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Executive Summary

This document comprises a detailed description of the outcomes of Task 5.4 – Major Pests and Diseases Monitoring and Control and it is aimed to design an expert system able to suggest possible interventions according to state-of-the-art agronomic procedures for hazelnut plants. These interventions are guided by indicators which identify the presence and the severity of certain pests/diseases and by the historical data present in the database.

Furthermore, in this document the following sub-tasks have been addressed:

1. Validation of remote sensing data with field data;
2. Identification of the best date to apply the IPM strategies;
3. Optimization of the quantities and the typology of pesticides.

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Abbreviations and Acronyms

ANOVA	Standard analysis of variance
API	Application programming interface
Chla+b	Chlorophyll a+b
CWSI	Crop water stress index
DEM	Digital elevation model
DN	Digital Number
DSM	Digital surface model
ELC	Empirical line claibration
FOV	Field of view
GI	Greenness index
GPS	Global Positioning System
IoT	Internet of Things
MCARI	Modified Chlorophyll Absorption in Reflectance Index
MGRVI	Modified Green Red Vegetation Index
NDCI	Normalized difference chlorophyll index or normalized difference red edge index
NDVI	Normalized differential vegetation index
NGN	Nut gray necrosis
NGRDI	Normalized green red difference index
NIR	Near-infrared
OSAVI	Optimized soil-adjusted vegetation index
PRI	Photochemical reflectance index
RECI	Red edge chlorophyll index
RDVI	Renormalized difference vegetation Index
RGB	Red Green Blue
RGBVI	Red green blue vegetation index
RTK	Real time kinematic
SCADA	Supervisory control and data acquisition
SfM	Structure from motion
SIPI	Structure insensitive pigment index
SR	Simple ratio index
UAV	Unmanned aerial vehicle
UGV	Unmanned ground vehicle
VARI	Visible atmospherically resistant index
VDVI	Visible difference vegetation index
VNIR	Visible and near infrared
WP	Work package
GPS	Global Positioning System
CNN	Convolutional Neural Network

1 Introduction

The aim of Task 5.4 – "Major Pests and Diseases Monitoring and Control" is to define an expert system to suggest possible interventions in accordance with state-of-the-art agronomic procedures for hazelnut plants. The interventions are based on indicators which identify the presence and the severity of certain pests/diseases and of the historical data present in the database.

The most important factors causing hazelnut biotic stress are some arthropods (such as mite, coleopteran and heteropteran) and phytopathogens as reported in Deliverable 4.5. The biotic stress factors considered in PANTHEON project are:

- *Phytoptus avellanae*, an acarine gall-mite, about 0.3 mm long, that infests the buds causing important reduction in the harvest in particular if female flower buds are affected; this mite migrates during the spring months as nymphs to new axillary buds. Mite population increases from July through the following spring, causing bud swelling and the appearance of typical big bud symptoms resulting in a loss of viability of the infested buds.
- True bugs (*Halyomorpha halys*, *Gonocerus acuteangulatus*, *Palomena prasina*, *Piezodorus lituratus*, *Rhaphygaster nebulosa*, *Nezara viridula* and *Dolycoris baccarum*) are widespread on hazel throughout the world and cause substantial losses. These species, which belong to the Rynchota order and Heteroptera suborder, cause a reduction in the quantity and quality of hazelnuts production. Their feeding activity on nuts causes, depending on the phenological stage of the hazelnut, the traumatic abortion damage, in which the growth of the kernel is interrupted, and the so-called "cimiciato", which consists of malformations of nuts, sometimes resulting in an unpleasant taste and a reduction in organoleptic characteristics
- *Monostichella coryli* is a plant pathogen infecting hazelnut crop and causing anthracnose. The disease has two different expressions during the same vegetative season. In early spring, necrotic spots can be observed on affected buds that quickly increase in size and lead to death whereas in late spring-summer, the fungus starts inciting necrotic lesions that can expand on a broad portion of the leaf.

Insect and mite pests as well as pathogens are major detriments to production of hazelnuts throughout the world [1, 2]. Thus, the management of phytosanitary emergencies in hazelnut production has always been of great importance in order to harvest a healthy product with appropriate organoleptic characteristics. Monitoring and control practices for these pests and diseases have always been based on the work of expert agronomists who must periodically check the presence and consistency of damage agents in the field to apply, when the level of presence of pests and diseases is getting close to predefined economic injury level, appropriate control strategies at the right time and in the right way [3].

These management activities are however very time-consuming, thus, the use of enabling technologies, such as the automatization of at least part of the monitoring or control activities would alleviate human effort, while preserving precise control of the plants state.

In this deliverable we will report:

i) the standard phytosanitary practices that are commonly applied to contrast the main entomological and microbiological problems;
ii) the results of the actions carried out within the PANTHEON project with the aim of the automatization for the management of these problems;
iii) the new guidelines aimed at reducing chemical inputs and implementing environmental sustainability, while maintaining high quality standards.

It is necessary to point out that these practices for managing phytosanitary problems are governed by a series of European, national, and regional regulations whose priority objectives are to protect human health and the environment. For this reason, a process of revision of plant protection products has been undertaken in recent years, which has contributed significantly to changing the European scenario of the chemical products market in agriculture, ensuring a very high level of safety for the European agricultural products.

At present, each farmer must choose between "**organic**" and **"integrated production"** (IPM).

The **organic production** approaches the farm management and the agri-food production considering the interaction between multiple factors, such as: the best environmental practices, a high level of biodiversity, the preservation of natural resources and the use of natural substances and processes when applying the standards of production.

Regarding the **integrated production**, in Italy, the Legislative Decree no. 150/2012, implementing European Directive no. 128/2009 and subsequently the "National Action Plan on the sustainable use of plant protection products" (PAN), identified two levels of application for integrated pest management (IPM):

- a **mandatory IPM**, which concerns the application of techniques for the prevention and monitoring of crop pests, the use of biological means to control them, the use of appropriate cultivation practices and the use of plant protection products that present the least risk to human health, from those available on the market.
- a **voluntary IPM** (Sistema di Qualità Nazionale di Produzione Integrata, SQNPI) that provides for the application of integrated production specifications, of which integrated pest management is a fundamental aspect.

In addition, the Latium Region, where the PANTHEON field is located, has implemented the European and Italian regulations (Directive n. 128/09/EU, Italian Legislative Decree no. 150 of 14 August 2012, Ministerial Decree of 22 January 2014 on the PAN (National Action Plan on the Sustainable Use of Plant Protection Products), Regulation (CE) no. 1107/2009, and subsequent acts, with particular reference to the list of active ingredients candidates for substitution in Reg. no. 2015/408 of 11/3/2015 and subsequent amendments) and has therefore produced "Technical standards" [4] for the integrated management of phytosanitary emergencies in hazelnut production.

All these aspects have been considered in the definition of the new guidelines.

2 Current agronomic strategies for detection and management of pests and diseases

2.1 True bugs

The true bugs (Rynchota Heteroptera) such as the box bug, *Gonocerus acuteangulatus* (Figure 1), the green shield bug, *Palomena prasina,* the southern green shield bug *Nezara viridula* and the newly introduced in Italy, the brown marmorated stink bug, *Halyomorpha halys* play a fundamental role in the hazelnut plantation as they can cause significant reductions in both quantity and quality of the product. All these pests, both at preimaginal and adult stage, insert their mouth parts into the nuts causing two different kinds of damages, depending on the moment when the trophic activity is conducted: i) the traumatic abortion at the beginning of seed development, occasionally with the premature nuts fall and ii) the "*cimiciato*", induced when the nuts are fully developed, which consists in the necrosis of the cotyledon tissues with the appearence of black or white spots and shrivelling, as well as an alteration of organoleptic characteristics with an unpleasant taste. These pests usually overwinter as adults outside the orchards, in natural shelters such as evergreen shrubs or more easily in artificial structures where they can find favorable environmental conditions to overcome the severity of winter. In late spring/early summer, the true bugs begin to move towards the hazelnut plantations in search of food sources and, in particular, of growing nuts.

Figure 1 Adults of Gonocerus acuteangulatus *feeding on nuts.*

2.1.1 Monitoring

Their monitoring, which is fundamental in the determination of effective control strategies, is usually conducted following at least two different methodologies.

When adopting the first one, usually called "*frappage*", the collection of insects present in the canopy relies on laying a white sheet on the ground under the canopy on which selected branches are shaken to cause the drop of bugs (Figure 2). More specifically, the branches of six half-plants per hectare (corresponding to three whole plants) are shaken over the sheet on a weekly basis, from May to September, in the early morning to prevent the adults from flying away. Then, all the material collected is examined in the laboratory for species identification and relative biological forms.

Figure 2 White sheet laid on the ground under the canopy for the collection of insects with the frappage *method*

When employing the second method, the presence of true bugs in the field is assessed by using commercially available traps. This approach recorded a major impulse in recent years, following the spread of the highly damaging and polyphagous true bug, *Halyomorpha halys*, throughout the world. The research of methods to contrast the populations of this phytophagous has led to the definition of an aggregative pheromone to bait traps and capture not only the above-mentioned bug species but also all the other bugs that can be found in the main crops, including hazelnuts. This method exploits the use of a pheromone which is coupled with a sticky cardboard, leading all the bugs attracted by the pheromonal stimulus to be captured. The traps are then examined by experts and the true bugs counted.

Both methodologies are time-consuming activities carried out by expert agronomists who, at fixed time intervals, usually on a weekly basis, have to resort to the frappage method or check the traps deployed in the field identifying the target insect species and counting the number of specimens. When the number of collected insects exceeds a predefined threshold, control measures must be performed.

2.1.2 Control

The chemical control of true bugs is required if their presence in the field reaches an average number of 2 specimens per plant. The possibility of using different active ingredients depends on the production system the farmer decides to adopt. The choice is limited to organic and integrated crop management. The latter is divided in turn into voluntary (SQNPI) and mandatory. The Latium region guidelines [4] for the true bugs integrated management in hazelnut plantation are summarized in the following Table 1.

Table 1 Integrated true bugs management according to Latium regional regulations

Pest	**Threshold**	**Crop management**	**Active ingredients (number of treatments allowed)**	**Note**
True bugs	2 specimens per plant	SQNPI (Voluntary IPM)	Azadirachtin Etofenprox (1) λCyhalothrin (2) Indoxacarb (1)	In any case, no more than 3 treatments between

			Deltamethrin (2)	Pyrethroids and Etofenprox
		Mandatory IPM	λCyhalothrin Mineral oil Indoxacarb Etofenprox	
		Organic	Mineral Oil	

2.2 Gall mites

The gall mite, *Phytoptus avellanae* is a crucial pest in hazelnut production. This mite, belonging to Arachinida class and to Eriophyoidea superfamily, causes severe symptoms on generative and vegetative buds. The resulting damage consists of swelling buds and the appearance of typical large bud symptoms (Figure 3) from winter until the first weeks of spring. During the spring, it migrates to new axillary buds, colonizing them. *Phytoptus avellanae* populations increase from the summer through the following spring, causing the colonized buds to lose their vitality. Common gall mite management practices in Latium Region are regulated, as mentioned above for true bugs, in accordance with regional laws [4]. The next paragraphs describe the operations that agronomists must carry out in the field for the monitoring and control of the gall mite.

Figure 3 Phytoptus avellanae specimens inside a gall (left); galls caused by the mite (right)

2.2.1 Monitoring

Usual mite monitoring requires that infestation levels are assessed by agronomists during the winter on 10 selected plant per hectare. In the winter months indeed, the buds compromised (galls) by the action of the mite can be easily discriminated from healthy and still closed buds. On the 10 plants, one branch per cardinal direction is selected and the infestation is evaluated as the ratio between the number of galls and the number of buds per branch. The intervention threshold above which chemical treatment is necessary is 15-20 % of buds affected by the mite.

2.2.2 Control

As in the case of true bugs, the use of active ingredients to control the mite is restricted by European, national, and regional regulations. Common practices for controlling this pest recommend a sulphur treatment when the mite migrates from the infested buds (galls) to the healthy ones, when the new buds have 3-4 leaves that have completely opened. In the northern hemisphere and particularly in Italy, this happens generally, for early varieties at the end of February and between April and June for other cultivars.

The following Table 2 shows the active ingredients that can be used in the Latium region, where the experimentation was carried out, according to the management that the farmer decides to adopt.

Table 2 Integrated gall-mite management according to Latium regional regulations

Pest	Threshold	Crop management	Active ingredients (number of treatments allowed)	Note
Gall-mite (*Phytoptus avellanae*)	15-20 % of buds affected by the mite	SQNPI (Voluntary IPM)	Sulphur Mineral oil	-
		Mandatory IPM	Sulphur Mineral oil Clofentezin Pyrethrum	
		Organic	Sulphur Mineral oil Pyrethrum	

2.3 Bud rot and leaf anthracnose

The disease is caused by *Gloeosporium coryli* (synonyms *Monostichella coryli, Labrella coryli*, *Piggotia coryli, Cheilaria coryli*) and has two distinct pathogenic expressions during the same vegetative season. In the early spring, at the beginning of the vegetative season, the pathogen causes a bud rotting, with necrotic spots that quickly increase in size and lead the infected buds to death, even before their sprouting (Figure 4). In some circumstances, the pathogen is also entitled for an early necrosis of male inflorescences. Later in the season, in late spring-summer, the fungus starts inciting necrotic lesions on the leaves that expand on substantial portions of the leaf lamina, affecting the photosynthetic activity of the plant.

