CHAPTER 4

ABSTRACT

Every year, different harmful organisms (pests and pathogens) significantly reduce the potential yield of agricultural crops and threaten the sustainability of the planet's forests. At present, this situation is compounded by the harmful effects of climate change. It is essential to generate new knowledge and technologies for a more efficient control and management of strategic diseases and pests that constitute a threat in key productive sectors for the world economy, and in particular the Spanish one.

KEYWORDS

plant health | pests | climate change | emerging diseases | resistance | genome editing | crop production | forest | plant pathogens

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CHAPTER 4

PLANT HEALTH. RESISTANCE TO PESTS AND DISEASES

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EXECUTIVE SUMMARY

Plant health is of global importance for sustainable agriculture, food security and environmental protection. To satisfy a growing demand for food, global agricultural production must increase by 70% by 2050. However, main agricultural crops are threatened by pests and diseases that put global food supplies at risk. Rates of introduction of exotic of pests and pathogens are increasing exponentially in recent years, driven largely by altered patterns and increasing rates of travel and trade. Climate change and global trade drive the distribution, host range, and impact plant pests and diseases, many of which can spread or reemerge after having been under control. Indeed, many historical and contemporary diseases are emerging as major threats to modern agriculture and food security. Forests, as main components of natural ecosystems that provide not only important primary resources (timber, resin, cork) but also crucial ecosystem services for the humanity (climate regulation, carbon storage, wildlife support, social use and recreation), are also at unprecedented sanitary risk.

Given this scenario, the present committee sees the following key challenges for the next 10-20 years in the field of plant health: 1. Globalization and increasing impact of climate change in plant health; 2. Control of emerging and re-emerging pests and diseases; 3. Strategic pests and diseases that constitute a threat in the key productive sectors for the Spanish economy; 4. Sustainable management of pests and pathogens resistance to phytosanitary treatments; 5. Achieving broad spectrum immunity through genome editing.

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The achievement of the key challenges described in this report will not only provide crops more resilient to different pathogens and pests, but will contribute also to develop robust and sustainable bio-economies while preventing environmental degradation and will likely contribute to empower high technological innovations and further high-impact revolutionary developments. Particularly, both synthetic biology and genome editing will provide the opportunity for developing useful tools to generate more resistant and safer crop plants. A better understanding of different issues described in this challenge compilation will be of clear direct or indirect benefit to the agronomic and forestry sectors.

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1. INTRODUCTION AND GENERAL DESCRIPTION

Plant health is of global importance for sustainable agriculture, food security and environmental protection. To satisfy a growing demand for food, global agricultural production must increase by 70% by 2050. However, main agricultural crops are threatened by pests and diseases that put global food supplies at risk. Accurate information on the extent of losses from pests and disease is difficult to estimate but 30-40% losses in developing countries annually from 'field-to-fork' are common (Flood, 2010). More specifically, yield losses caused by pests and diseases are estimated to average 21.5% in wheat, 30.0% in rice, 22.6% in maize, 17.2% in potato, and 21.4% in soybean; these crops account for half of the global human calorie intake (Carvajal-Yepes et al., 2019). According to the latest estimates from the Food and Agriculture Organisation of the United Nations (FAO), 840 million people, 12% of the global population, were unable to meet their dietary energy requirements (http://www. fao.org/publications/sofi/en), with even greater numbers lacking minimal requirements of essential vitamins and minerals. Previous European Union (EU) agricultural policy had focused on constraining food production but there is a new understanding that the EU should now increase its biomass production for food, livestock feed and other uses, including renewable materials to support the bioeconomy. Agriculture will face in the near future major challenges to deliver food and nutrition security at a time of increasing pressures from climate change, social and economic inequity and instability, and at the same time facing the continuing need to avoid further losses in biodiversity and ecosystem services.

Rates of introduction of exotic pests and pathogens have increased in recent years, driven largely by altered patterns and increasing rates of travel and trade (Brasier, 2008). Under the present constraints of limited availability of arable land, climate change, increased seasonal weather instability, and intensive global trade, the threat posed by plant pests and diseases to humankind may become even more serious, particularly because these conditions favor the emergence or re-emergence of harmful organisms that have high impact. Many historical and contemporary pests and diseases that were under control are now reemerging as threats to modern agriculture and food security. The European Food Safety Authority (EFSA, 2012) states that emerging risks can result from: i) a newly identified plant threat for which a significant probability of introduction and geographical spread may occur; ii) an unexpected new or increased probability of introduction or spread of

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an already known agent, for example as a consequence of new trade or new agricultural policy and iii) a new or increased susceptibility of host plants to a known agent or to an agent with altered virulence (including development of resistance to previously effective control measures mainly of chemical nature) or extended host range. Tackling the issue of existing, evolving as well as invasive insect and pathogen species is of crucial importance as they are a major threat to plant health and are considered to be one of the main direct drivers of several detrimental effects on biodiversity, human and animal health, and crop production.

