Euphresco

Final Report

Project title (Acronym)
Phytosanitary risks of newly introduced crops (PRONC)

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Contents

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2. Short project report

2.1. Short executive summary

The increasing introduction of new crops in Europe are an opportunity for growers to produce for niche markets. These newly introduced exotic crops, as well as “forgotten” crops, are mainly grown and marketed outside the general, large-scale commercial agriculture. This local production, and the associated short food supply chain, obtain their planting materials (seed, tubers, cuttings) from different sources, e.g. multipliers in the South of Europe or directly from overseas areas, internet shops, etc. The phytosanitary status of this material is very rarely checked. Entry and spread of possible plant pathogens and pests could pose a threat to traditional crops, besides hampering the cultivation of the new crops. Especially when planting material is obtained from non-European countries, phytosanitary risks can be high. The project focussed on gathering information on a selection of vegetatively propagated tuber producing crops that are gaining a lot of interest, but for which only limited information on their phytosanitary status is available. A survey was performed on yacon (*Smallanthus sonchifolius*), ulluco (*Ullucus tuberosus*), sweet potato (*Ipomoea batatas*), crosne (*Stachys affinis*), mashua (*Tropaeolum tuberosum*), oca (*Oxalis tuberosa*) and Jerusalem artichoke (*Helianthus tuberosus*). The survey was complemented with a desktop study to map the distribution, pathways and extent of these niche crops. The focus of risk identification was put on viruses and nematodes due to their high potential for introduction without being noticed due to lack of typical symptoms. In addition, specifically for viruses, screening through HTS (high throughput sequencing) was performed to detect targeted and untargeted viruses. A limited number of biological assessments was performed for selected viruses and nematodes (e.g. host status of other crops) to gather information in support of pest risk analyses. Literature studies were performed to identify control options for selected pests.

2.2. Project aims

There is an increasing interest in growing and commercializing new and “forgotten” crops. This means an opportunity for growers to produce for niche markets. The planting materials (seed, tubers, cuttings) for these crops could introduce new plant pathogens and pest. Several questions raise from a phytosanitary viewpoint.

We will address the following questions, focusing on tuber producing crops which are vegetatively propagated: yacon, ulluco, sweet potato, crosne, mashua, oca and Jerusalem artichoke:

- Where are these crops cultured, what are the varieties and how is the planting material distributed?
- What is the origin of the planting material?
- Which viruses and nematodes are associated with propagation material and crops?
- What are the phytosanitary risks of these crops?
- Which phytosanitary measures can reduce the introduction and distribution of some of these plant pathogens?
- Can biological characterization data be generated for the contemporary Papaya mosaic virus isolate “PapMV-U” to include transmissibility by contact, host range and symptom expression data across a range of experimental and crop hosts?

- Can biological characterization data be generated for *Ullucus* potyvirus-1 to include transmissibility by UK clones of the aphid species *Myzus persicae* from *U. tuberosus* to a range of experimental and crop hosts?

2.3. Description of the main activities

In a first phase of the project, an inventory was made on the variety and origin of exotic tuber crops in our horticulture. How is the production of these crops evolving, where does the propagation material come from and what are the different pathways of introduction, where are the main producers located and what type of cultures can we recognize.
All information on newly introduced crops (such as types, varieties, origin), on propagation material suppliers, commercial growers and main food stores commercializing these crops was collected through internet search, contacts and interviews.
The Belgian partner produced an extensive (national) questionnaire to ask the commercial growers of the different tuber crops about their experience on the quality and phytosanitary status of the propagation material, the crop in the field and the tubers at harvest. The questionnaire was sent to over 60 growers specialized in growing these new, innovative tuber crops. In addition, a semi-structured interview was performed with the propagation material suppliers for sweet potato. For sweet potato in particular, we found that a lot of different suppliers supply propagation material (slips, tubers) from different origin and quality which required the gathering of additional info. Information about the origin of their propagation material, the method used for propagating the material and the phytosanitary controls performed by the suppliers was also collected through the questionnaire.
In a second phase of the project, the phytosanitary status of the newly introduced crops was assessed. The majority of the project partners focused on the status of the virus diseases. However, the Belgian partner also looked at the presence of nematodes on the propagation material.
The survey and sampling strategy were based on the inventory that was drafted in the initial phase of the project. In addition, for virus analyses, the choice was made to use a limited pooling or even collecting individual samples. This was done, because for the subsequent sequencing strategy, total RNA sequencing (+ rRNA depletion) was chosen, not allowing high pooling (limited sensitivity). In Belgium, samples were taken both for virus and nematode detection and symptomatic samples were preferred. If not available, a random sampling was performed. Additionally, some symptomless samples were also taken and included in the survey. In addition to the samples of crosne, sweet potato, yacon, oca, ulluco, mashua and Jerusalem artichoke, some samples of yam and ginger (initially not foreseen in the project) were included. Besides field samples, also some *in vitro* plants (sweet potato), and tubers bought from seed companies were also included. Some of the material of sweet potato, ulluco, crosne, yacon and mashua were planted in the greenhouse (insect proof) in view of biological characterization experiments. In addition, the plants were also used to assess symptom development. At the end of the project, plants grown from tubers of sweet potato, yacon and crosne were uprooted to take tuber, root, and soil samples for nematode analysis.
In the project, the methodology that was used to asses the virus status, namely Illumina sequencing, was further evaluated. Basically, RNA sequencing on ribodepleted total RNA was performed, followed by a bioinformatic pipeline using VirusDetect as a final step to identify the