Figure 4 Symptoms of Monostichella coryli *on buds and twigs in springtime and on leaves in summertime.*

2.3.1 Monitoring

Despite the significant detrimental effect of this disease, it is difficult to estimate the exact yield losses due to the pathogen because of the complicated relationship between the reduction of photosynthetic activities and the shrinking in fruit production. Also, the pathogen is able to overwinter as mycelium in dormant buds so that the incidence of the disease in a given year is, among the other variables, related to the abundance of the disease in the previous year. All these biological facets make this pathogen particularly problematic to be scrutinized and eventually anticipated, so that the monitoring is basically committed to the personal experience of the agronomist.

2.3.2 Control

As a result of the uncertainties described above, the control of the disease usually relies on preventive treatments with copper salts, in different formulations and in different moments from the leaf fall to the bud sprouting, to prevent the occurrence of new infections. Also, treatments with methyl thiophanate, a fungicide with a wide spectrum against several fungal diseases, are effective. Given the systemic action of the fungicide, it must be distributed, in autumn, before the complete fall of the leaves to be phloematically translocated inside the plant. However, this active ingredient is no more allowed in Latium region nor in IPM and organic management (Table 3). The following table shows the only active ingredient, the copper, that can be used in the Latium region, where the experimentation was carried out, according to the management that the farmer decides to adopt.

Table 3 Integrated anthracnose management according to Latium regional regulations

Disease	Threshold	Crop management	Active ingredients (number of treatments allowed)	Note
Anthracnose	-	SQNPI (Voluntary IPM)	Copper (max 4kg/ha/yr)	Max 28 kg/ha in 7 years
		Mandatory IPM		
		Organic		

3 New approaches to pests and diseases management

3.1 True bugs

3.1.1 Early automated detection of true bugs

The possibility of intercepting the beginning of the colonization phase of the orchard by true bugs can be extremely useful and it act as a trigger for more effective monitoring activities. In particular, the *frappage* which allows the identification of the population density present in the field.

Thus, we developed an automatic pest detection system, presented in [5], by exploiting the visual information of the sticky traps deployed around the hazelnut field to ascertain the beginning of the colonization phase in the hazelnut plantation. This makes it possible to activate the monitoring activity (*frappage*) only and exclusively when the traps indicate the presence of the true bugs, thus allowing optimization of human activities in the field.

Given the complexity of manually defining relevant features for identifying bugs and distinguishing them from other possible insects and/or objects stuck on the trap, we resorted to a data-driven approach based on Convolutional Neural Networks (CNNs). We resorted to You Only Look Once (YOLO) framework [6] which allows for real-time processing.

For this purpose, adults of *Palomena prasina* were collected in autumn before they could find shelters where to overwinter. These specimens were put on 50x50 cm white plastic boards on which a non-water-soluble glue (Temocid) was applied (Figure 5).

Figure 5 Example of two Palomena prasina *specimens glued on the cardboard.*

We collected a dataset in outdoor environments to reproduce realistic operating conditions. As in real-world field applications, other objects, such as leaves or dirt, and/or no target insects got trapped on the sticky boards. Examples of images in the collected dataset are reported in Figure 6. Images were recorded with resolution 1280×720 with a 3D camera MYNT EYE D120 which also allows capturing the depth map with a nominal accuracy of 5 mm. A total of 3777 images were collected and manually labeled. The dataset has been made public at the following link: https://tinyurl.com/y4rv9wo5 to boost reproducibility and results comparison.

Figure 6 Examples of dataset images with different levels of complexity for detecting bugs.

Furthermore, data augmentation techniques were implemented to synthetically generate additional data based on the collected ones, thus improving the generalization capabilities of the network. More specifically, we applied color and geometric transformations, mosaic augmentation (patching four images into one), background blurring and Gaussian noise addition. At training time, the YOLO architecture was fed with images and annotated bounding boxes and was trained to minimize a loss function based on the Intersection over Union (IoU) index, i.e., the ratio between the area of overlap and the area of union between ground truth and predicted boxes. In addition, the influence of the depth map, synthesizing distance measures, on the bugs detection performance was investigated.

The activity resulted in a mean average precision of 94.5% on a holdout dataset composed of 611 novel RGB images. Best performance was obtained when using color and geometric transformations, as well as mosaic augmentation. Examples of the predicted bounding boxes along with confidence scores are shown in Figure 7. False positives, generated by very similar insects, are highlighted with red x-marks, while the single false negative is marked with red circles. Similar accuracy was also obtained with RGB-D data, for which, however, the nominal accuracy of the camera does not allow to fully sense different distances with insects/objects of such small dimensions. Finally, the real-time effectiveness of the approach was also validated by deploying the detection system on a NVIDIA Jetson Xavier which can be integrated onboard any robotic platform. In particular, the detection system works at 49.5 fps.

Figure 7 Examples of predicted bounding boxes with respective confidence scores. The false positives are highlighted with red x-marks and the only false negative with a red circle.

Methods and results

The presence of true bugs in the hazelnut orchard does not necessarily lead to the occurrence of damages. The nuts must be in a phase of susceptibility to the action of the phytophagous for damages to arise. Pinpointing the moment of maximum nuts susceptibility to the true bugs activity is crucial for identifying the most effective pest control strategies. To do that, a preliminary investigation was conducted in 2018 in a hazelnut plantation of IRTA Research Center (Mas Bové, Constantì, Catalogna, Spain). This activity was carried out by UNITUS staff as part of a collaboration with the IRTA research institute and concerned a species, the *Palomena prasina*, which is commonly harmful to hazelnut production in Italy too. The study provided a useful starting point from which further experiments, in the PANTHEON field, were conducted. Thus, in the IRTA orchard, 5 branches per plant were selected from 12 hazelnut trees. All these branches were wrapped with sleeves in May to preserve them from the trophic action of the bugs. A total of 60 sleeves were put on the selected plants. Five *Palomena prasina* specimens, collected in the same field, were inserted inside each sleeve, at different timing (Time1, Time2, Time4 and Time5) and kept inside for one week in the thesis Time1 and Time4 and two weeks in the thesis Time2 and Time5, to induce the described different damages (Table 4). The thesis Time3 was considered as control and no bugs were inserted into the sleeves.

Table 4 Treatments, targeted damage, number of sleeves and date of introduction and removal of Palomena prasina *specimens during the preliminary experimentation conducted in Spain.*

Thesis	Targeted Damage	N. of Sleeves	Date of Introduction	Date of Removal
Time1	Traumatic abortion	12	17/05/2018	24/05/2018
Time2	Traumatic abortion	12	24/05/2018	06/06/2018
Time4	*"Cimiciato"*	12	11/07/2018	19/07/2018
Time5	*"Cimiciato"*	12	18/07/2018	31/07/2018
Time3	Control	12	-	-

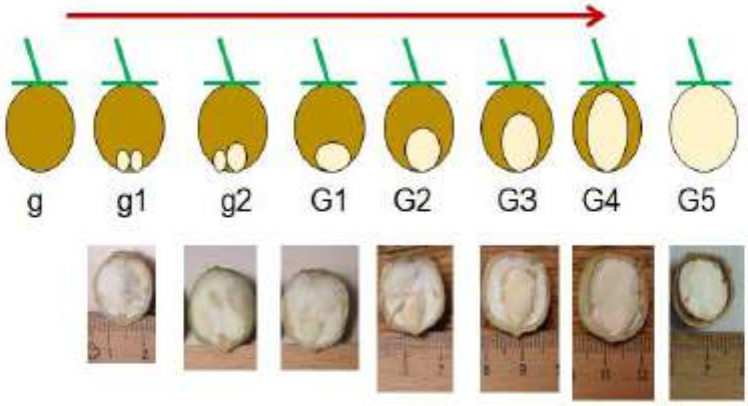

Figure 8 Graphical scheme adopted by IRTA Research Center (Spain) to individuate the phenological stage of nuts on the basis of the seed development

At the same time, 50 nuts from the same field were collected weekly, brought to the laboratory and, after opening them in half, they were classified by the seed development according to a protocol adopted by IRTA Research Center (Spain), to highlight the mean phenological stage (Figure 8). An increasing numerical value has been assigned to each phenological stage, so that the results can be quantitatively compared:

g =1; g1=2; g2 =3; G1 = 4; G2 = 5; G3 = 6; G4 = 7; G5 = 8.

Table 5 reports the considered nut phenological stages. During the season, the sleeves have been checked weekly and when dropped nuts were detected, they were collected and further analyzed in the laboratory. On the 27th of August, just before the harvesting time, the branches enclosed were cut and the nuts were brought to the laboratory where they were opened, and the infestation assessed for the nuts belonging to all the thesis tested.

Table 5 Nut phenological stages

g	No ovule observed in the medullary tissue of the ovary
g1	Two ovules of the same size are observed
g2	One ovule more developed than the other is observed: beginning of growth
G1	The kernel occupies more than ¼ of the nut
G2	The kernel occupies between ¼ and ½ of the nut
G3	The kernel occupies between ½ and ¾ of the nut
G4	The kernel occupies more than ¾ of the nut but not all of it
G5	The kernel occupies the entire nut

The results of this preliminary activity showed that thesis Time2 was the period during which nuts revealed the highest level of traumatic abortion damage with premature dropping of nuts (Table 6). This is also confirmed by the statistical analysis (p<0,0001) (Table 7). Thus, according to the assessment of nut phenological stage, the Time2 period coincides with the g2/G1 phases (Table 8).

Regarding the "*cimiciato*", the Time5 thesis showed the highest level of altered nuts (Table 9) (Figure 9), suggesting that this damage can be caused from the moment when nuts reached the G5 phenological stage. In addition, the longer the duration of the contact between the phytophagous and the nuts, the higher the bug damage activity.

Table 6 Total nuts per treatment and those that dropped in the experimentation conducted in Spain.

Thesis	Total nuts	Dropped nuts
Time1	202	19
Time2	291	128
Time4	308	20
Time5	271	10
Time3 (Control)	1264	187

Table 7 Percentage of dropped nuts per thesis in the experimentation conducted in Spain. Different letters indicate significative difference among the treatments (Duncan's Multiple Range Test, p<0,05)

Thesis	Percentage of dropped nuts
Time2	50,014 **a**
Time1	10,349 **b**
Time4	5,681 **b**
Time5	3,616 **b**
Time3 (control)	4,862 **b**

Table 8 Number of nuts per treatment at the different phenological stage

Thesis	Nut phenological stage								
	g	**g1**	**g2**	**G1**	**G2**	**G3**	**G4**	**G5**	**Total**
Time1	5	2	2	4	1	2	1	2	19
Time2	14	22	38	18	2	1	0	0	125
Time4	4	3	6	0	0	0	2	5	20
Time5	0	3	4	1	0	0	0	2	10
Time3	0	1	1	4	2	1	0	1	10
Total	53	31	51	27	5	4	3	10	184

Table 9 Number of total nuts per treatment and those with "cimiciato"

Thesis	Total number of nuts	Nuts with formed kernel	Nuts with "*cimiciato*"
Time1	202	166	2
Time2	291	87	1
Time4	308	262	108
Time5	271	237	163
Time3 (Control)	192	155	3
Total	1264	907	277

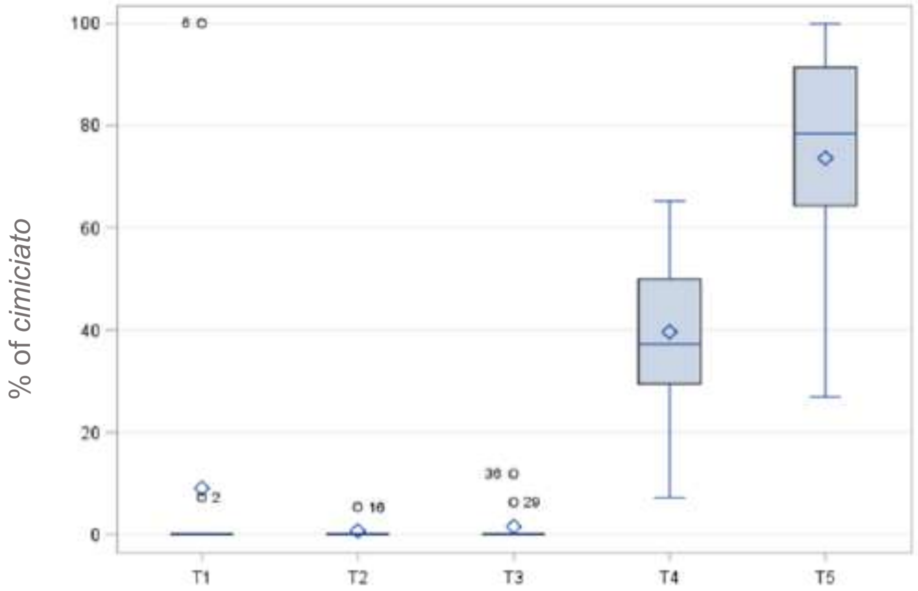

Figure 9 Percentage distribution of "cimiciato" in the nuts from the different treatments (T). Two-way ANOVA (treatment and plant) for one variable (percentage "cimiciato"). Duncan's Multiple Range Test (P<0.05).

Once such preliminary information about the most susceptible nut phenological stage were obtained, we needed to validate this acquisition in order to identify the best date to apply IPM strategies against true bugs. Thus, from 18 of June to 1 of August in 2019 and from 3 of June to 16 of July in 2020, 50 nuts were randomly collected every week from the plants of PANTHEON field (Figure 10Figure 11). The collected nuts were brought to the laboratory and, after opening them in half, were classified by the seed development according to the protocol adopted. At the same time, specimens of true bugs were collected using the *frappage* method. In the area of study, Viterbo district in north of Latium, the most damaging true bug is *Gonocerus acuteangulatus* so we decided to use it in both year of experimentation, 2019 and 2020. In both years, the true bugs have been introduced in the sleeves selected on the plants belonging to the three theses:

- T1 for the traumatic abortion;
- T2 for the "*cimiciato*";
- Control.