Forests, as main components of natural ecosystems that provide not only important primary resources (timber, resin, cork, etc.) but also crucial ecosystem services for the humanity (climate regulation, carbon storage, wildlife support, social use and recreation), are also at unprecedented sanitary risk (Trumbore et al., 2015). Recent anthropogenic influences such as climate warming, drought intensification, wildfires and the continuous expansion of new pests and pathogens beyond biogeographical regions, are dramatically challenging the resilience of natural and planted forest, riskily pushing some forest ecosystems to a point of no return (Seidl et al., 2017). Prevention, mitigation and management of forest health decay is a main challenge for the scientific community, especially given the intrinsic complexity of forest ecosystems and the difficulties associated to their investigation and management. Both the large spatial and temporal scales of forest ecosystem impose conceptual questions and methodological challenges that need to be addressed. Research must do a stronger effort to combine long-term time series of forest monitoring, broad-scale tools (e.g. remote sensing) for landscape-scale health assessments, and mechanistic fine-scale investigations in order identify the drivers of forest decay, how they interact and the recovery trajectory of forest functioning. Concomitantly, the low profitability of forest systems and their environmental context prevent the use of intensive phytosanitary tools, making the search of alternative environmentally-friendly and cost-effective ways to preserve forest health an urgent need. Finally, while for agricultural crops, yield and crop quality are clear objectives to be pursued, forest ecosystem health needs a more holistic definition, in which fundamental attributes of forest ecosystems (e.g. species biodiversity, soil protection, vegetation structure, carbon and nutrient cycling) must be considered (Millar and Stephenson, 2015).

Given this scenario, the present committee identifies the following key challenges for the next 10-20 years in the field of plant health:

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1. Globalization and increasing impact of climate change in plant health
2. Control of emerging and re-emerging pests and diseases
3. Strategic pests and diseases that constitute a threat in the key productive sectors for the Spanish economy
4. Sustainable management of pests and pathogens resistance to phytosanitary treatments
5. Achieving broad spectrum immunity through genome editing

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

Currently, our society demands longer, healthier and safer life. Plant health has a relevant role that must be promoted and sponsored by the corresponding public authorities. Existing a close linkage between plant health, food security and societal stability, the spread of plant pests and diseases among crops and forests, has significant global consequences for farmers, the seed industry, policy-makers, the general public, and the environment and landscape.

Research in plant health has contributed to science in a broader way, beginning with the work of the plant pathologist Anthony de Bary, and others, who helped to establish the germ theory of disease. This served as the foundation for innovations that improved the human condition by establishing the etiology of diseases affecting people and also by limiting the impact of plant pathogens on the food supply. Other significant contributions of basic science in plant health have been the identification of bacteria and viruses as disease-causing agents, demonstration of RNA as a genetic molecule, and the discovery of novel phenomena such as the autocatalytic properties of the RNA, the ice-nucleating bacteria and RNA silencing (Fraenkel-Conrat and Williams, 1955; Lindbo and Dougherty, 2005; Lindow et al., 1982).

The achievement of the key challenges described in this report will not only provide crops more resilient to different pathogens and pest, but will contribute also to develop robust and sustainable bio-economies while preventing environmental degradation and will likely contribute to empower high technological innovations and further high-impact revolutionary developments. Particularly, both synthetic biology and genome editing will provide the opportunity for developing useful tools to generate more resistant and safer crops. A better understanding of the different issues described in this challenge compilation will be of clear direct or indirect benefit to the agronomic and forestry sectors.

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3. KEY CHALLENGING POINTS

3.1. Globalization and increasing impact of climate change in plant health

Climate change and globalization act as main drivers of the increasing sanitary risks. The complex nature of plant-pathogen and plant-pest interactions make difficult to predict the impacts on crop yields and quality due to changes in climate, as well as to generalize the appropriate strategies to minimize them. Outcomes of climate change in plant pathosystems include, among others, modifications in host resistance, altered stages and rates of pathogen and pests (including vectors) development and changes in the physiology of host-pathogen-vector interactions. Likewise, climate change may have a direct effect on the phenology and physiology of both host and pests, as well as indirect effects on the interaction of pests with their hosts, natural enemies and competitors. These effects have been predicted to result into shifts in the geographical distribution of hosts, pathogens and pests, changes in pest and disease incidences and severity of damage, altered crop losses due to harmful organisms and modifications in the efficiency of management strategies (Canto et al., 2009; Sharma, 2014; Jones et al., 2016; Forrest, 2016).