viruses. In addition, the open-source cloud based metagenomics pipeline CZ ID (http://www.czid.org) was used for global pathogen detection.
For nematode identification, standard protocols for nematode extraction from potato tubers or plant roots were applied. Briefly, peels were taken from each tuber and macerated in a blender. The mixture was then subjected to centrifugal flotation using the automated zonal centrifuge to extract nematodes. The obtained nematode suspension was analyzed using microscopy and the number and type of nematode was recorded. Most plant-parasitic nematodes were determined up to species level, unless this was not relevant (low numbers, common ectoparasitic nematodes). For some genera of interest (mainly *Meloidogyne*, *Pratylenchus*) identification was always performed up to species level. Molecular analysis was performed once for confirmation of *M. hapla* and to rule out *M. chitwoodi* and *M. fallax*, which are morphologically similar nematode species.
For some of the newly identified viruses, biological characterisation experiments were set up. This was the case for *Physostegia* chlorotic mottle virus, papaya mosaic virus - *Ullucus* and *Ullucus* virus 1. For *Physostegia* chlorotic mottle, the role of leafhoppers (*Empoasca pteridis*) in the transmission of the virus was assessed through insect transmission experiments. Also for *Ullucus* potyvirus-1, insect transmission trials were set up with aphids under strict quarantine conditions. In the insect transmission experiments, virus acquisition and virus transmission were evaluated over a period of 6 weeks. The biological characterization for papaya mosaic virus – *Ullucus* focused on the host range assessment through inoculation studies on a range of experimental host plants (*Chenopodium* spp., *Nicotiana* spp., *Physalis floridana*, *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato), plant to plant and transmission via skin (gloves) contact experiments.
Lastly, towards the end of the project, guidelines were drawn up to help informing and warning the growers with respect to the phytosanitary risks that some of these tuber crops hold. The guidelines were drafted based on the results of the survey and biological characterisation results.
In Germany, plants and planting material were ordered from online nurseries using a private address. The parcels were opened in the diagnostic laboratory and propagated under greenhouse conditions. In total, 65 plants were ordered including potato, topinambur, apios, yacon, mint, patchouli, Jerusalem artichoke (topinambur), sweet potato, oca, wasabi, hop and kantikari. Samples were take and dsRNA extracted as a viral sequence enrichment method and processed by Ilumina sequencing. Out of those 65 plant samples, viral/viroid sequences could only be detected in 8 samples indicating a high proportion of healthy planting material.

2.4. Main results

Many viruses were detected in the tuber crops analysed during the project. In sweet potato, potyviruses are almost always found in any type of sample (from stores, growers, internet shops). In some cases, the combination of sweet potato feathery mottle virus (potyvirus) and sweet potato chlorotic stunt virus was found. It is a combination of particular phytosanitary concern that is often found throughout all sweet potato growing areas and it is causing quite some damage in the crop. The number of viruses found in sweet potato was always high, and this applied to all types of samples (from import, internet, growers). It makes it clear that the vegetatively propagated starting material really needs more attention before it is entering our region. One virus (*Physostegia* chlorotic mottle virus; PhCMoV) drew attention, since it is a

relatively new virus to the EPPO region, and because it has been reported to induce serious disease symptoms in tomato and pepper. This virus was also found in crosne (*Stachys affinis*). In oca (*Oxalis tuberosus*), only one virus of importance was found, namely *Arabis mosaic virus* (ArMV). In mashua and Jerusalem artichoke (*Helianthus tuberus*), the virus diversity was also limited, whereas in yacon (*Smallanthus sonchifolius*) and ulluco (*Ullucus tuberosus*) the virus diversity was quite high. The viruses that were found in ulluco include a tymovirus similar to Andean potato latent virus (APLV) which is a specific phytosanitary concern.