In 2019, 25, 25 and 23 sleeves were adopted for theses T1, T2 and Control, respectively, while in 2020, the sleeves were 30, 25 and 25 for T1, T2 and Control, respectively. The bugs have been kept for one week into the sleeves and then removed, maintaining the proportion of 1 adult per 5 nuts or, when it was necessary, 2 pre-imaginal stages per 5 nuts. The introduction was carried out in both years when the average phenological stage had reached the level of g2/G1 in the T1 sleeves and G4/G5 in the T2 ones. Specifically, in 2019 these actions were carried out on June 27th and July 18th respectively, and in 2020 on June 18th and July 6th, respectively. At the end of August, just before the harvesting time, the enclosed branches were cut, and the nuts were brought to the laboratory where they were opened. The infestation for the nuts belonging to all the theses was thus tested.

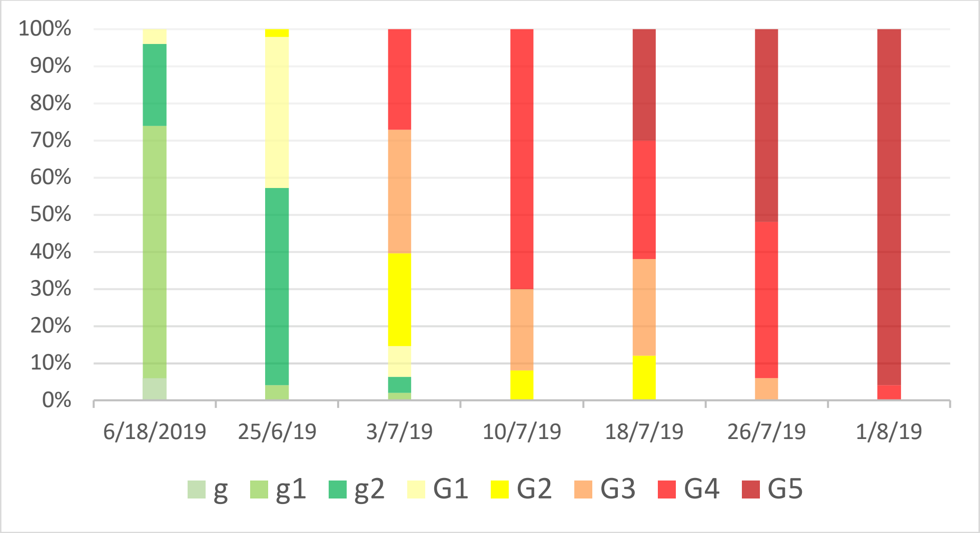

Figure 10 Percentage distribution of the phenological stage during the 2019 season

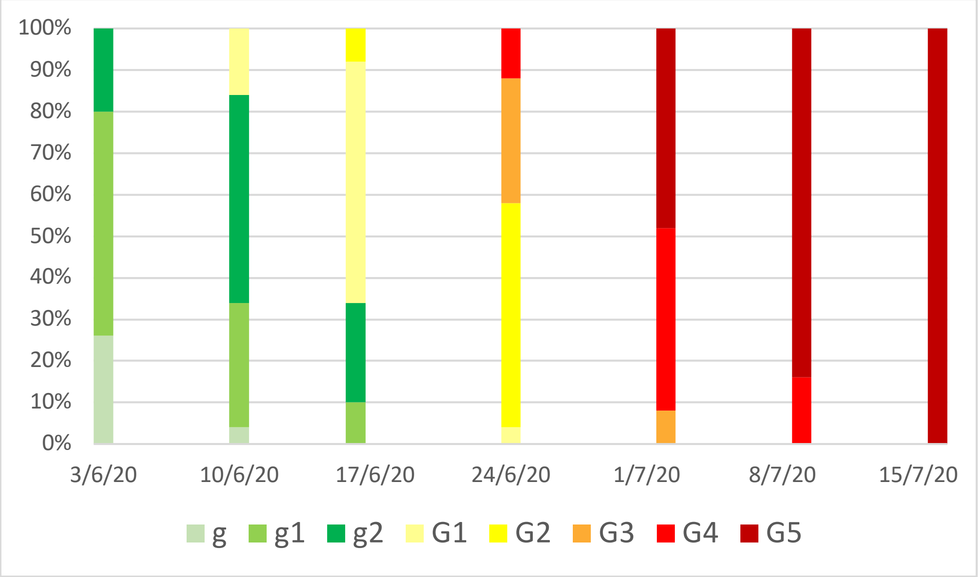

Figure 11 Percentage distribution of the phenological stage during the 2020 season

The results confirmed the ability of the bugs, inserted inside the sleeves at the defined moments, to cause the mentioned damages, traumatic abortion and "*cimiciato*". Regarding traumatic abortion, the introduction of bugs during the phenological development phases of hazelnuts equal to g2/G1 caused about 60% damage in 2019 (Figure 12), while in the second year this damage was about 35% of the nuts present in the sleeves of the T1 thesis (Figure 13). Regarding the “*cimiciato*”, our results confirmed what we have observed in the preliminary investigations conducted in Spain. The true bugs can induce the “*cimiciato*” (that we kept divided in the graphs 16 and 17 in two columns on the basis of different types of symptoms as spot b/n and shrivelled nuts) when nuts are at the G4 and G5 phenological stage.

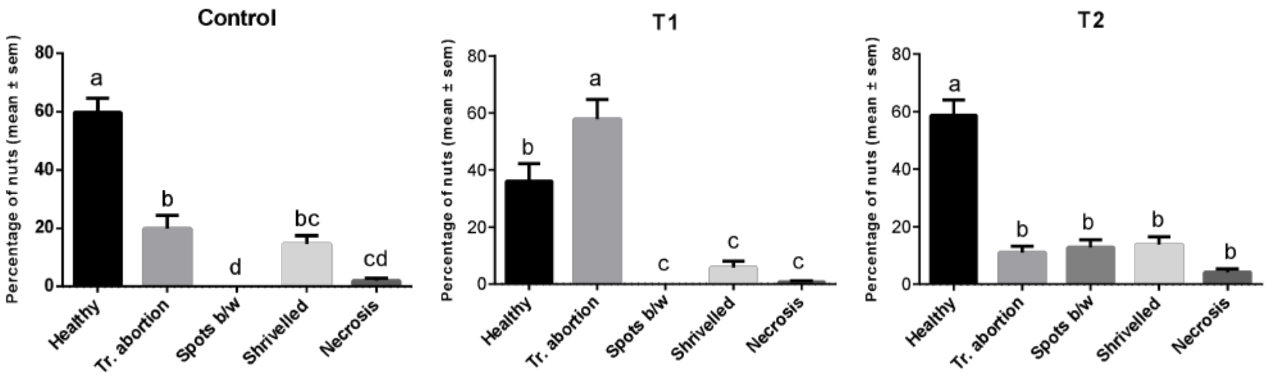

Figure 12 Percentage (mean ± sem) of nuts with the different damages in the three theses, in 2019. The cimiciato is the sum of spot b/w and shrivelled nuts. Different letters indicate significative difference among the treatments (Duncan's Multiple Range Test, p<0,05)

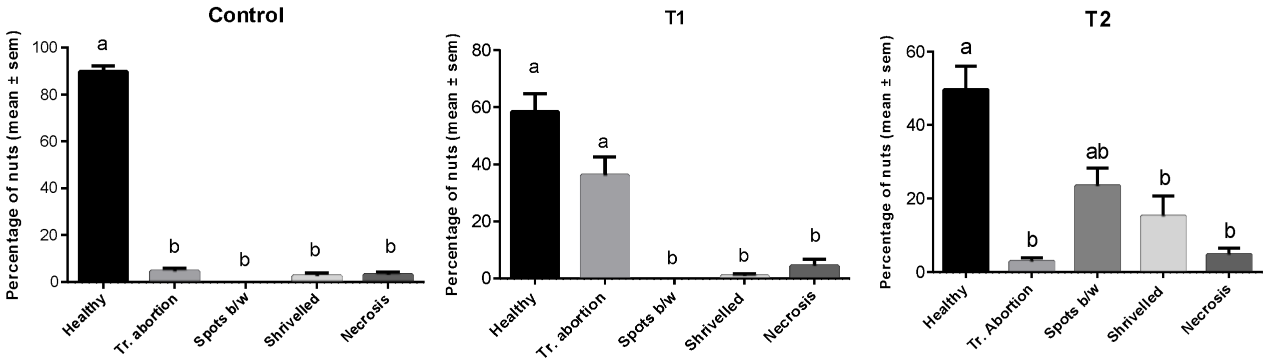

Figure 13 Percentage (mean ± sem) of nuts with the different damages in the three theses, in 2020. The cimiciato is the sum of spot b/w and shrivelled nuts .Different letters indicate significative difference among the treatments (Duncan's Multiple Range Test, p<0,05)

Findings

The experimentation carried out within the PANTHEON project confirmed the capacity of true bugs to cause the described damages (i.e., traumatic abortion and *cimiciato*) to hazelnut production in the Viterbo area. In addition, trials conducted in the IRTA Research Center in Spain and then in the PANTHEON experimental field, showed that the damage activity of these phytophagous is closely associated with the phenological development of the hazelnut. In fact, both abortion and *cimiciato* only occur if the bugs act at well-defined stages of the hazelnut's phenological development.

The importance of identifying the correct moment when the damage is caused is fundamental in the management of true bugs. The monitoring indeed must involve the phenological development of the plant, and the kernel in particular, in order to associate the presence of the insect with the susceptibility level of the plant. This useful finding has been implemented in the newly developed guidelines and can certainly lead to a reduction in chemical input in true bugs control, which is the aim of both the PANTHEON project and European policies, increasingly aimed at ecologically sustainable management of agricultural production.

3.2 Gall mite

3.2.1 Automated detection of gall mite

Driven by the good performance for detection of true bugs, we also developed a YOLO-based pest detection system, presented in [7], able to recognize gall-mites. In fact, for this task as well, it is necessary to resort to data-driven approaches due to the impossibility of manually defining discriminating features of the gall-mites.

As first step for the detection system, we collected images containing examples of gall-mites in the hazelnut orchard. In particular, we acquired images in two different moments of the year, winter and early spring, in order to individuate the best moment for monitoring activities. We will refer to these datasets as winter (D_w) and spring (D_s) datasets, respectively. In our hypothesis, in winter, the galls compromised by the action of the mite can be less easily discriminated from healthy and still closed buds. During the spring, on the other hand, the emergence of the first leaves from healthy buds makes it easier to discriminate the healthy buds from the galls. Figure 14 shows examples of buds colonized by Phytoptus avellanae in winter (on the left) and in spring (on the right).

Figure 14 Buds colonized by Phytoptus avellanae in two different seasons of the year, winter (left) and spring (right).

Note that, differently from existing work focusing on laboratory setups, the collected datasets are fully representative of real-world conditions as they were directly acquired on the field on contaminated plants. This implies that different trees are captured at different distances and with different orientations, leading to very different backgrounds in the images. Examples of images in the winter and spring datasets are reported in Figure 15 and Figure 16, respectively. Images were recorded with resolution 1280×720 with a SONY A5100 camera. A total of 122 and 488 labeled images composes the winter and the spring datasets, respectively. Note that each image comprises several examples of gall-mites, especially in the winter dataset in which images with about 50 gall-mites are present. The labeling was performed in two phases: *i)* first, a subset of the datasets was manually labeled by an expert agronomist and used to train preliminary YOLO-based NNs, *ii)* next, these NNs were used to predict the presence of the gall-mites on the remaining images providing a rough labeling on these images and *iii)* these labels were manually inspected and refined. The datasets have been made public at the following link: https://tinyurl.com/yy9efsmu to boost reproducibility and enable results comparison.

Figure 15 Examples of images from the winter dataset.

Figure 16 Examples of images from the spring dataset.

We considered the same architecture used for the true bugs detection and, similarly, we resorted to data augmentation techniques which allow to synthetically increase the datasets size. The detection systems resulted in mean average precision of 60.7% and 82.2% for the winter and spring datasets, respectively. As expected, significantly lower accuracy is obtained with the winter images in which the galls are visibly much more difficult to identify. However, further investigations will be made considering a larger training set. The accuracy was evaluated on 25 and 90 novel images for the winter and spring datasets, respectively.

Coherently with the true bugs results, best performance was obtained when using color and geometric transformations, as well as mosaic augmentation. Examples of the predicted bounding boxes along with confidence scores are shown (pink rectangles) in Figure 17 for the winter (left) and spring (right) datasets. Ground truth values are also reported (green rectangles). We can observe that a higher number of false negatives, i.e., missed predictions, is recorded for the winter case in which the gall-mites are significantly less visible.

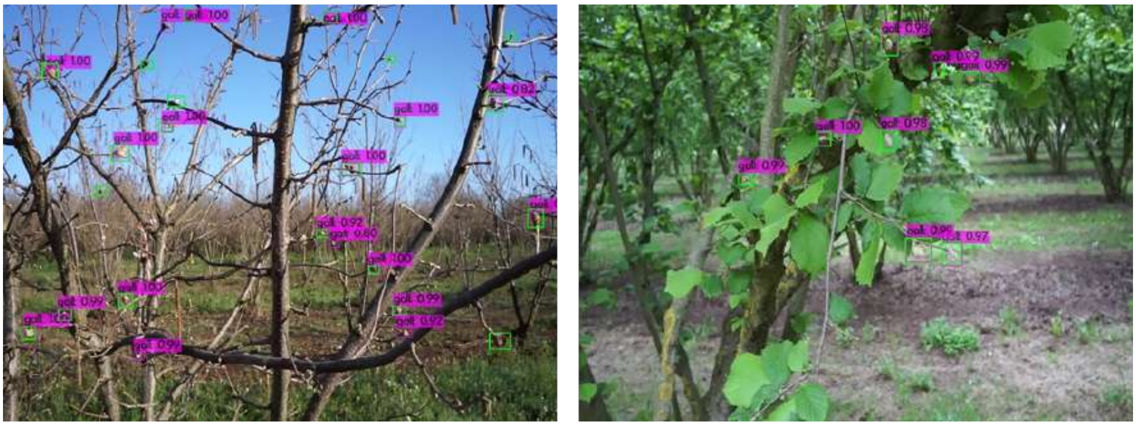

Figure 17 Examples of predictions on winter (left) and spring (right) data samples.