Despite the efforts in the last decades to prevent biological invasions, agricultural pests and pathogens continue to spread at unprecedented rates (Strange and Scott, 2005; Dehnen-Schmutz et al., 2010). The extent and speed at which the geographical distribution of pest and pathogens is spreading is determined by the processes that facilitate their dissemination to new areas (e.g. global trade), favored by extreme climatic events and by the ability to survive in them that is determined primarily by the minimum winter temperatures. Between 2010 and 2015, ProMED (http://promedmail.org) has listed approximately 140 new records of plant pests and pathogens. Most common are reports of newly arrived viruses and viroids (36.6%), followed by fungi (28.2%). ProMED reports primarily on infectious diseases, including plant-pathogenic nematodes, and thus new reports of insect pests are rare. The majority of new observations are in the Northern Hemisphere, although several records are from Australia. The most commonly affected crops are maize (9.1%), bananas (8.5%), citrus (7.7%), and potato (7.0%) (Bebber, 2015), while almost all forest systems are at risk (Santini et al., 2013).

The effects of variations in climate on cycles of pests and pathogens are influenced by the effect of such variations on crop development. Available information suggests that the elevated CO_2 (eCO_2) in the atmosphere will increase

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the plant photosynthetic rate, but the extent of the increase will depend on the levels of other environmental factors such as temperature or relative humidity, plant type (C3 or C4), or irrigation efficiency.

The increase in temperature projected by climate models can favour shorter reproductive and infection/infestation cycles by plant pathogens and pests, as well as an increase in their reproduction rate. Both effects can operate in favour of the evolutionary potential and virulence gain processes of plant pathogens, which may be associated with both the increase in the size of the resulting populations and the highest expectation of survival of such populations between crop stations. Pests, including those that comprise the main groups of virus vectors such as aphids, whiteflies, cycads and thrips, are very sensitive to changes in temperature, wind and precipitation. The likely increase in winter temperatures in the coming years will modify the biological cycle and behavior of these insects, as well as their ability to expand to other geographic regions. Since 1960 there has been a displacement of more than 600 pests and diseases towards the poles at an average of 2.7 km / year (Bebber, 2015). Also, changes in the temperature regime alter the plant´s physiology affecting the two better-known types of antiviral resistance mechanisms, those based on RNA interference and dominant resistances based on protein-protein recognitions (Canto et al., 2009). Other interactions with stress response pathways in plants may also have consequences for disease expression and progression.

Pest and pathogen control will be foreseeably more complicated, due to the difficulties to predict the response of pest, pathogen and vector populations to the physiological alterations of the plants and the changes in ecosystem functioning that may have effects on host susceptibility. On the positive side, we must count on recent technological advances that have improved significantly our ability to react. For example, the use of high throughput sequencing is greatly facilitating the discovery of the etiology of new diseases, and is providing the information necessary to design effective and affordable detection tools. Similarly, advances in breeding technologies for cultivated plants, including genome editing, are providing responsiveness in terms of genetic improvement never before experienced.

Challenges

It is crucial to intensify research on globalization, climate change and their consequences. Over the past few years, some notable shortcomings have been corrected, but many aspects remain poorly explored. The effects of the most

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important parameters (essentially temperature and eCO_2) have been studied in detail for a certain number of systems, but there are other parameters little studied, including the effect of greenhouse gases other than CO2, the effect of increases or decreases in precipitation, relative humidity and unusual events such as tornadoes, floods, extreme droughts or massive wildfires. On the other hand, real situations are complex, and therefore future lines of research should intensify the study of factors —both biotic and abiotic— combined, under controlled experimental but also upscaling to situations closer to reality. Additionally, it is essential to advance in the modeling of trade and climate change scenarios in relation to the emergence, incidence and severity of pest and pathogens, both for medium- and long-term predictive purposes and to help in the application of short- and medium-term control strategies.