An overview of the commonalities in viruses found between the PRONC partners (Fera (United Kingdom), ILVO (Belgium), JKI (Germany) and WUR/NVWA (The Netherlands)) can be found below. They are grouped per crop and when found in multiple data sets, this is indicated in brackets.

Mashua (*Tropaeolum tuberosum*)

Viruses found by several partners

- Mashua virus Y (potyvirus): WUR/NVWA, ILVO
- Verbena latent virus (carlavirus): WUR/NVWA, ILVO
- Red clover mosaic carlavirus : WUR/NVWA, ILVO

Viruses found by one partner:

- *Physostegia* chlorotic mottle virus (alphanucleorhabdovirus): WUR/NVWA
- Turnip yellows virus (polerovirus): ILVO

Unknown ('new') viruses:

- Potexviruses: FERA (3), WUR/NVWA (1); Polerovirus: FERA (4); Enamovirus: FERA (1); Marafivirus: FERA (1); Umbravirus: WUR/NVWA (1); Reo-like virus: WPR (1); Potyvirus: ILVO (1); Carlavirus: ILVO (1)

Ullucus (*Ullucus tuberosus*)

Viruses found by several partners

- Papaya mosaic virus (potexvirus): WUR/NVWA, FERA, ILVO
- Ullucus potyvirus 1: WUR/NVWA, FERA, ILVO
- Ullucus polerovirus 1: WUR/NVWA, ILVO
- Ullucus comovirus 1: WUR/NVWA, ILVO
- Ullucus tobamovirus 1: WUR/NVWA, ILVO
- Ullucus tymovirus 1: WUR/NVWA, ILVO

Viruses found by one partner:

- Tomato spotted wilt virus (orthotospovirus): ILVO
- Turnip yellows virus (polerovirus): ILVO

Unknown ('new') viruses:

- Comovirus: FERA (2); Polerovirus: FERA (5); Tymovirus: FERA (1); Potyvirus: FERA (1)

Oca (*Oxalis tuberosa*)

Viruses found by several partners

- Arabis mosaic virus (nepovirus): FERA (3), ILVO

Viruses found by one partner:

- Ullucus polerovirus 1: FERA (2)
- Blueberry leaf mottle virus (nepovirus): FERA (2)

Unknown ('new') viruses:

- Potexvirus: FERA (6); Polerovirus: FERA (4); Cytorhabdovirus: FERA (6) ; Ophiovirus: FERA (4); Allexivirus: FERA (2); Enamovirus: FERA (1); Capulavirus: FERA (1)

Yacon (*Smallanthus sonchifolius*)

Viruses found by several partners

- Potato yellowing virus (ilarvirus): FERA (2), ILVO, JKI (1)
- Yacon necrotic mottle virus (badnavirus): FERA (2), ILVO (2), JKI (1)

Viruses found by one partner:

- Yacon virus (capillovirus): ILVO (1)
- Cucumber mosaic virus (cucumovirus): ILVO (1)
- Fragaria chiloensis latent virus (ilarvirus): ILVO (1)
- Pepino mosaic virus (potexvirus): ILVO (1)
- Dahlia mosaic virus (caulimovirus): JKI (1)

Unknown ('new') viruses:

- Capulavirus: FERA (1)

Sweet potato (*Ipomoea batatas*)

Viruses found by several partners

- Sweet potato badnavius B: ILVO, JKI (1)

Viruses found by one partner:

- Sweet potato symptomless virus (mastrevirus): ILVO
- Sweet potato caulimo-like virus (caulimovirus): ILVO
- *Physostegia* chlorotic mottle virus (alphanucleorhabdovirus) : ILVO
- Potato yellowing virus (ilarvirus): ILVO, JKI (1)
- Sweet potato chlorotic stunt virus (crinivirus): ILVO
- Cucumber mosaic virus (cucumovirus): ILVO
- Tomato mosaic virus (tobamovirus): ILVO
- Radish mosaic virus (comovirus): ILVO
- Sweet potato leaf curl virus ? (begomovirus)
- Sweet potato feathery mottle virus (potyvirus): ILVO
- Sweet potato virus C (potyvirus): ILVO
- Sweet potato virus G (potyvirus): ILVO
- Sweet potato virus 2 (potyvirus): ILVO
- Sweet potato badnavirus A: ILVO
- Sweet potato badnavirus C: ILVO

