Characterisation and control of sour rot caused by *Geotrichum* spp. on peach trees in the Baix Segre area
Acronym GEOPEACH

Summary

During the last decade, a great many resources have been dedicated to understanding the epidemiology and control of rot caused by *Monilinia spp*., the main pathogen in stone fruit. However, the spread over the last 5-6 years of *Geotrichum spp*. which causes sour rot, with incidence even higher than *Monilia spp*. in some cases, makes it essential to study the epidemiology and infection process of the fungus, as well as control measures in the field and/or post-harvest to reduce its incidence as far as possible. At present, there is no specific treatment for this fungus and although the efficacy of certain active substances has been reported in other countries, in Spain they are either not authorised for farming or have been used and not proved truly effective. Consequently, the emergence of this new scenario in which *Geotrichum* is increasingly gaining strength will be a major challenge for companies in the sector, which will have to face a new enemy whose epidemiology and tools for its control are still unknown. It is true that the source of the inoculum is the field, but it is during post-harvest that its aggressiveness causes most of the losses and complaints, hence the financial cost of a consignment affected by sour rot reaching the destination market, e.g. containers exported to Brazil, the United Arab Emirates, etc., not only has a major economic impact, but also a major impact on customer confidence and on the environment.

Objectives

The general objective is to obtain a better understanding of the *Geotrichum* spp. infection process in peach trees, factors affecting the spread of the disease and possible means of control. The specific objectives are to:

1. Determine the presence and distribution of the inoculum in stone fruit farms and the effect of factors involved in its infection process
2. Determine the presence and distribution of the inoculum in different areas of the plant
3. Determine which products are effective for the control of *Geotrichum* spp. in order to establish a control strategy
4. Determine which post-harvest practices are effective in reducing the central inoculum and slowing the development of the disease

Description of the actions carried out in the project

The following actions have been carried out:

- **Action 1:** determining the presence of *Geotrichum* spp. on farms with a history of the disease by sampling soil, leaves and fruit (sources of the inoculum in the field) and relating this to agrometeorological and nutritional factors on each farm, to correlate them with the incidence of sour rot at harvest. Studying different pathogen and host-related factors involved in the infection process: inoculum concentration, pectinolytic enzyme production, fruit maturity stage, etc.
- **Action 2:** determining the presence of *Geotrichum* spp. in different areas of the plant (sources of inoculum in the plant) and checking the real risk of infection posed by the plant by monitoring the sour rot in entries vs packed product. Assessing a collection of *Geotrichum* spp. isolates from field and central isolates. Assessing the influence of temperature (T) and relative humidity (RH) storage conditions on post-harvest survival of *Geotrichum* spp.

- **Action 3:** determining the efficacy of synthetic fungicides and alternative products on isolates of *Geotrichum* sp. in vitro and in vivo.
- **Action 4:** studying the effect of storage conditions (normal cold, controlled atmosphere or modified atmosphere product packaged in bags) on the development of the disease and assessing the efficacy of different soaps and disinfectants to reduce the inoculum in the plant.

Final results and practical recommendations

1. Population of *Geotrichum candidum* was found in the soil of the farms and on the leaves of the trees, at very different levels among the four farms, without being directly related to the final incidence of sour rot in harvested fruit. It therefore seems clear that the final incidence of the disease depends on other factors, such as the presence of damage to the fruit, maturity at harvest, chemical treatments in the field, cultural practices and agroclimatic factors. The farm is a source of the inoculum for the pathogen and these results indicate that part of the solution to control the disease should include reducing the inoculum in the field, through such measures as treatments, soil management practices and use of plant cover.
2. An artificial inoculation methodology was defined, which is absolutely essential for carrying out efficacy studies, among others. After studying different incubation T and RH conditions, inoculation methodologies, conditions with or without damage and the inoculum concentration, among other factors, the following points were established as the artificial inoculation protocol: inoculate 20 µL of a spore suspension adjusted to 10^6 sp/mL into damaged fruit, and incubate for 24h at 30°C + 6 days at 20°C, at a high RH at all times.
3. Pectinolytic (PG) enzymes contained in the juice generated by the rot have been found to play a key role in the capacity to infect, spreading the disease and causing an exponential increase in the shrinkage due to peeled (non-commercial) fruit. This juice also contains spores, making it a very dangerous weapon.
4. The fruit and vegetable plant is also a source of inoculum, especially the packing line surfaces and, to a lesser extent, the pallets. The *G. candidum* population at these sites is able to survive for 24-48h. Cleaning and disinfection procedures are essential and have proven fully effective in reducing contamination if applied correctly.
5. Water from pallet tipping pools or the hydrocooler are not a source of inoculum as long as sufficient levels of disinfectant are used. ClO_2 and the peracetic acid + hydrogen peroxide formulation provide satisfactory control with only 1 minute's contact, both at the commercial dose and at half dose. However, in the case of using hypochlorite as a disinfecting agent, care must be taken to maintain an adequate and stable dose to minimise the risk of infection. Storage and/or shipping chamber environments would not be considered a source of inoculum.
6. In the sampling carried out by the companies incubating fruit from entries and after packaging, the incidence of sour rot increases by over 50% between these two points. It should be stressed that the real problem caused by this disease is not reflected in this type of control, since the main cause of loss is peeling that the pectinolytic enzymes (PG) generated by rotten fruit cause in the healthy fruit in its vicinity. It is essential to inform harvesters of the characteristics of this type of rot, to ensure they avoid putting rotten fruit in the pallet, clean the bins, etc.