3.2. Control of emerging and re-emerging plant pathogens and pests

Emerging and re-emerging plant infectious diseases and pests are major threats to food security. Their incidence increases due changes in the epidemiology frequently associated with invasion of new pest or pathogen populations, and their impact might be particularly severe. Alteration of the equilibrium between harmful organisms and host plants present in natural ecosystems results into the emergence or re-emergence of pests and diseases. Human activity facilitates new damaging encounters between plants and pathogens-pests. Moreover, the alteration of plant landscapes (e.g. widespread cultivation in monocultures, introduction of new plants or new plant genotypes) or the intensification of global trade of plants and plant products increases the instability favouring emergence of pests and diseases. Furthermore, climate change is also severely modifying co-evolved equilibrium, potentially leading to increased problems associated to harmful organisms of plants. Epidemiological theory predicts that the emergence of new pests and diseases is the result of complex interactions among these multiple factors. Diseases and pests reduce global agricultural production by about 10-15% each, and the highest losses often hit food-deficit regions with fast-growing populations where frequently emerging or re-emerging problems occur. Understanding the processes that lead to the emergence or re-emergence of pathogens and pests would help preventing the serious agricultural problems that they pose.

In this scenario, plant health practices should help to guarantee food security and safety. For that, pest and pathogen control measures are required to prevent crop yield losses. The European legislation promotes the use of control methods that

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are environmentally friendly and respectful with human health, and aims to implement Integrated Pest Management (IPM) strategies (EC, 2020). Sustainable biological, physical and other non-chemical methods must be preferred to chemical methods, and the pesticides applied shall be as specific as possible for the target and shall have the least side effects on human health, non-target organisms and the environment. However, resistant crop varieties, biocontrol agents and/or pesticides efficient against key pests are not always available, especially for those recently introduced. The development and improvement of novel technologies (e.g.- pest resistant varieties generated by CRISPR/Cas and other editing technologies, microbiome-primed plants, geolocation and molecular tools for pest monitoring, remote sensing technics for early detection, mathematical modelling for the establishment and spread, IPM, etc.) are expected to have an impact in the near future. In addition, the complexity of agroecosystems, together with the threats derived from climatic change and the growing social demands relative to food security emphasize the need of generating basic knowledge and its transference to the productive sector.

Emerging exotic pests and diseases are of particular relevance in forest systems. While native antagonists co-habit with natural forests in a dynamic equilibrium and rarely cause alarming outbreaks, exotic pests and diseases, once established into a new geographical range, may cause devastating effects on forest ecosystem functioning. The Dutch elm disease and the ink disease of chestnuts are good examples of how exotic invaders may decimate native forests when expanding to new habitats. Many other pests and diseases are nowadays initiating its spread across Iberian forests posing unprecedented problems to many if not all native forest trees. Lack of natural enemies and lack of efficient resistance mechanisms may be behind the high susceptibility of native forests to these imported enemies. However, knowledge on these newly established interactions is still very limited. As natural extensively-managed systems with low economical return, research efforts for improving early diagnosis and large-scale monitoring, fine-tuning integrated management control, management actions that favor resistance enhancement or minimize the impact of damage on tree fitness (tolerance) and assessing long-term and broad-scale impacts in ecosystem services are urgently needed.

Challenges

- Improve approaches and large-scale methodologies for pests and pathogen surveillance and diagnosis aimed to facilitate the rapid implementation of contingency plans.

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- Fine-tune methods for landscape-scale assessment of agricultural and forest health, in general, and incidence of exotic enemies, in particular, using remote sensing tools.
- Understanding factors driving emergence and evolution of pathogens and pests. Modelling and forecasting.
- Improve the mechanistic understanding and the ecophysiological basis of the high susceptibility of the native flora to alien enemies.
- Insights into global warming as driver of the emergence or re-emergence of pests and diseases.
- Enlarge the range of tools for integrated, sustainable and effective management of emerging and re-emerging pests and diseases.
- Understanding the impact of emerging and re-emerging harmful organisms on crops, wild plant communities, and the ecosystem services they provide.

The expected impact will be obtaining adequate responses to emerging disease or pests that may cause agricultural problems. In the longer term, acquired knowledge will help the agricultural/forestry sector to remain productive and contribute to agriculture sustainability and/or forest health. Adequate management of emergent pests and diseases will help to avoid large-scale transformation in the landscape and maintain socially valued ecosystem services of forests. Major needs should be solved to attain the challenges, especially increasing investment in: i) biosecurity containment facilities and specialized personnel to work with exotic and quarantine pathogens and pests; ii) permanent positions for highly qualified young scientists that could integrate different approaches and research disciplines; iii) permanent positions for qualified lab technicians; and iv) singular equipment shared in network frame (such as large scale phenotyping and genome sequencing platforms, etc.).

3.3. Strategic diseases and pests that constitute a threat in the key productive sectors for the Spanish economy

As indicated in previous sections, the threats posed by re-emerging or new plant pests and diseases are now greater than ever. The number of plant pests establishing in Europe has been predicted to increase significantly in the next 10 years based on current trends (EU project DAISIE at www.europe-aliens.org). Recently, the European Commission established a list of 20 priority quarantine pests (EC, 2019), based on their potential economic, environmental or social impact in respect of the EU territory. The purpose of this chapter is not to provide a detailed revision of all the harmful pests and pathogens

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(quarantine and indigenous) that currently pose a threat to Spanish agriculture and forestry, but to focus on those for which the CSIC is playing or may play a crucial role on gaining basic knowledge and contributing to develop innovative management approaches for minimizing their impacts.