Finally, the real-time effectiveness of the approach was also verified by these detection systems on the NVIDIA Jetson Xavier, reaching 49.5 fps. This enables the future integration of this detection system on any robotic platform for online processing.

3.2.2 Gall mite infestation and hazelnut plant

Gall mite monitoring on different agronomic management: methods and results

During the PANTHEON project the mite population was monitored in the selected field to identify differences of susceptibility in plants subject to different agronomic management. This information can be helpful in the determination of the more effective management acting in the reduction of infestation.

In addition, understanding where the mite's presence on the plant is concentrated can provide a restricted target for observation during the monitoring phases and a reduced area in which to concentrate any chemical treatments and consequently the release of pesticides in the environment.

Our activities were carried out in winter and consisted of counting the galls (buds modified by the colonisation and trophic action of *P. avellanae*) and buds present on randomly selected portions of branches. Thus, the infestation level has been assessed as the ratio between the number of galls and the number of buds per branch. The count was carried out in January 2019, 2020, and 2021 on 10 hazelnuts belonging to the following thesis:

- adult irrigated;
- adult not irrigated;
- young irrigated;
- young not irrigated.

On each of these plants, 3 branches of approximately 50 cm were selected and the count was carried out.

An increasing number of galls caused by the mite has been observed over the years of observations (GLM followed by Sidak post hoc test, P<0.05) (Figure 19) while we reported a constant number of buds and no statistical difference, available for colonisation by the mite (Figure 18)

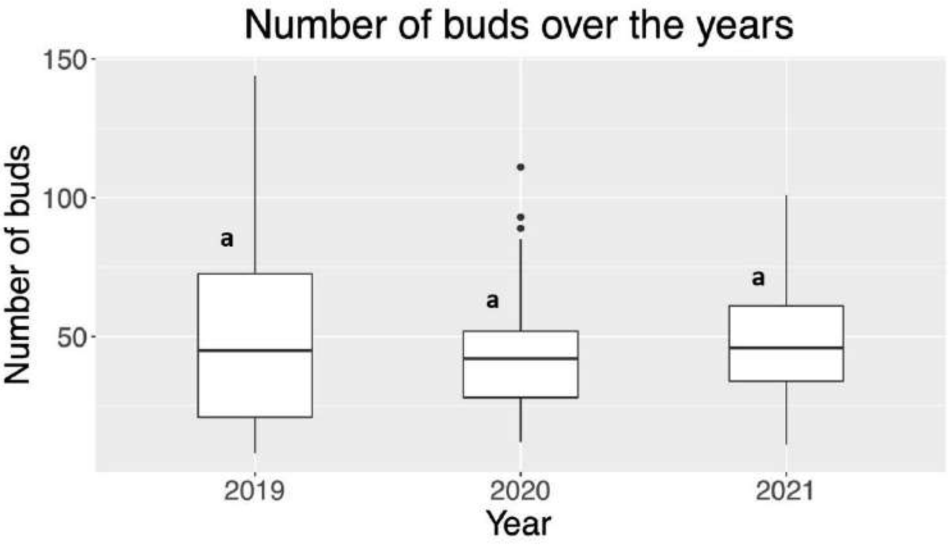

Figure 18 Number of buds over the year of experimentation. Different letters indicate significative difference among the years (GLM followed by Sidak post hoc test, P<0.05)

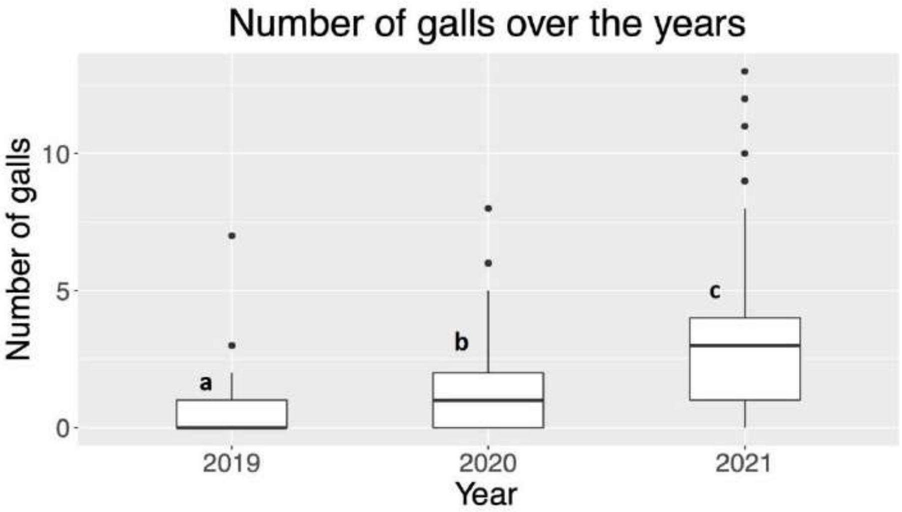

Figure 19 Number of galls over the year of experimentation. Different letters indicate significant difference among the years (GLM followed by Sidak post hoc test, P<0.05)

Regarding the experimentation useful to individuate the agronomic management that reduce the presence of *P. avellanae* thus the chemical input for the control of this pest, the only differences were found between the years of observation, as reported above, but not between irrigated and not-irrigated plants nor between young and adult plants. No statistically significant difference was observed between the theses considered, suggesting that the mite does not benefit from any of the managements considered (irrigation and no irrigation) nor from the age of the host plant.

Gall mite infestation at different plant heights: methods and results

In addition, in the 2021, the distribution of galls at different heights on the plant was evaluated for the two adult plants thesis, irrigated and not irrigated. This observation was carried out as described above and regarded the 10 plants per thesis, which have been divided into three sections from the ground to the top.

The first section corresponds to the range from 0 to 150 cm from the ground, the second from 150 cm to 300 cm and the third from 300 cm to the top of the plant.

The monitoring conducted in winter 2021 shows that the ratio galls on buds is statistically higher in the height 2 (150-300) than in the 1 (0-150cm) and 3 (>300cm) (Figure 21). In addition, it should be noted that in the height 1, the level of infestation, which is higher in comparison to height 3, is accompanied by a reduced number of available buds (Figure 20). In particular, in this zone, probably because of the favorable environmental conditions, the mite colonizes buds on young suckers, which then are often subjected to mechanical and/or chemical removal. In conclusion, the statistical analysis carried out on the number of galls shows that zone 2 is the one where the mite's presence is the greatest. No statistical differences have been observed between plants irrigated and not irrigated.

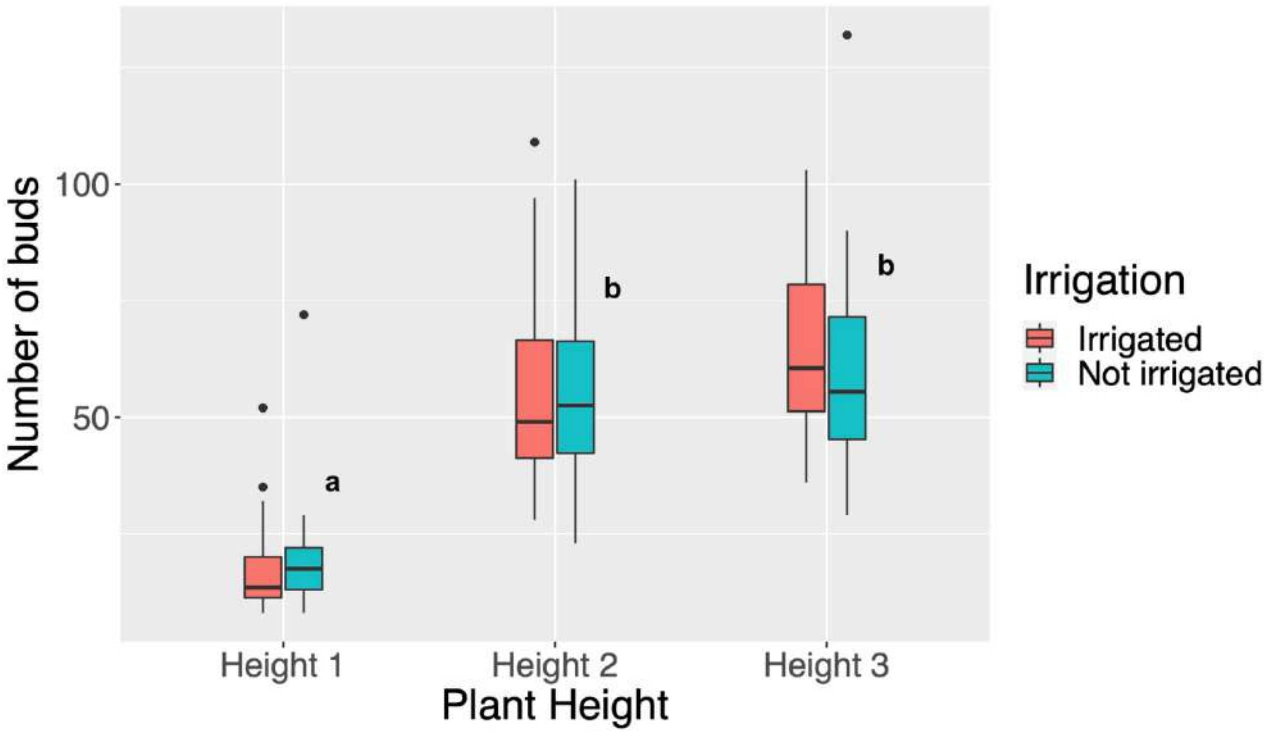

Figure 20 Number of buds recorded at different heights of the plants (height 1, 0-150 cm; height 2, 150-300 cm; height 3, >300 cm) and on plants with different irrigation management. Different letters indicate significant difference among the heights (GLM followed by Sidak post hoc test, P<0.05)

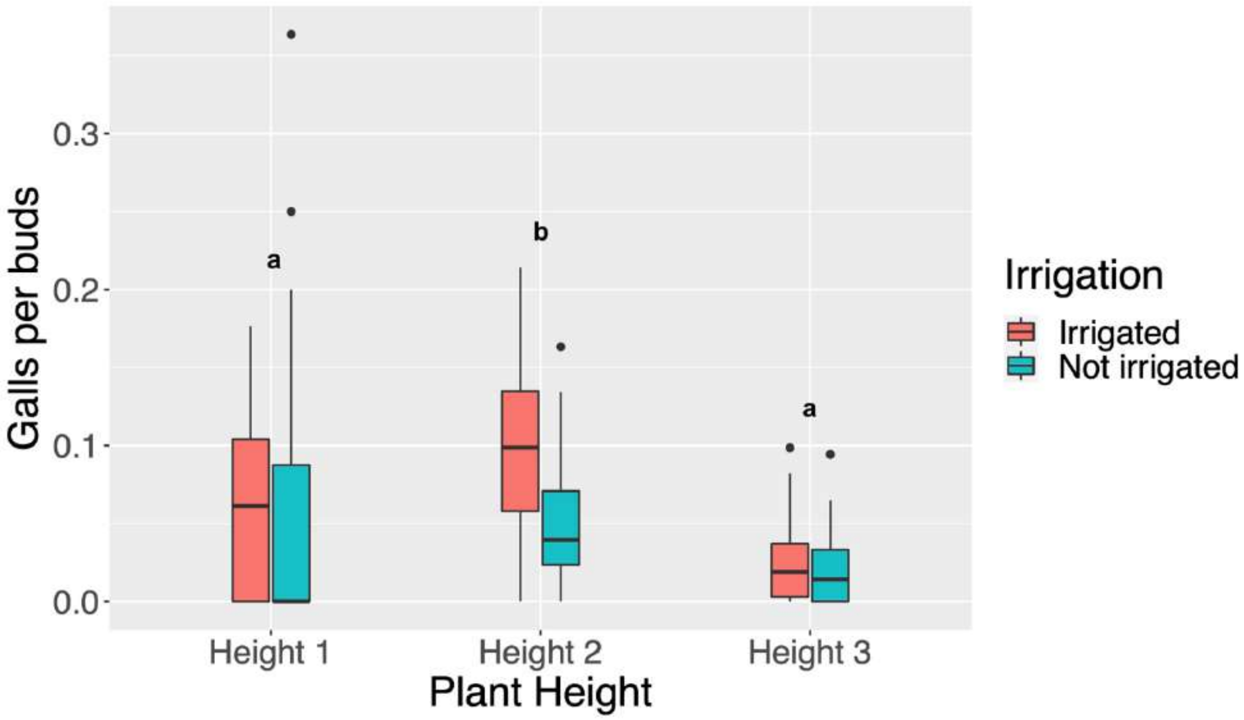

Figure 21 Ratio between galls and buds recorded at different heights of the plants (height 1, 0-150 cm; height 2, 150-300 cm; height 3, >300 cm). Different letters indicate significant difference among the heights (GLM followed by Sidak post hoc test, P<0.05)

Findings

The findings of the experimentation on gall-mites highlighted that irrigation management has no effect on the ability of this pest to cause different levels of damage. As a matter of fact, irrigated and non-irrigated plants did not show different numbers of galls on the branches. There was also no difference in infestation between young and adult plants. On the other hand, differences in the presence of galls were observed between the 3 plant height zones with a greater concentration recorded in the intermediate section (150-300 cm). The fact that galls are only concentrated on a part of the canopy is of great importance in the planning of strategies to contrast this pest. The integrated production rules that regulate the number of treatments and the active ingredients that can be used refer, at least in the Latium Region, to sulphur-based products that can be used in the control of *P. avellanae*. This experiment shows how both monitoring and control strategies can be applied not to the whole canopy but by concentrating the action in the range between 150 and 300 cm from the ground. This is the reason why this useful finding has been implemented in the recently developed guidelines. This certainly leads to a reduction in the dispersion of chemical substances, with positive economic and ecological results. In addition, the possibility of knowing the part of the plant where the mite-induced galls are concentrated provides valuable information that can be exploited by the developers of the robotic platforms on which these automated systems are going to be set up.