Conclusions

Probably the most important finding from the project was the discovery that 1 out of 3 pieces of fruit showing symptoms of sour rot were caused by a pathogen other than *G. candidum*. Different species of the genus *Pichia (P. kluyveri, P. kudriavzevii* and *P. manshurica*) were identified at the molecular level. This result led to the redesign of the remaining studies, since if sour rot was caused by a variety of micro-organisms, the effectiveness of chemical treatments, disinfectants, the effect of the type and conditions of storage, etc., needed to be assessed on at least these two pathogens: *G. candidum* and *Pichia kluyveri*.

These two species have optimum growth rates at 30-35°C and at very high water (a_w) or RH levels, a fact that was already suspected, since their incidence increased after periods of rain with heat waves. However, at 20°C their growth in fruit is slowed down considerably when the RH is low, at 60%, these being the usual conditions in a packaging room or supermarket. This leads us to think that contamination on the surfaces of the packaging lines may not pose a very high risk, because even if the fruit is infected, the conditions for development would not be optimal.

It is clear that sour rot will not be an easy disease to control using chemical treatments in the field or post-harvest, since, of the total of 13 synthetic fungicides and 15 alternative products assessed, only tebuconazole (Konan), two garlic extract-based formulations (Proalllium and Agroallium EVO), one based on essential oils (Araw) and calcium polysulphide (Curatio) showed a significant reduction when assessed in vitro. However, when tests were carried out on artificially inoculated fruit, none provided a satisfactory result.
The usual conditions of preserving stone fruit (normal cold, controlled atmosphere and modified atmosphere packaging) would not have, *a priori*, any effect on the development of *G. candidum* since the incidence and severity in artificially inoculated fruit did not show significant differences between the different types of preservation over a period of 30 days. In contrast, *P. kluyveri* does appear to be more adapted to CA or MAP bag conditions, as its incidence and severity rise substantially as the level of CO_2 increases.

Leader of the Operational Group

ORGANISATION: Cooperativa Agropecuària de Soses, SCCL

Coordinator of the Operational Group

ORGANISATION: IRTA - Institute of Agrifood Research and Technology

Other members of the Operational Group (grant recipients)

ORGANISATION: Agrícola ESPAX, S.L.

Other members of the Operational Group (not recipients of the grant)

ORGANISATION: -

Geographical area(s) of application

PROVINCE(S)	REGION(S)
Lleida	Segrià

Dissemination of the project (publications, conferences, multimedia, etc.)

The results were communicated and disseminated through yearly meetings with the participating companies, and Dr Carla Casals gave a presentation on the results to date at the IRTA Post-harvest Workshop 2021, which had to be held online due to COVID-19 restrictions. The presentation was entitled "*Geotrichum* and *Rhizopus*, emerging diseases in stone fruit. What do we know about them? (GEOPEACH and GOMORI)".

An explanatory article was published in the journal *Horticultura*: Casals, C.; Plaza, P.; Vilanova, L.; Sisquella, M.; Torres, R.; Teixidó, N. 2021. *Geotrichum* spp. y *Rhizopus* spp. enfermedades emergentes en fruta de hueso. ¿Qué sabemos de ellas? Horticultura. www.interempresas.net/A354049.

Project website

More information on the project

PROJECT DATES	TOTAL BUDGET
Start date (month-year): July 2020	Total budget: €91,319.00
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