3.3.1. Main pest of agriculture and forest Iberian systems

Indigenous pests, either endemic or immigrant species that have been established for a long time in our country, constitute a persistent threat for some key productive sectors. Examples of this type of pests in our country are the Mediterranean fruit fly, *Ceratitis capitata,* for fruit trees and the *aphids Myzus persicae* and *Aphis gossypii* for vegetable crops, though there are many others. In addition to these prevalent pests, some of the threats that have raised the most serious concerns in the last years correspond to recently introduced exotic species, such as the red palm weevil, *Rhynchophorus ferrugineus,* or the tomato moth, *Tuta absoluta*. In the EU priority quarantine list (EC, 2019), a total of 16 pests of vegetable, arable and fruit crops and forest trees has been included: fruit flies (*Anastrepha ludens, Bactrocera dorsalis, B. zonata Rhagoletis pomonella*), curculionids (*Anthonomus eugenii, Conotrachelus nenuphar*), lepidoptera (*Spodoptera frugiperda*, *Thaumatotibia leucotreta, Dendrolimus sibiricus*), psyllid (*Bactericera cockerelli*), and six coleopteran (*Aromia bungii, Popillia japonica, Agrilus anxius, A. planipennis, Anoplophora chinensis, A. glabripennis*). Additionally, other exotic pests not included as EU priority pests such as *Leptoglosus occidentalis* in stone pine orchards or *Dryocosmus kuriphilus* on chestnuts are all causing or are predicted to soon cause serious problems in Iberian forests. Finally, although some pests do not cause significant direct feeding damage *per se,* they are vectors of important plant pathogens (mainly virus and bacteria).

3.3.2. Main pathogens in agricultural and forestry Spanish systems

In Spain, besides native pathogens that have affected or continue affecting strategic crops causing severe diseases and important yield losses, at least half a hundred of new pathogens have been introduced, and other pathogens are spreading or increasing in virulence promoting the emergence and reemergence of plant diseases. Among those pathogens threatening the Spanish agricultural and ornamental plant sector include: 1) oomycete and fungi (diverse *Aspergillus* spp. and *Fusarium* spp. producing mycotoxins, *Botryosphaeria* spp., *F. mangiferae, F. oxysporum* f. sp. *basilici, F. solani* f. sp. *cucurbitae* race 1, *F. sterilihyphosum, Monilinia fructicola, Mycosphaerella nawae, Phytophthora*

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hedraiandra, P. tentaculata, Verticillium dahliae, several fungi causing grapevine trunk diseases); 2) bacteria and phytoplasmas (*Candidatus* Liberibacter *solanacearum, Clavibacter michiganense* pv. *sepedonicus, Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Erwinia amylovora, Pseudomonas viridiflava, Ps. syringae* pv. *actinidae, Ralstonia solanacearum, Xanthomonas arboricola* pv. *pruni, X. vesicatoria, Xylella fastidiosa*, Flavescence dorée or 'Stolbur'); 3) several viruses and viroids (e.g., Cucumber green mottle mosaic virus (CGMMV), Citrus Tristeza Virus (CTV), Cucumber vein yellowing virus (CVYV), Cucurbit yellow stunting disorder virus (CYSDV), Sharka of stone fruit trees (PPV), Tomato chlorosis virus (ToCV), Tomato torrado virus (ToTV), Tomato brown rugose fruit virus (ToBRFV), begomovirus etc.); and 4) nematodes (*Aphelenchoides besseyi,* several *Meloidogyne* spp. on fruit trees). Among those pathogens, the nematode, *Bursaphelenchus xylophilus* and the bacteria *Xylella fastidiosa* and *Candidatus* Liberibacter spp., are listed as priority pests for Europe (EC, 2019).