3.3 Bud rot and leaf anthracnose

3.3.1 Detection and monitoring of bud rot and leaf anthracnose

To rationalize control treatments against the pathogen, different complementary approaches, both human mediated and automated, have been evaluated during the Project. Each of these approaches has specific aims.

- **Evaluation of the presence of the pathogen during plant dormancy**. Given that the most probable source of fungal inoculum for the incoming year resides in plant tissue infected, mainly buds, during the previous year, we set up a new molecular assay based on qPCR able to detect and quantify the presence of the pathogen in any plant tissue.
- **Assessment of environmental parameters influencing the growth of the mycelium and the conidial germination.** Understanding the environmental parameters influencing the biological activities of the pathogen is essential to define the best timing for control treatments. In the project, a series of lab testing have been carried out to assess the influence of temperature on the mycelial growth and on the conidial germination. The first parameter allows to determine the moment when the resting mycelium restart to growth and colonize plant tissues at the beginning of the vegetative season, to assess the period of highest activity and when the conditions lead to the stop of biological activity. The second parameter describes if the temperature allows or not the conidia, the main way of spread of the disease, to germinate and infect new plant tissues.
- **Correlation between leaf symptoms and vegetational indices acquired by UAV**. Ground truth data about the occurrence of leaf anthracnose were collected during vegetative seasons to be compared with the vegetational indices describing plant health (Univ. of Trier) as obtained by processing images acquired by different cameras mounted on by UAV (Univ. of Bruxelles). Finding a correlation between these data should have led to the definition of an automated warning of the onset of disease symptoms and the evolution of plant health condition during the season.

Evaluation of the presence of the pathogen during plant dormancy

In 2019-2020, a molecular assay that allows to detect very low amount of the pathogen’s DNA within plant tissue, was developed and its effectiveness was tested. The assay consists in a quantitative PCR (qPCR) that uses highly specific primers targeting a specific region of the DNA of *Monostichella coryli* based on SYBR Green technology (Figure 22). The assay can assess and quantifying the presence of the pathogen quickly, even in asymptomatic tissues, without the need to pass through the isolation/cultivation step, and at a reasonable cost. The assay is the subject of a paper under preparation that will be submitted to a scientific journal.

Besides its main aim to detect and quantify the presence of the pathogen in dormant plant tissue during winter, the developed assay allowed to confirm the causal relationship between foliar symptoms and the pathogen in the samples collected during the ground truth monitoring.

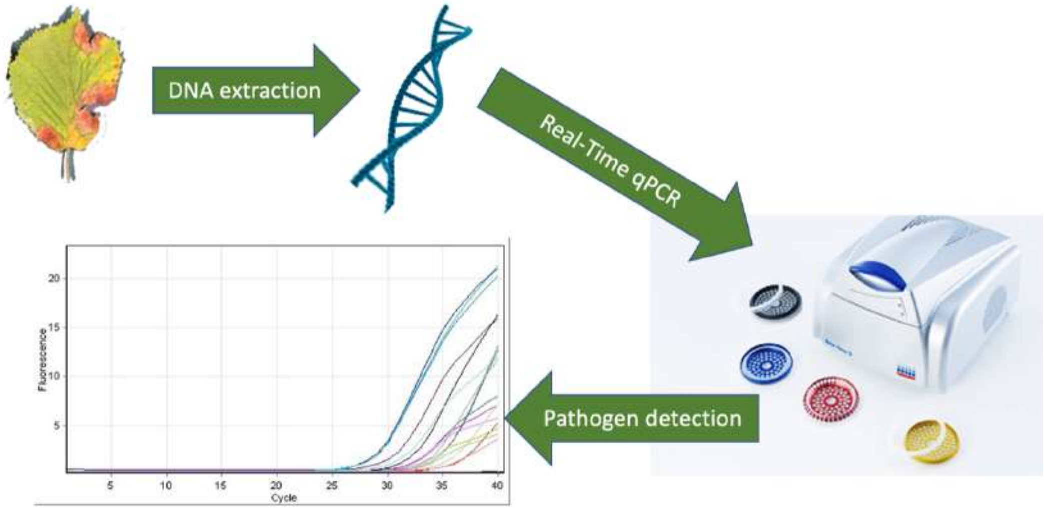

Figure 22 Scheme of molecular assay for Monostichella coryli detection and quantification

The assay was applied for the first time in the vegetative season 2021. By analyzing qPCR results on a sample of 100 dormant buds collected randomly from the hazelnut plants under investigation at the very beginning of the season, April 6th, it was demonstrated that the pathogen was already present in about 35% of the samples. This confirms the preferential overwintering strategy for the pathogen, providing relevant information about the disease incidence expected for the incoming season.

To further confirm this datum, we visually measured the incidence of buds attacked by the pathogen (no sprouting) in the canopy during the first field survey (April 24th) in which this symptom was appreciable. A 0–5 assessment scale was established and used for in field evaluation: 0) = No disease; 1) = 1-5 % of dead buds; 2) = 5-10 %; 3) = 11-20 %; 4) = 21-50 %; 5) = > 50 %.

The incidence of necrotic buds ranged from 1 in the adult plants (both irrigated and not) to a slightly higher value in young plants: 1.2 for irrigated and 1.7 for not irrigated, suggesting a higher susceptibility to infection for young plant vs adult plants (Figure 23).

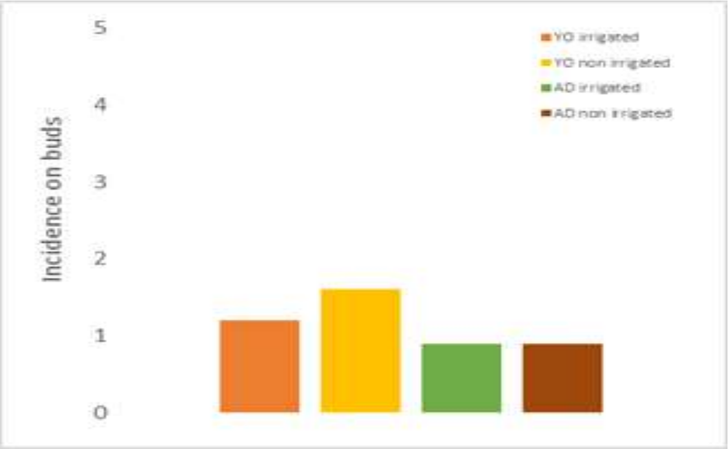

Figure 23 Incidence of necrotic buds caused by Monostichella coryli in early spring (24th April 2021).

Assessment of environmental parameters influencing the growth of the mycelium and the conidial germination

The activities of the project are aimed to develop a method to a more precise and correct use of phytosanitary treatments. In such sense the knowledge of biological features of the pathogen, and more specifically the relationship with the environmental parameters, is fundamental to prospectively set up models able to predict the disease occurrence and spread. The formulation of a reliable model needs a series of experimental steps aimed to explore the different aspects of the biology and ecology of the species.

As a first approach, two major physiological features of the pathogen were investigated in vitro: the mycelial growth and the conidial germination in response to different temperatures. The mycelium growth rates of 9 isolates of the pathogen were explored at 6 different constant temperatures (5, 10, 15, 20, 25, 30 °C), while the germination rates of the conidia were explored at 3 different constant temperatures (15, 23, 27 °C). The experimental data were then used to provide a mathematical interpretation of the phenomena.

Mycelial growth and temperature

Agar plugs (5 mm diameter) were taken from the edge of actively growing cultures on PDA and transferred onto the center of 9-cm PDA Petri dishes. Three replicate plates were incubated at 5, 10, 15, 20, 25, 30°C in the dark and measurements were taken after 7, 14 and 21 days at right angles along two lines intersecting the center of the inoculum and the mean growth rates plus and minus the standard deviation (±SD) were calculated. To estimate the effect of temperature on the pathogen's mycelial growth rate, the Briére equation was used to define T_L and T_M that are respectively the lower and upper thermal thresholds below and above which, the mycelial growth is hampered:

$$R[T] = aT(T - T_L)(T_M - T)^{\frac{1}{m}}$$

Given that the expression is continuous in its range of definition, it is possible to calculate the coordinates of the maximum placing at zero the first derivative in:

$$T_{opt} = \frac{2mT_M + T_L(m+1) + \sqrt{4m^2T_M^2 + T_L^2(m+1)^2 - 4mT_MT_L}}{4m+2}$$

This information is highly relevant from a biological point of view since it represents the optimal temperature for the mycelium growth (Figure 24). This operation has been repeated for all the strains tested (Figure 25).

The T_L values ranged between -9 °C and 0 °C, with the last value shared by most of the isolates. For the T_M, namely the upper temperature threshold for the mycelium growth, all the values are included in the range of 30-36 °C. These behaviors are also coherent with the different optimal temperatures, T_{OPT}, calculated among the isolates, all between 21 and 24 °C

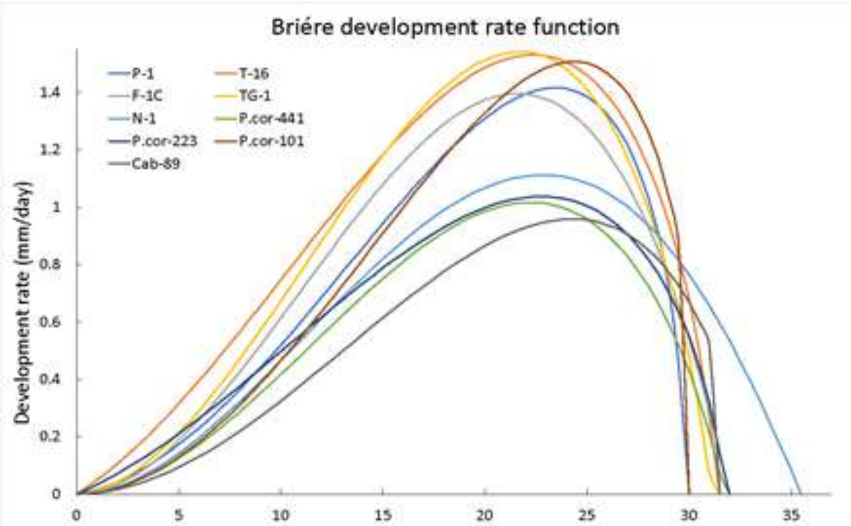

Figure 24 Best representing development rate functions for Monostichella coryli mycelium growth

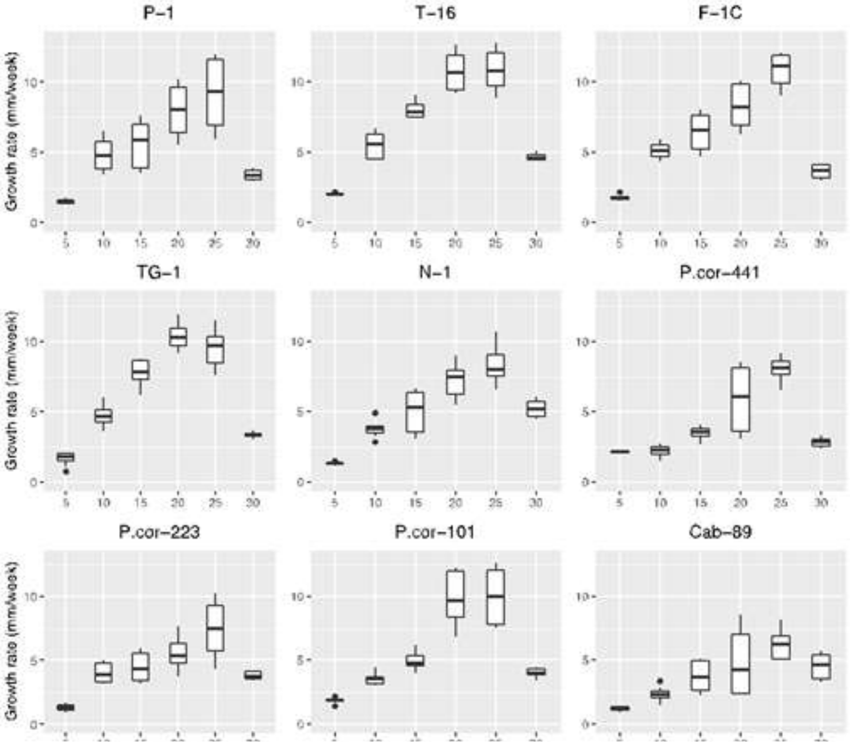

Figure 25 Experimental mycelium growth rates of the different Monostichella coryli isolates.

Effect of temperature on conidial germination

A conidial water suspension was quantified and diluted to a final concentration of 10^5 spore/ml. Spore germination was evaluated by placing 5 µl of spore suspension on a thin layer of water agar medium on a glass slide. The glass slides have been incubated for 48 h at 15, 23, 27°C and 100% RH. Germinated conidia were counted after 6 h and 24 h using a minimum of 100 conidia per replicate, four replicates were accessed.

