxidise, as every organic material. Oxidation can also be induced for instance by a hot water, steam or air treatment. Damage repair can only start once the seeds are getting wet, as enzyme activity is needed for this and enzymes need water. More oxidation results in more damage and weaker seeds and seedlings. These seedlings will emerge slower or not at all and are more sensitive to drought stress and pathogens (see picture).

How to reduce vigour loss

Harvest seeds with maximum stress tolerance, dry them well, keep them stored under optimal conditions and be cautious with physical seed sanitation treatments.

Carrot seedlings exposed to spores of Alternaria radicina (Black rot disease). Upper picture is from high vigour seeds. Bottom picture from seeds of the same seed lot but stored at higher humidity and temperature.

Practical recommendations

- Harvest the seeds, if possible, at full maturity, since less mature seeds are more sensitive to induction of damage.
- Dry the seeds soon after harvest, preferably to an equilibrium with 30 -40% relative humidity and keep them dry.
- Store the seeds under optimal conditions: 30-40% RH, cool and preferably without oxygen. Do this also with left-over seeds.
- Be careful with sanitation treatments. Perform test treatments with a small sample.
- Speed of germination is a good indicator of seed vigour. More damage needs more time for repair.

Further information

Read more on seed storage and vigour: http://library.wur.nl/WebQuery/wurpubs/534005

Authors: Steven P.C. Groot & Jan Kodde (Wageningen University & Research)
Contact: steven.groot@wur.nl
Publisher: ÖMKi Hungarian Research Institute of Organic Agriculture
Date: May 2020
LIVESEED: Boosting organic seed and plant breeding across Europe. LIVESEED is based on the concept that cultivars adapted to organic systems are key for realising the full potential of organic agriculture in Europe. Research project 2017-2021.
Social Media: Facebook [LIVESEED] & Twitter [@LIVESEEDeu]

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7.5. Seed health in potatoes

PRACTICE ABSTRACT No. 28

Seed health in potatoes

Problems

The potato crop is susceptible to many pathogens. Potato virus and bacterial soft rot are most actual -but definitely not the only- problems in seed production. Yield losses can go up to 50-70% or complete crop failure.

Solutions

Organic seed potatoes

A typical variety for organic farming allows for moderate fertilization levels, has a stable product quality under stress conditions, is broad resistant against Late Blight and virus and has a short field period.

Virus

Potato virus X and Y are spread by aphids or by cross-contamination. They can show symptoms, like 'squeezed' or rolled leaf growth, yellowing or mosaic patterns, mostly on top of the plant. However, the expression is dependent on variety, crop maturity and growing conditions. Rogueing basic seed lots is key, which takes experience. A diseased plant can be missed, particularly in varieties that show no symptoms; causing 'secondary disease' next season.

Figure 1: *Crinkled leaves with chlorotic spots due to Potato virus Y.*

Bacterial soft rot or blackleg

Pectobacterium and *Dickeya* (Erwinia): plants fall due to stem rot or wilting, with creamy tuber spots and a fishy smell. **Virus** rogueing is a notorious path for **Erwinia** spread. Like virus, infested tubers may be symptomless, enabling 'invisible' spread through a seed lot. Farm hygiene is the only control measure.

Figure 2: *Bacterial soft rot causing black stem rot and stem wilting.*

Practical recommendations

- grow a virus resistant variety
- rogue diseased plants, don't rogue in a wet crop
- rogue from 'healthy' to diseased plots
- remove diseased plants (marginal effect) plus all tubers
- aphid control (in OF, one has to rely on natural predators)
- remove 'Solanaceae' weeds and 'volunteers'
- a diseased seed crop may go for consumption
- at harvest: remove suspicious tubers
- allow rotten tubers to dry in storage

Further information

1. https://wiki.groenkennisnet.nl
2. A. Mulder and L.J. Turkesteen (Eds.), 2005. Potato Diseases. Den Haag, The Netherlands. ISBN 90-802036-4-5

Authors: Abco de Buck (LBI)
Contact: a.debuck@louisbolk.nl

Publisher: ÖMKi Hungarian Research Institute of Organic Agriculture
Date: April 2020
LIVESEED: Boosting organic seed and plant breeding across Europe. LIVESEED is based on the concept that cultivars adapted to organic systems are key for realising the full potential of organic agriculture in Europe. Research project 2017-2021.
Social Media: Facebook [LIVESEED] & Twitter [@LIVESEEDeu]

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7.6. Application of acetic acid as a seed treatment in organic cereal seed

PRACTICE ABSTRACT No. 41

Target audience: farmers, farm advisors, seed producers

Agrologica

Application of acetic acid as a seed treatment in organic cereal seed

Problems

Common bunt is a devastating seed borne disease in wheat. If a seed lot is contaminated with just a few spores, there is a high risk that the disease will develop and reduce yield and quality of the crop. Acetic acid is very effective to control common bunt in wheat, but there is a high risk of negative effects on germination. Therefore the procedure of application is crucial for a successful treatment.

Solutions

The crucial point in seed treatments with acetic acid is to make sure that the entire seed surface is covered, to affect all bunt spores. It is crucial that the application is as uniform as possible and as fast as possible.

It is easier to cover all the kernel surface with acid, if a higher amount of acid are applied, but if so, the seed needs to be dried after 30 to 60 seconds to avoid negative effects on germination.

Figure: *Vinegar treatment in a cement drum (Photo: Matteo Petitti)*

Practical recommendations

- Small seed samples (0-2kg) can be treated in a box with high amounts of acetic acid (<20ml/kg) and drying with a hair dryer or similar after 30 seconds.
- Seed samples of 5-20kg can be treated in a cement drum by applying acetic acid just enough to make the seed humid. 20ml/kg is optimal, but a slightly higher amount can be applied if the seed after treatment is spread on a clean surface in the sun or wind for drying.
- If huge amount of seed need to be treated, it is crucial not to exceed the limit of 20ml/kg, as it will be difficult to dry the seed quickly enough after treatment before germination is affected.
- If you are uncertain whether your treatment is optimal, it is better to use a lower dose, and then repeat the treatment after the seed has been properly dried.

Further information

1. Borgen, A. og B.J.Nielsen 2001: Effect of seed treatment with acetic acid in control of seed borne diseases. In: Proceedings from BCPC Symposium No. 76: "Seed Treatment: Challenges & Opportunities", eds. A. J. Biddle. BCPC, Farnham, 135-140.
2. PA#2: Managingcommon bunt in wheat seed lots
3. LIVESEED video on bunt treatment methods

Authors: Anders Borgen (Agrologica)
Contact: borgen@agrologica.dk

Publisher: ÖMKi Hungarian Research Institute of Organic Agriculture
Date: May 2020
LIVESEED: Boosting organic seed and plant breeding across Europe. LIVESEED is based on the concept that cultivars adapted to organic systems are key for realising the full potential of organic agriculture in Europe. Research project 2017-2021.
Social Media: Facebook [LIVESEED] & Twitter [@LIVESEEDeu]

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