There are several outstanding examples of pathogens threatening Iberian crops, among which some of them deserve special attention due to the dramatic economic consequences that its entrance or spread could cause to Spanish agriculture, or because there are several CSIC's research groups working on them: 1) Huanglongbing (HLB) or Citrus greening disease caused by *Candidatus* Liberibacter spp. is considered the most devastating citrus disease worldwide. The lack of resistant citrus varieties and economically feasible treatments for infected trees, and absence of durable control mechanisms makes HLB disease a threat to more than 516 million trees throughout the EU. The African citrus psyllid, *Trioza erytreae,* that transmits HLB, has recently been found in northwestern Iberian Peninsula (Spain and Portugal) with increases the threat that HLB poses to the Spanish citriculture. 2) In Europe, *X. fastidiosa* has emerged as a pathogen of global importance associated with a devastating epidemic in olive trees in Italy in 2013. But since then, several other outbreaks were discovered in the EU, affecting other important economic crops such as grapes and almonds in Spain, but also species of cultural/patrimonial importance, landscape and ornamental plants. These outbreaks evidence that major EU crops can be severely damaged by *X. fastidiosa* infections, affecting not only EU agricultural productivity but the environment and cultural heritage (e.g., Over €5.5 billion in agricultural production could be affected annually and nearly 300,000 jobs involved in olive, citrus, almonds and grapes production could be at risk if *X. fastidiosa* becomes widely spread in the EU; EFSA, 2018). 3) Verticillium wilt of olive caused by the soilborne fungus *V. dahliae* is currently considered the

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main soilborne disease threatening olive production worldwide, and is becoming an increasing concern in olive production because is causing devastation in young and old olive orchards in different regions of Andalusia, with a prevalence on those areas higher than 40%, and is also spreading to all other major olive-growing areas of our country. 4) Several fungal species belonging to the Botryosphaeriaceae are becoming important emerging diseases mainly damaging stone and pome fruits, olive, walnut and avocado in many countries including Spain (e.g., 70-90% loss has been reported in olive trees; Moral et al., 2019), which highlight the need for a focus on these pathogens. 5) Global spread and increase of populations of whiteflies as a result of climate change and changes in agricultural practices is resulting in the emergence or re-emergence of whitefly-transmitted viruses that severely damage economically important crops, including DNA viruses of the genus *Begomovirus* and RNA viruses of the genera *Crinivirus, Ipomovirus,* and *Torradovirus* which are associated to severe outbreaks, such as CVYV, CYSDV, ToCV and ToTV and for which no effective control is always available (Navas-Castillo et al., 2014). 6) Several Tobamovirus have re-emerged in the last few years causing important problems such as CGMMV in cucurbits and ToBRFV in tomato, probably due to their extreme persistence and infectivity, global seed and fruit trade and the widespread use of tolerant varieties (Dombrovsky et al., 2017). 7) Sharka disease caused by PPV is one of the most devasting *Prunus* spp. diseases, which causes a high percentage of apricot and plum EU production being unmarketable every year. The costs of sanitary controls, surveys and eradication programs against PPV worldwide in the last 30 years exceeds 10,000 million euros (Cambra et al., 2006).

As occurring for strategic agricultural crops, the most problematic and concerning sanitary risks of temperate forests are those originated by exotic invasive species to which native or planted trees lack efficient resistance and tolerance mechanisms (Wingfield et al., 2015). Most, if not all, native forest species in the Iberian Peninsula are or will be soon threatened by exotic organisms, some of which are already causing devastating damage and large-scale transformations in our forest systems. Exotic pathogens such as *Bursaphelencus xylophilus, Pestalotia stevensonii* or *Fusarium circinatum* on pines, several *Phytophthora* spp. (including *P. ramorum* on oaks and *Phytophthora alni* in alders), *Hymenoscyphus pseudoalbidus* on ashes, *Cryphonectria parasitica* on chestnuts and *Ophiostoma novo-ulmi, Brenneria quercina* are all threathening Iberian forest. Although is not easy to select one harmful organism as the most relevant for Iberian forest, considering the vast area occupied by the iconic oak open lands 'dehesas' in the Iberian Peninsula (ca. 7 Mha),

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their high environmental and social value and their strong link with humans since ancient times, we stand out the oak decline as one of the most dramatic forest health problems currently affecting Iberian forests. Although other pathogens may be involved, chronic root pruning by the oomycete *Phytophthora cinnamomi,* an alien invasive species widely distributed in Iberian soils, appears to be the main biotic trigger of oak decline. The extent of oak decline is not yet well known, but by 2010, 8000 ha of holm oak stands were estimated to be being lost annually only in Spain, and climate projection models forecast even a worse scenario in the near future.

3.4. Sustainable management of pests and pathogens resistance to phytosanitary treatments

Plant protection products (PPP) include synthetic pesticides derived from a chemical synthesis or biopesticides, from a biological origin (animals, plants, bacteria, minerals, etc). The number of PPPs used has doubled since 1980. However, more and more synthetic PPP are being banned due to environmental and human health concerns and the development of new conventional (synthetic) PPPs has decreased significantly. In contrast, the number of biopesticides has increased in the last decades (EPRS, 2019). The effectiveness of pesticides is threatened by the evolution of harmful organisms resulting in resistant pathogens, weeds, or pests (Hawkins et al., 2018).