Conidia were counted as germinated when the length of the germ tube was at least one half the length of the spore. The experimental data were interpreted in mathematical keys, analyzing the germination of the conidia through a logistic function. From a mathematical point of view, it is possible to assume that as time passes the number of germinated conidia reach even more than the total number of 100, suggesting a logistic interpretation of the phenomenon. The results of the non-linear regressions are reported in Figure 26.

The logistic function demonstrated a good reliability in representing the germination of conidia in function of time. Moreover, there are two relevant information deriving from this interpretation of the data, namely the two germination rates G0 and r. The former, more specifically, becomes relevant above all when prompt control actions are required. The knowledge of the percentage of conidia which promptly germinates after 6 hours at a given temperature is the first rough estimation of the incidence of the disease on the field.

Surely these results need additional future studies, focusing, as example, on the influence of the water activity on the germination. The idea, indeed, is to reach a pool of information that can be enclosed in a mathematical model, aimed to predict the periods with the higher risk of incidence of the disease, and, as a logical consequence, the most suitable timing for treatments in a correct control strategy: in such sense, these results represent the basics for the implementation of future decision support system for integrated Pest Management of this pathogen.

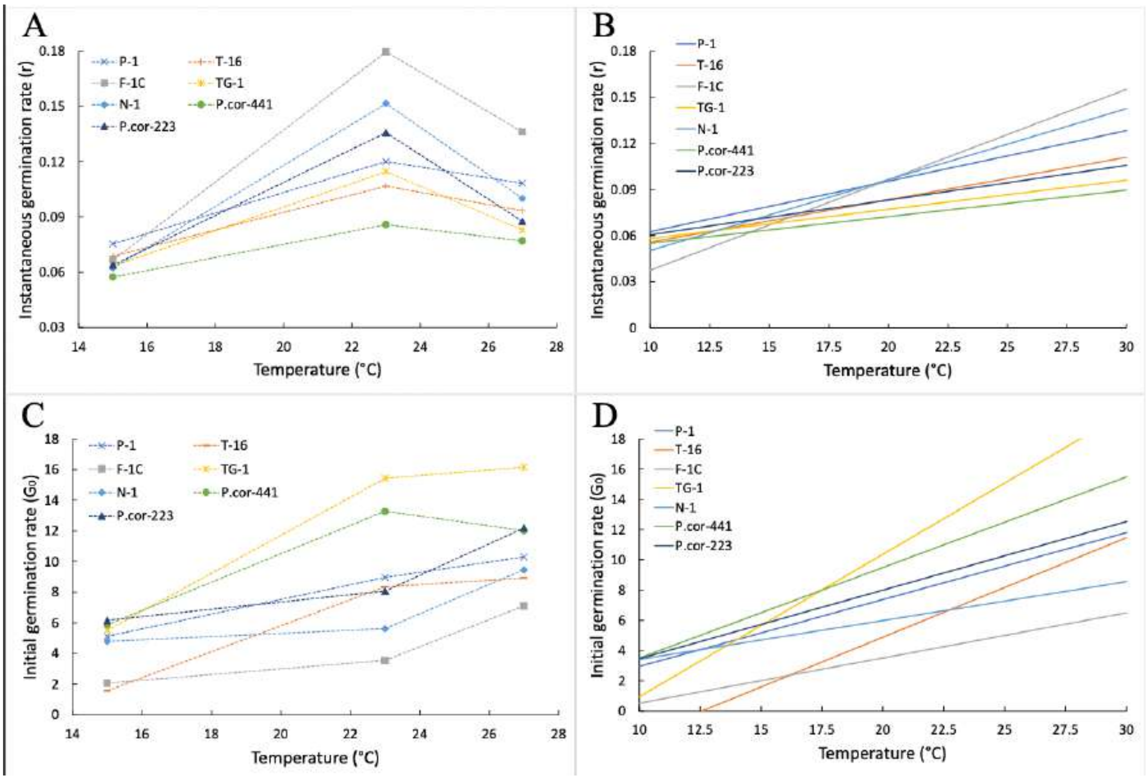

Figure 26 Instantaneous (r) and initial (G0) germination rates in function of temperature. A) Instantaneous germination rates r estimated by fitting the experimental data with the logistic function plotted in function of the three temperatures investigated. B) Best fit line interpolating the values plotted in A. C) Initial germination rates G0 estimated by fitting the experimental data with the logistic function plotted in function of the three temperatures investigated. D) Best fit line interpolating the values plotted in C.

Correlation between leaf symptoms and vegetational indices acquired by UAV.

Given the numerous issues, both technical and COVID-related, as the natural variability in the occurrence of diseases, only in 2021 it was possible to carry out all the scheduled activities, here reported up to the date of the writing of the Deliverable. However, the lack of reliable indices related to disease presence as a result from the investigations carried out during 2019 and 2020, hampers the data validation. Nonetheless, we expect to have valuable indications at the end of the ongoing campaign.

The monitoring campaign 2021 started on April 6th with plants still dormant, continued on April 24th, May 8th, May 22nd and June 12th, and it is scheduled for the rest of the vegetative season (June 24th, July 8th, July 22nd and August 5th). At each sampling date, from each of 20 trees (Young trees not irrigated: 5 plants NI2, NI4, NI6, NI8, NI10; Young trees irrigated: 5 plants I2, I4, I6, I8, I10; Adult trees not irrigated: 5 plants NI2, NI4, NI6,NI8, NI10; Adult trees irrigated: 5 plants I2, I4, I6, I8, I10) 50 leaves were chosen randomly and taken to the laboratory to measure the percentage of necrosis observed on each leaf. An image analysis software (ImageJ) was utilized to determine the percentage of necrotic area due to the attack of the pathogen. The mean value across the 50 leaves was then taken as the representative value for each tree.

At the date of writing, data from both ground surveys and UAV images are available for 4 dates: April 24th, May 8th, May 22nd and June 10th.

On April 24th all leaves from all the selected trees were asymptomatic, despite the pathogen was already present, according to molecular qPCR assay, in randomly tested leaves in about 50% of the investigated plants. The first anthracnose symptoms were observed starting from 8th May, but only on the leaves of young trees, both irrigated and not (YoI and YoNI), where small necrosis on the margin of the leaves occurred occupying about 1.5% of the leaf surface. The percentage of necrotic leaf area steadily increased to about 3% on May 22nd and up to 7% on June 12th (Figure 27)

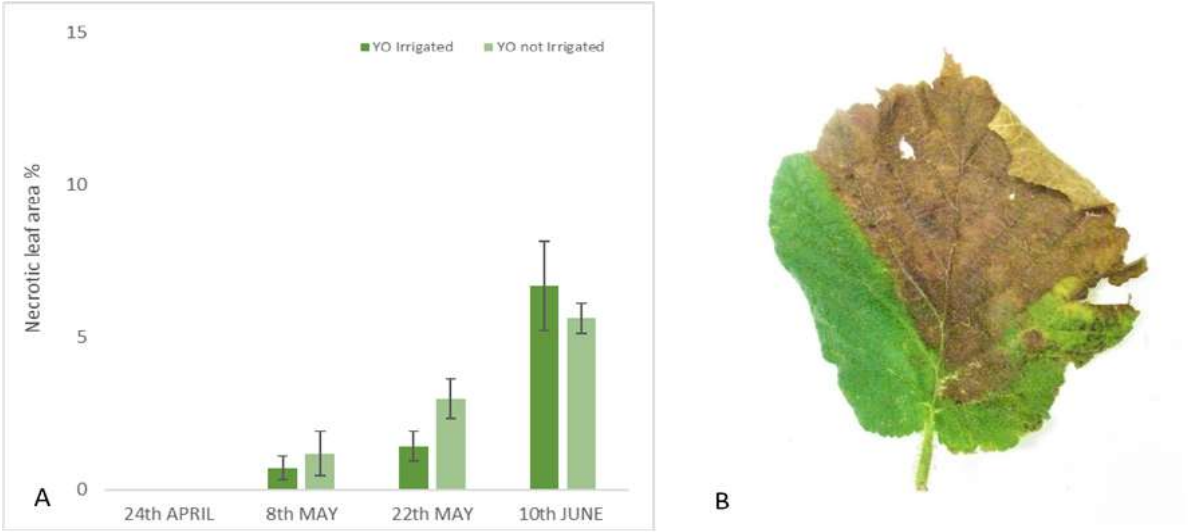

Figure 27 A) Average percentage of necrotic leaf area on irrigated and non-irrigated young trees in the four sampling dates. B) symptoms of Monostichella coryli on hazelnut leaf.

On the adult trees, instead, foliar necrosis appeared only on 12th of June with an average percentage of 2.2%. According to the results obtained from molecular testing, it has to be highlighted that the pathogen was detected in asymptomatic leaves of both young and adult plants, confirming the presence of the pathogen within host tissues before the onset of leaf necrotic symptoms. Furthermore, as expected, *M. coryli* was also molecularly detected in all the leaf samples showing the distinctive symptomatology of the disease.

<u>Hyperspectral image data collection</u>. Three cameras were mounted on the UAV: *i)* an RGB camera (Sony α5100), *ii)* a customized six-band multi-spectral camera (Tetracam MCAW-6) and *iii)* a thermal camera (TeAx ThermalCapture-2.0) (Figure 28**Error! Reference source not found.**). For the collection of UAV imagery, the

drone was flown at an altitude of about 25 meters up to May 22nd, then The UAV was flown at 50 m and at a speed of about 1 meter per second. The technical features of the cameras are reported in Table 10.

Table 10 Sensors mounted on the UAV

Band Name	Center Wavelength (nm)	Bandwidth FWHM (nm)
	Sony α5100	
Blue	~400-530	~100
Green	~460-630	~100
Red	~570-700	~130
	Tetracam MCAW-6	
Green	530.7	3
Green-Yellow	550.0	10
Yellow-Green	570.0	10
Red	680.0	10
Red-Edge	720.0	10
Near-infrared	900.0	10
	TeAx ThermalCapture 2.0	
Thermal	7.5-13.5 µm	N/A

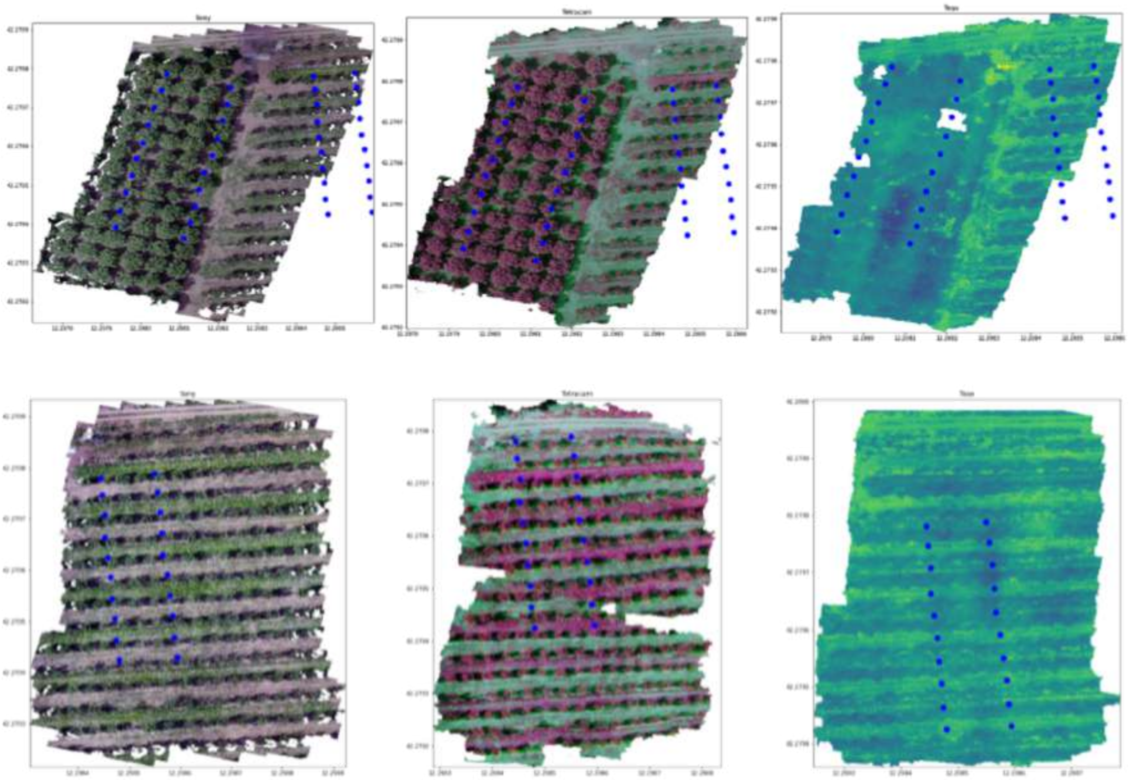

Figure 28 Example of orthoimages from UAV flying at 25 m of altitude as acquired by Sony α5100 (left), Tetracam MCAW-6 (center) and TeAx ThermalCapture-2.0 (right) on adult (above) and young (below) plants on May 8th.)

Among the vegetative indices extrapolated from orthoimages, NDVI and SR seem to give clearer trends. Both the indices show distinct values between young and adult plants at the same sampling dates, with the adult plants maintaining a steady value with low variability always higher than the young plants, as reported in Figure 29.