The following crops were only analysed by ILVO

Yam (*Dioscorea cayenensis/alata*)

- Potato yellowing virus (ilarvirus)
- Yam virus Y (?, Betaflexiviridae))
- Yam mild mosaic virus (potyvirus)
- Yam necrosis associated virus (?, Secoviridae)
- Dioscorea bacilliform virus (badnavirus)
- Potato yellowing virus (ilarvirus)

Chinese artichoke (= crosne; *Stachys affinis*)

- *Physostegia* chlorotic mottle virus (alphanucleorhabdovirus)

Jerusalem artichoke (*Helianthus tuberosus*)

- new caulimovirus

- Plum pox virus (potyvirus)

The following crops were only analysed by JKI

Potato (*Solanum tuberosum)*

- Potato virus Y (potyvirus)

Hop (*Humulus lupulus*)

- Hop latent virus (carlavirus) (3)
- Hop latent viroid (cocadviroid) (1)

No nematodes of major concern were found in tubers from the field and from planting material acquired from suppliers, nor in the pots with plants and new tubers grown from sampled planting material. However, we detected relatively high numbers of *Pratylenchus* species (mainly *P. penetrans/ P. fallax*) in several field samples of sweet potato. Similarly, the root-knot nematode *Meloidogyne hapla*, was extracted from pots planted with yacon. The tropical nematodes *M. incognita* and *M. javanica* were found in high numbers, infecting ginger rhizomes and roots in one greenhouse. Although none of these nematode species have a quarantine status and are commonly found in Belgium, attention should be paid to the spread of plant-parasitic nematodes via planting material. This also warrants for caution when including new tuber crops in rotations with susceptible field crops, such as potato, carrot, bean, pea.

The survey, questionnaire and interviews revealed that except for sweet potato, most planting material of these niche tuber crops is sold without plant passports (and ginger rhizomes were even obtained from the supermarket). Even though for sweet potato plant passports are available, the interviews with the suppliers reveal that the planting material is often not subject to any external quality control, or only a visual control.

2.5. Conclusions and recommendations to policy makers

The results of the virus screening in the different crops definitely indicate the need to pay more attention to the presence of viruses of phytosanitary risk in these crops. Our survey only checked a limited number of samples of each tuber type, and already revealed a lot of viruses, as well as some nematodes. We think that chances to find more infected plants and spread of diseases via uncertified planting material is quite high.

This goes for all of the tested crops. Taking into account that the tuber crops are propagated in a vegetative manner, these viruses will not disappear from the crops and will be continuously spread along the distribution channels. The crop that is gaining the most interest in the last years, and also growing rapidly in terms of production, is sweet potato. Therefore, a lot of attention needs to go to testing imported propagation material of this crop. In parallel, research is needed to remove the viruses of phytosanitary concern of the starting material. This is currently ongoing, and there is good hope that in the near future, clean starting material for sweet potato will be available. The two other crops that still require a lot of attention are ulluco and yacon. These are also, after sweet potato, the two crops in which there is the most interest by amateur and professional growers. Also for these two crops, testing imported propagation material is required, and as long as the phytosanitary risks for some of the viruses are not clear

(e.g. the tymoviruses in ulluco) it is advised not to use planting material that contains these viruses.
For mashua, oca, crosne, and Jerusalem artichoke monitoring the situation in the future is still advisable (particularly for the presence of ArMV (oca), PhCMoV (crosne), and PPV (Jerusalem artichoke)), yet the phytosanitary risks are generally considered as not high.
No nematodes of major concern were found in tubers from the field and tubers sold as planting material. However, plant-parasitic nematodes were found in half of the samples, and consisted mainly of root-lesion nematodes (*Pratylenchus* spp.). Mashua, yacon and crosne even contained very high numbers of *P. penetrans* and *P. fallax*, indicating that they might be good host plants. Although only *P. penetrans* has an RNQP status, all *Pratylenchus* species are commonly found in Europe and attention should be paid not to spread these damaging nematode species through planting material between growers' fields.
The finding of 3 *Meloidogyne* species (*M. hapla, M. javanica, M. incognita*), the most widely spread nematode genus, on 5 samples of yacon and ginger, indicates how easy this sedentary endoparasitic nematode travels along with planting material (rhizomes, tubers, roots). This is also true for nematodes with similar biology and hosts (*Nacobbus aberrans*). International trade should only be allowed for certified planting material, more specifically for *Meloidogyne* spp.
The nematodes found in some of the tubers indicate that tubers as planting material can spread *Pratylenchus* spp. and *Meloidogyne* spp. However, this is also the case for other tuber crops e.g. potato. Quality of planting material is here an issue, rather than phytosanitary control.