Pesticide-resistant individuals are natural variants selected by intensive application of PPP that carry genetic (i.e., nucleotide polymorphisms) or epigenetic modifications leading to biochemical, physiological, and ultimately phenotypic differences (R4P Network, 2016). Strategies should be implemented for a correct manage of resistance in order to prevent or at least slow down the accumulation of resistant individuals in pest populations and to preserve the effectiveness of available pesticides (FAO, 2012). The major resistance management strategies currently recommended for all types of pesticides include the use of a mixtures of pesticides with different modes of action or resulting in different resistance mechanisms and rotations or alternation of pesticides to avoid single sense selection processes. Specific resistance management strategies have been developed for the various fungicide chemical groups such as: i) implement integrated disease (pest) management (IPM); ii) restrict the number of treatments per season, iii) applying pesticides only when strictly necessary; iv) use low effective (recommended) doses; or v) avoid eradicant (biocides) uses. For durable insecticides managements, tactics include i) the avoidance of overuse of a single insecticidal mode of action; ii)

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avoidance of generic insecticides focusing on a single crop-pest combination; iii) use in the frame of integrated pest management (IPM) approaches; iv) protect beneficial organisms; v) rotate unrelated compounds; vi) use of mixtures with caution; and vii) monitor problematic pest to detect first shifts in sensitivity (FAO, 2012). Accurate, sensitive and reliable tools for both monitoring and modelling of pesticide resistance are critical for the correct implementation of resistance management strategies (Kole et al., 2019).

As an alternative to synthetic PPP, biopesticides and related alternative management products are increasingly used. Third-generation pest control agents reduced the risk of synthetic pesticides, and biobased pesticides is a term that is increasingly used interchangeably with biopesticides. New tools, including semiochemicals and plant-incorporated protectants (PIPs), as well as botanical and microbially derived chemicals, are playing an increased major role in pest management (Seiber et al., 2014). Ideally these products are target-specific, with low toxicity to nontarget organisms, reduced in persistence in the environment, and potentially usable in organic agriculture (Seiber et al., 2014). Most of the biopesticides, especially the microbial products, have multiple modes of action and usually do not target a single site or gene, thus reducing the risk of development of resistance (Dimock and Ockey, 2017).

Challenges

- Improve the sustainability of crop production through the intensification in the implementation of IPM systems, coping the reduction of phytosanitary products by combining the new technologies, precision farming, or development of resistant varieties by means of classical and new breeding techniques.
- Combine the development of accurate laboratory pest monitoring techniques with field monitoring to provide useful on-time guidance to growers.
- Effective application of theoretic modelling to environmental conditions present in field populations to minimise resistance risk.

Impact of research

Basic and applied research on sustainable use of pesticides based on the understanding of the mechanisms underlying the development and evolution of resistance in target harmful organisms will have a great impact on durable control of pests and pathogens and then on food security and environmental protection.

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3.5. Achieving broad spectrum immunity through genome editing

Breeding crops for resistance to pests and diseases has tended to be a lower priority than breeding for yield and quality when control chemicals have been available. Traditionally, growers use chemical compounds to reduce the disease incidence in crops. Conversely, new environmental concerns, chemical safety of growers and the emergence of different resistance mechanisms from pathogen are reducing the use of pesticides and their applications (Gullino and Kuijpers, 1994). The new European Union guidelines aimed to progressively limit the use of chemical compounds are forcing to join efforts to achieve more durable resistance to pathogens and pests trough new molecular approaches.

The development of 'omic' technologies has revolutionized studies and approaches in Plant Pathology and in other areas of Plant Biology. But the technique that will have a greater impact on plant health in the coming years is undoubtedly that based on the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas technology. This technology is the more accessible alternative for genome editing of eukaryotes described until now. Genome editing has radically shake-up the biological world by providing a means to edit genomes of living organisms, including humans, plants, animals, and microbes. The CRISPR/Cas system can induce sequence-specific mutagenesis to alter or modulate genes and that can be used for trait improvement in crops. CRISPR/Cas has the potential to make more robust and efficient crops, more resilient to climate change alterations (Zhang et al., 2019). Robustness of CRISPR/Cas and specially its versatility and accessibility have made it possible that in a few years it has become a very promising tool for generating resistance against plant pathogens and pests.