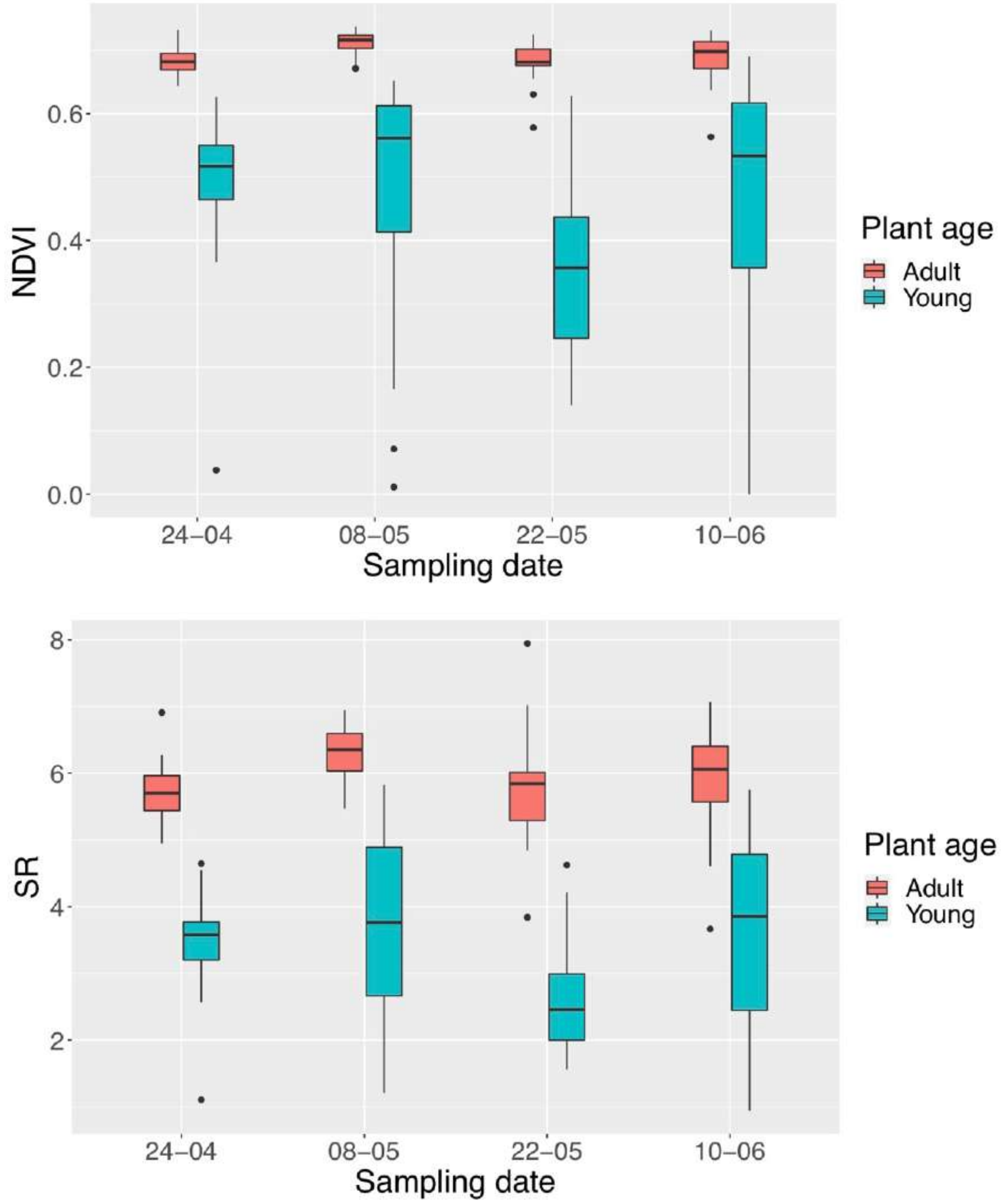

Figure 29 The development of SR and NDVI indices over the course of the data collection season for both young and adult treatment groups.

Moreover, both indices show a slowly decreasing trend during time for young plants, particularly evident in the last sampling date (June 10th). This trend seems to be inversely related to the measured increase of necrotic leaf areas, below reported as box plot (Figure 30).

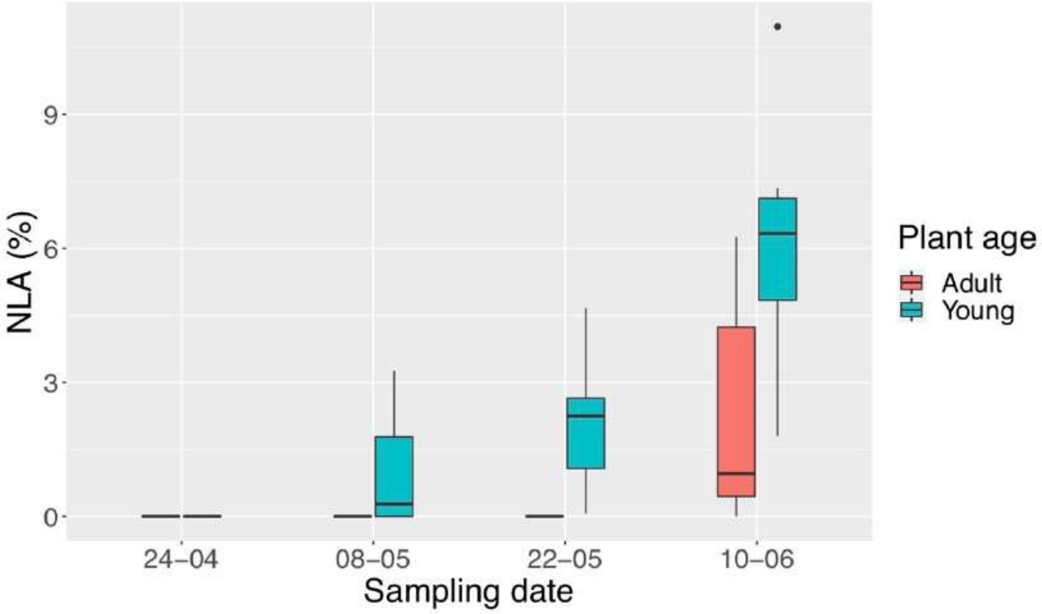

Figure 30 Percentage of necrotic leaf area on irrigated, non-irrigated young and adult trees in the four sampling dates.

However, at the last sampling date available, June 10th, the NLA was still quite low, so that is expected to raise further in the following samplings. Thus, these preliminary indications will require the data from the remaining part of the vegetative season 2021 to be confirmed.

4 New Guidelines to control insects phytophagous and plant pathogens

In the light of the usual field practices applied by agronomists in the phytosanitary management of hazelnut plantation, we propose below a new set of guidelines, which considers the valuable results in the automation obtained within the PANTHEON project and that can be of high support in the reduction of both time-consuming activities and of the chemical input for the contrast to the described pests and diseases.

4.1 True bugs

For the definition of new guidelines for partially automated monitoring and control of true bugs, we propose the following alert system based on the early detection, with the YOLO system, of the phytophagous approaching the orchard attracted and collected by pheromone baited traps positioned around the field. This system, integrated with standard pest management practices, reduces human time and effort and it is an innovative approach for dealing with these harmful organisms.

As shown in the flow chart (Figure 31), starting in April, in concomitance with the vegetative restart, sticky traps baited with sexual and aggregative pheromone traps, commercially available, must be placed around the hazelnut plantation and not inside. After positioning, a robotic platform integrated with the described acquisition tool (YOLO system) inspects the traps distributed around the field on a weekly basis. The objective of this action is to intercept the true bugs that, once they have overwintered in artificial or natural shelters, move into the hazelnut orchard to begin the feeding phase causing damage to the nuts. If the system detects the presence of true bugs, a human-led monitoring (frappage) should start, which will determine, on a weekly basis, the presence and numerical consistency of true bugs in the field. On the other hand, if the automated system does not detect any bugs, it will continue to acquire new information every week.

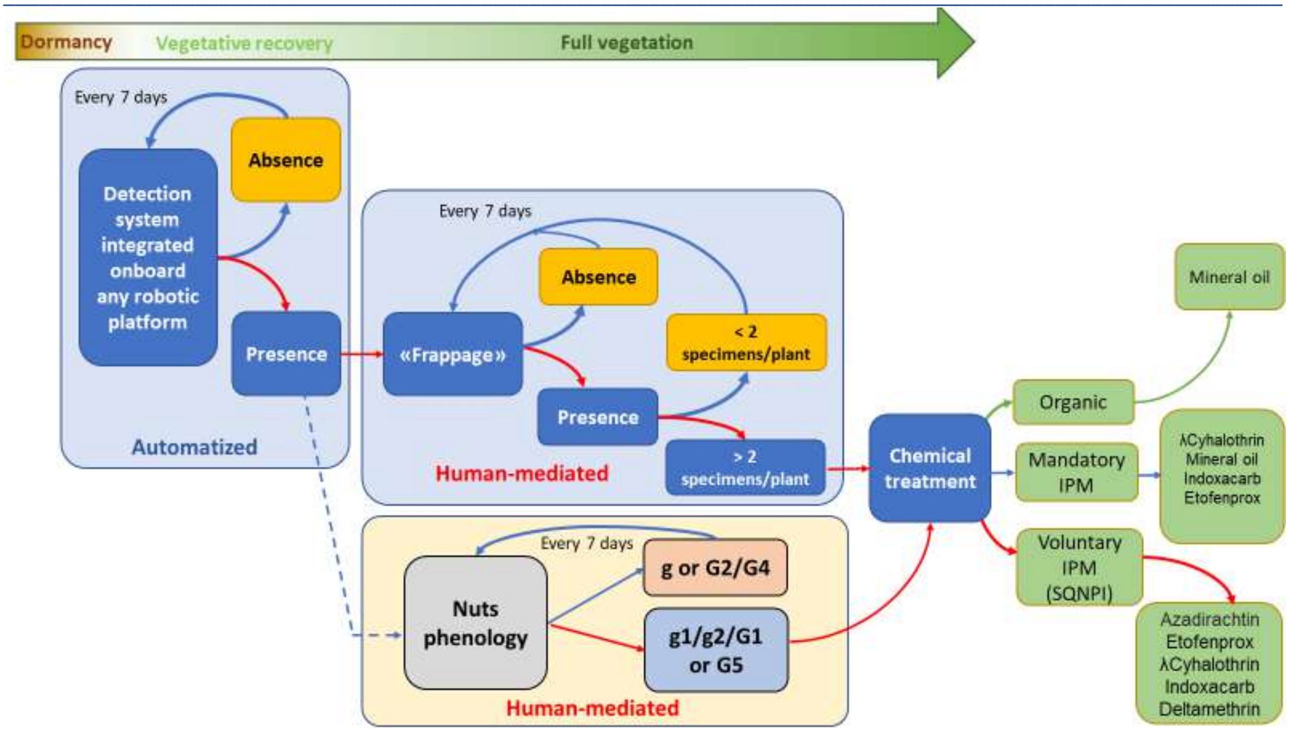

Figure 31 The flow chart represents the new guidelines for the management of true bugs in Latium Region Italy, integrating an automatized detection with usual human practices.

Thus, once the automated system detects and counts the presence of true bugs on the traps around the field, the frappage should start since this is still the most accurate and effective method to determine the real consistency of pests within an orchard. Further activities based on the findings of this project will be aimed at identifying the relationship between the number of true bugs counted by the automated system on the traps and the consistency of these phytophagous in the field. In the Latium region, where the experimentation has been conducted, 2 specimens/plant collected weekly on 10 plants is the threshold above which chemical treatment must be applied, depending on the production system that the farmer has decided to adopt (organic, mandatory IPM, voluntary IPM). However, the presence of bugs in a hazelnut orchard is not the only factor that can lead to damage. In fact, another factor is involved in the traumatic abortion or *cimiciato* damage occurrence and it is the susceptibility of the nut, which is temporary, especially in the case of abortion. The relationship between nuts susceptibility (as showed in the section 3.1.2) and the numerical consistency of true bugs in the field are therefore the information that human activity must obtain at least weekly. The chemical treatment for the control of these phytophagous are those showed in the flow chart and are regulated by Regional regulations.

4.2 Gall mites

The definition of new guidelines for partially automated monitoring and control of gall mite, followed the example provided for the true bugs. Thus, the results obtained confirmed the suitability of introducing an alert system based on the YOLO technology, able to detect, during the winter or at the beginning of spring, the presence of galls induced by the mite. The flow chart in Figure 32 shows the procedures triggered by the automated detection of the presence of galls on plants that lets start a series of human-led activities for the refining of information on the mite population size in the field. In fact, our experiments showed high effectiveness (average accuracy equal to 82.2%) in detecting galls but not healthy buds, which makes it impossible to determine the 20% ratio of galls to total buds identified as the threshold for intervention.