<u>The consortium recommends the following</u>:
The results of the virus screening in the different crops definitely indicate the need to pay more attention to the presence of viruses of phytosanitary concern in all of these tuber crops. When importing these tuber crops, as well as when trading these tubers, the phytosanitary status with respect to the presence of viruses as well as the phytosanitary risk that is involved, should be known better.
The same recommendation can be extended to the nematodes, although we did not find any species of phytosanitary concern in the samples. Still, international exchange of tuber material should be monitored as a preventive measure, to prevent import of quarantine nematodes such as *M. enterolobii*.
Growers should be discouraged to plant tubers or rhizomes sold without a plant passport, or, even worse, acquired from grocery stores. These materials pose a great risk for introduction of damaging pathogens, mainly viruses, but also nematodes, into their fields. In addition, it is worth mentioning that in several EU countries, a lot of attention is now given to assessing the starting material of sweet potato.
Further recommendations for decision makers and research are the following:

- Growers should be made aware of the dangers of imported planting materials, including tubers or rhizomes, especially from outside EU. The advantages of plant passports should be highlighted.
- Training of inspection services and growers to recognize symptoms of viral and nematode infection.
- Include sampling of traded planting materials by inspection services (NPPOs), especially for the detection of viruses in sweet potato, ulluco and yacon.
- Impose plant passports in trade of these new crops

- If production of new tuber crops becomes increasingly popular, it is advised to investigate their host status for pathogenic viruses and nematodes such as *Pratylenchus* spp. and *Meloidogyne* spp. which are regularly found in fields and /or quarantine nematodes (*P. penetrans, P. fallax, M. hapla, M. chitwoodi*).
- Fields meant for production of planting material should be checked for the presence viruses and endoparasitic nematodes, especially if the tuber crops are hosts.

2.6. Benefits from trans-national cooperation

Recent advances in high-throughput sequencing (HTS) technologies and bioinformatics have generated huge new opportunities for discovering and diagnosing plant pathogens, in particular viruses and viroids. Plant health has undoubtedly benefited from these new methodologies, but at the same time, faces now substantial bottlenecks, namely the biological characterization of the newly discovered viruses and the analysis of their impact at the biosecurity, commercial, regulatory, and scientific levels (Massart et al. 2017).

Also in PRONC, the project benefited greatly from a research collaboration effort between the transnational partners. However, a huge amount of data was generated and several novel (undescribed) viruses were detected and it is now particularly challenging to continue this collaboration effort and continue to characterize new viruses and to assess the risk they pose, with genome sequence information as the unique starting point. The amount of information needed to assess the risk posed by a new virus species to a certain commodity or region is huge. Scientists may indeed have to work for years to provide the answers needed to conduct a thorough PRA according to international phytosanitary standards (ISPM 2 and ISPM 11). Therefore, continued intensive collaboration between the plant health laboratories is extremely important. This starts with exchanging initial findings in eg. data sharing initiatives. However, this needs to be complemented by biological characterization experiments and assessment of biological properties not forgetting appropriate communication with the regulatory authorities, since the increasing identification of new viral sequences by HTS technologies may complicate the decision-making process for certification programs, quarantine processes, and more generally, the trading of plant materials. The only information available may be the full or partial genome sequence with the additional complexity that it may have been detected in asymptomatic samples or in combination with other viruses. It is increasingly important to understand the biology of any new viral sequence to provide a basis for assessing the risk they pose and take scientifically-based decisions. Scientists are therefore now challenged to provide biological data on these newly described viral sequences, in a short timeframe and with limited funding. In that context, transnational cooperation is of utmost importance to efficiently generate the required data.

3. Publications

3.1. Article(s) for publication in the EPPO Bulletin

In preparation.

3.2. Article for publication in the EPPO Reporting Service

None.

3.3. Article(s) for publication in other journals

In preparation.

4. Open Euphresco data

None.