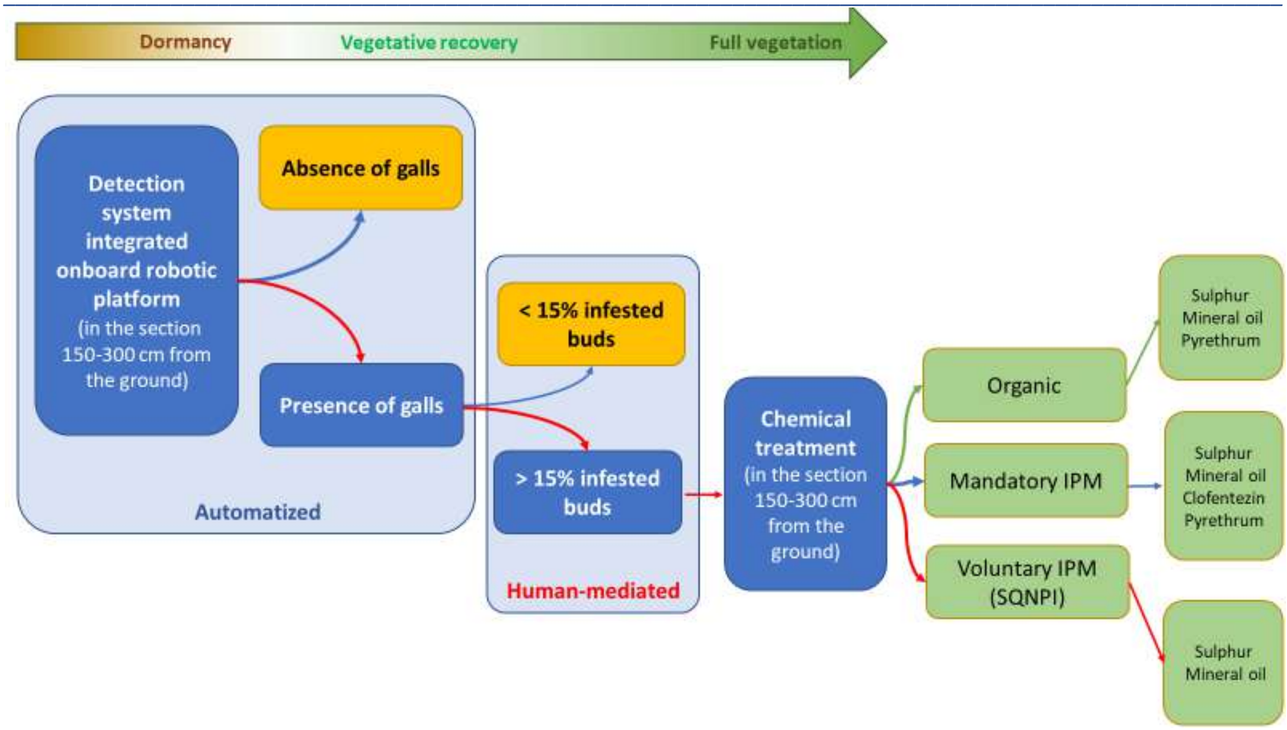

Figure 32 The flow chart represents the new guidelines for the management of gall-mite in Latium Region Italy, that integrates usual human practices with an automatized detection of the pest.

More specifically, in the guidelines we propose, the automatized activity must be conducted at the beginning of spring (end of march/early April) and it requires that a robotic platform integrated with the described acquisition tool (YOLO system) inspects the hazelnuts in the orchard. Our findings highlighted that the galls are concentrated in a belt comprised between 150 and 300 cm from the ground, thus it is effective to focus monitoring actions in this area. In case of the presence of galls, the agronomist must apply the methods described in section 2.2.1, which require the count of galls and buds on four branches, one per cardinal direction, selected from 10 plants per hectare. This allows to determine the effective level of infestation caused by the mite and if this is higher than the intervention threshold (15/20% of the infested buds) above which chemical intervention is necessary. If monitoring shows that the threshold is reached, a chemical treatment must be applied during the mite migration phase. This normally occurs in April, when the average temperature is 11/12 °C and when the new buds have 3-4 leaves completely opened. The selection of the active ingredient is ruled by regional regulations and is based on the phytosanitary management that the farmer has decided to apply (organic, mandatory IPM or voluntary IPM/SQNPI). In any case, the dispersion of the active ingredient must be concentrated not to the whole canopy, as we highlighted in section 3.2.2, and as already mentioned for monitoring, but in the zone between 150 and 300 cm above the ground.

4.3 Hazelnut anthracnose

To the current status of the knowledge, the hazelnut anthracnose can be controlled only by preventive scheduled treatments with copper in both IPM and organic farming in Latium region. However, these treatments are scheduled regardless of the real occurrence of the disease or not. In the project, we attempted to organize a decisional workflow based on both human-mediated and automated information, aimed to determine the timing and significance of disease presence, and consequently providing a warning alert indicating that phytosanitary treatments are required and their best timing. It is based on 3 steps:

i. evaluation of the presence of the pathogen in dormant buds (%), assessed by species-specific qPCR (HUMAN-MEDIATED) on a sample of 20 buds per plants (10 plants/ha). If the pathogen is present, a visual evaluation of bud rot incidence should be carried out by trained technicians. Even if a clear correlation between the bud rot incidence and the incidence of the disease during the season wasn't still defined, a threshold of 20% of damaged buds seems to be plausible.

ii. Model prediction of the evolution of the disease in field according to meteorological data (AUTOMATIZED). When the threshold above is exceeded, a prediction model should be run to forecast the growth of the fungus in plant tissue, its sporulation the spread of the spores in the environment and the germination of spores (conidia). This model is at its first steps of definition, with details only about the influence of temperature on mycelial growth (T_M, T_L and T_{OPT}) and on conidial germination.

iii. Early assessment of plant disease on canopy (AUTOMATIZED). This step implies the assessment of anthracnose incidence from vegetational indices acquired from UAV and it should flank the indications provided by the predictive model. When a disease index threshold (to be defined) is exceeded, the system suggests the execution of a phytosanitary treatment to the farmer. As things currently stand, NDVI and CR are the most promising vegetational indices, but further experiments are needed to corroborate the results

The basic concept for the implementation of future decision support system for integrated Pest Management of this pathogen is represented in Figure 33. The chemical treatments for the control of these fungal pathogen are subject to European, National and Regional regulations and are also dependent on the production system chosen by the farmer (organic, mandatory IPM, voluntary IPM).

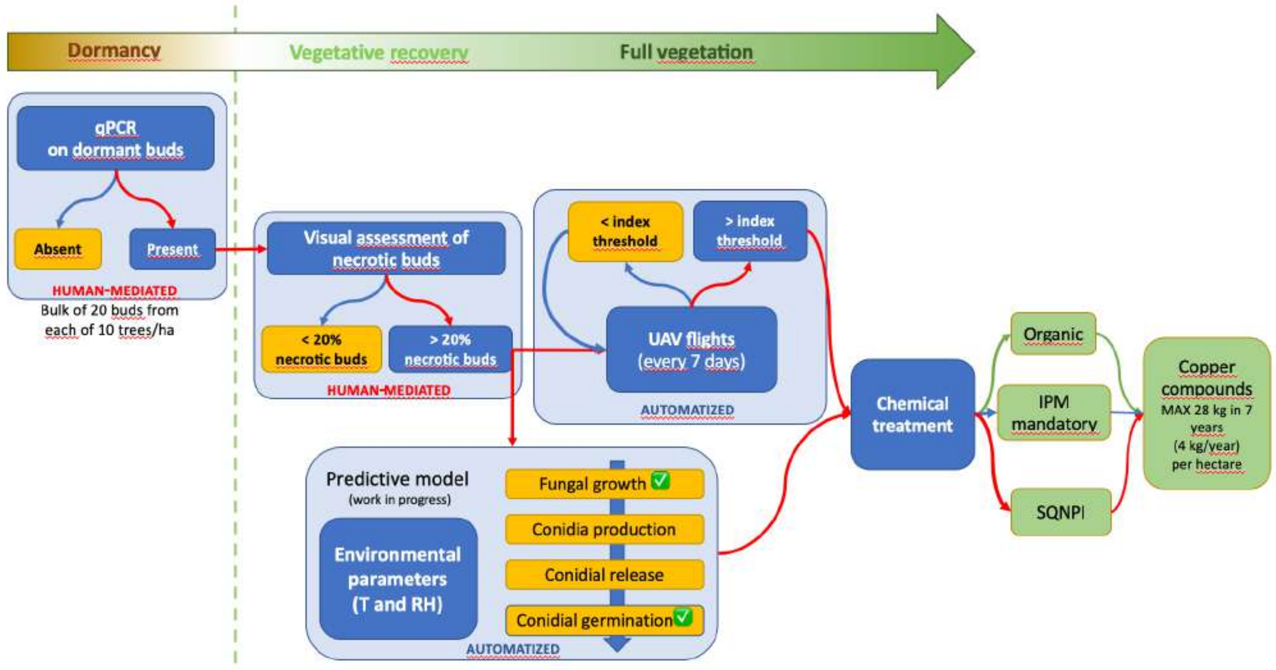

Figure 33 The flow chart represents the concept guidelines for the management of anthracnose, integrating human mediated and automatized protocols. Treatments are in agreements with regulation in Latium Region, Italy

5 Conclusions

This document summarizes the development of automated methods for assessing the phytosanitary status of the plantation and it suggests automated and human mediated interventions to farmers for some operations to monitor and manage pests and plant diseases. New guidelines useful in the reduction of both time-consuming operations and chemical input are presented.

5.1 Completed tasks

5.1.1 Pests

The data collected during the years of experimentation has permitted the definition of new guidelines for the management of pests on hazelnut, that apply technological advances in the automation of some practices, commonly carried out by humans, for the reduction of time-consuming activities. Specifically, we demonstrated that our data-driven pest detection system integrated on robotic platforms, provides the automatic detection of true bugs on pheromone traps, while these pests are approaching the hazelnut orchard, thus triggering further activities, this time human mediated, to determine the effective consistency of the phytophagous in the field. The data-driven detection system has also been applied effectively for the detection of galls caused by the mite *Phytoptus avellanae* and even in this case, the simple detection of these structures on the plant triggers further monitoring activities by agronomists.

The studies carried out also allowed the identification of the exact time when the control of the various pests must be conducted. In the case of true bugs, in fact, it has been verified that the phenological stage of the hazelnut is fundamental in the occurrence of the two types of damage that these pests can cause. For this reason, we suggest that the monitoring of true bugs population consistency (with frappage) must be integrated with weekly observations on nut phenological development, to identify the precise high susceptibility stage of the nut to the feeding action of the phytophagous.

Regarding the optimization of the quantities and the active ingredients of pesticides, the identification of a band between 150 and 300 cm from the ground where the mite-induced galls are concentrated, allows both monitoring and chemical control to be focused on this band and no longer on the whole canopy. This allows a clear reduction of pesticides dispersion, thus increasing the sustainability of this crop.

5.1.2 Anthracnose

The first step concerning the molecular method for detection of *M. coryli* in plant tissue, both symptomatic and not, has been achieved and it is going to be submitted for publication. The relationships between pathogen growth and conidial germination have been described and they are the subject of another ongoing paper. However, they constitute only the preliminary step for the definition of a predictive mathematical model. Finally, despite the huge efforts since the beginning of the Project, even if the data about disease assessment by ground surveys and UAV remote sensing collected during the current vegetative season are giving interesting indications, they are not yet sufficient to draw consistent results on automated detection of disease occurrence.

5.2 Future Research

Many further investigations should be carried out in the future based on the findings described in this deliverable.

5.2.1 Pests

The high potential of the data-driven pest detection system made it possible in our experiments to identify *Palomena prasina* specimens on pheromone sticky traps placed in the field. This technology can be tested in the future to distinguish on the traps, different species of true bugs: it will be extremely important, as the intensity of the damaging action caused by these organisms is not the same. Some of these bugs, particularly the newly introduced in Italy *Halyomorpha halys*, showed a greater ability to induce the damages to hazelnuts. Moreover, future experimentation will be aimed at identifying the relationship between true bugs counted by the automated system and their consistency in the field. This will allow the complete automation of the monitoring of these harmful organisms.

Furthermore, we have also demonstrated that the data-driven approaches applied to the detection of galls caused by *Phytoptus avellanae* provides encouraging results as well. This experimentation will need to be refined in the light of the correct identification and counting of healthy buds. This would make it possible to obtain, with an automated system, the ratio of galls on healthy buds that is the basis of the economic intervention threshold for this pest. This would further reduce the number of field human mediated activities.

5.2.2 Anthracnose

Future research on biological features of the pathogen as related to environmental variables will be addressed to complete all the modules required for a robust predictive model, capable of simulate and predict the evolution of the disease according to specific environmental conditions. If the results about vegetational indices and ground truth data of the entire 2021 season will confirm the current trend, a validation could be attempted during next vegetative season 2022.

5.3 Criticalities and Mitigation Actions

5.3.1 Pests

Criticalities. In the Deliverable 4.5 “Pest and disease detection” one of the major objectives of pests detection task was to identify spectral indices which are sensitive to plant damage caused by pests. Thus, it was attempted to assess the damage caused by true bugs by means of spectral analysis of the trees at the branch level. However, the analysis of multispectral images did not identify significant spectral response at the branch level. COVID19 pandemic caused delay in some field activities.

Mitigation actions. The unsatisfactory results of the approach described in Deliverable 4.5 prompted the design, experimentation, and application of a completely different approach, i.e., a data-driven system for the detection of the damage agents, e.g., true bugs, even before they cause the damage. Indeed, this provides the farmer with the possibility of preparing a series of measures to contain these pests and their damage. Furthermore, motivated by the encouraging results obtained in true bugs monitoring, we attempted to replicate this data-driven architecture also for early spring detection of *Phytoptus avellanae* induced galls in order to detect a triggering condition, i.e., if the intervention thresholds were exceeded, for the application of the control treatments allowed by the various phytosanitary regulations.

5.3.2 Anthracnose

Criticalities. The project aimed to the definition of an automated assessment of disease onset based on the orthoimages acquired by different sensors mounted on UAV and the consequent vegetational indices obtained from those images. Unfortunately, during 2019 and 2020 campaigns, several difficulties, such as inconsistencies in the type of data acquired from UAV and related technical issues, along with the enormous impediments imposed by COVID19 pandemic, seriously affected the research activities.

Mitigation actions. A first mitigation action was the delay of this Deliverable to include field and UAV data from 2021 campaign. This is ongoing and the possibility to obtain useful indication remains, depending on the results that will be obtained till the end of the 2021 season. However, both the definition of the molecular assay (qPCR) for reliable detection of the pathogen in plant tissue and the study of the influence of environmental parameters on biological behaviors of the pathogen were aimed to provide auxiliary elucidations to the final goal of early detection of the pathogen.

5.4 Ongoing activities

5.4.1 Anthracnose

At the time of this Deliverable submission, the collection of both remote sensing and ground truth data is not yet complete and it will continue up to the end of vegetative season 2021 (middle August). The results, once available, will be integrated with new data according to the methodology presented here.

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