CRISPR/Cas-based technologies have successfully been used for improving resistance to fungal, bacterial and viral pathogens (Borrelli et al., 2018). The main strategies employed in model species such as *Arabidopsis thaliana* and *Nicotiana benthamiana*, include the integration of CRISPR-encoding sequences that target and interfere with the pathogen genome. Also, CRISPR/Cas can be used to mediate targeted mutations in the host plant genome to increase resistance. In most of the cases these have been achieved by targeting a single locus. One of the most promising capabilities of this technology is the possibility of acting on different loci at the same time. Thus, for example, Zsögön et al. (2018) were able to *de novo* domesticate wild tomato plants, *S. pimpinellifolium*, by targeting a set of six key genes using a multiplex CRISPR/Cas9

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approach to generate loss-of-function alleles. Multiplex genome editing in plants initially focused on input traits such as herbicide resistance, but has recently been expanded to hormone biosynthesis and perception, metabolic engineering, plant development and molecular farming, with more than 100 simultaneous targeting events reported (Najera et al., 2019).

CRISPR/Cas has successfully been used to confer resistance/tolerance against single important fungal, bacterial and viral diseases (Chen et al., 2019). A future challenge will be to evaluate the feasibility of the CRISPR/Cas-based approaches for engineering broad-spectrum resistance against multiple pathogens and pests. The main limitation to deploy genome-edited crops in the fields rely on the legal and procedural uncertainties regarding the status of genome edited crops in Europe. While the CRISPR/Cas technology is being adopted at an unprecedented speed, the current regulatory framework remains outdated. Moreover, the European Court of Justice (ECJ) ruling from 2018 (C-528/16) brought even more confusion because of the interpretation that crops obtained by precision breeding are subject to the GMO regulatory provisions. The network EU-SAGE[1] was launched. aiming to provide information about genome editing and to promote the development of European and EU member state policies that enable the use of genome editing for sustainable agriculture and food production.

Impact

Although multiplexed CRISPR/Cas-based approaches have been already described, their routine and extensive implementation is limited by important technological developments. One that can be worth highlighting is the way in which the different ribonucleoprotein complexes are delivered to the plant, avoiding genetic transformation. Research in this area will require a significant progress in single-cell genomics and transcriptional profiling. These technological advances may reveal molecular details of pathogen restriction, cell autonomous and non-autonomous pathogen responses, and the influence of environmental factors and host developmental stage on pathogen infection.

1. EU-SAGE is a network representing 131 European plant science institutes and societies that have joined forces to provide information about genome editing and promote the development of European and EU member state policies that enable the use of genome editing for sustainable agriculture and food production. This network is coordinated by Prof. Dr. Dirk Inzé, Science Director at VIB-UGent Center for Plant Systems Biology.

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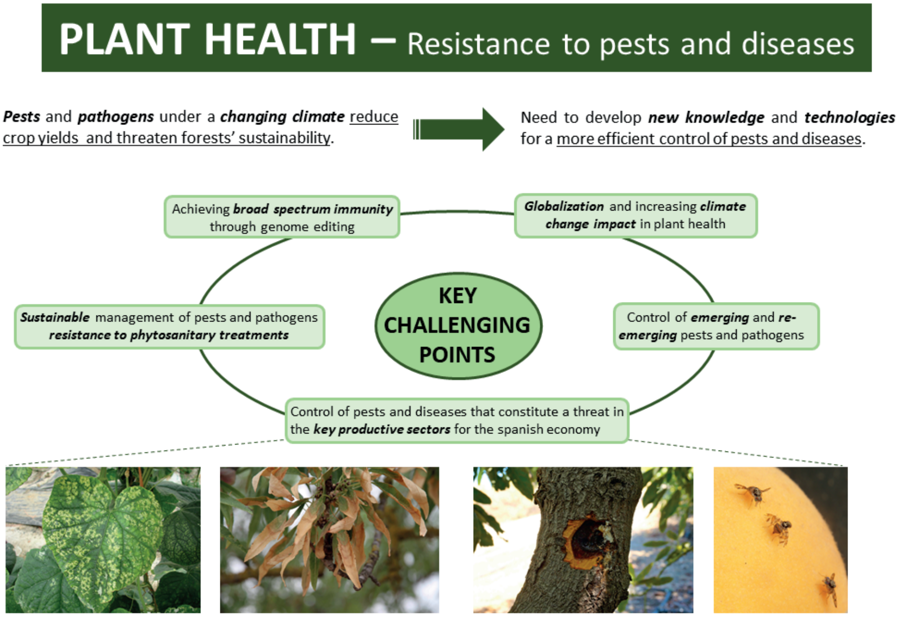
SUMMARY FOR EXPERTS



Cucumber green mottle mosaic virus in cucumber (Almería, Spain)

Xylella fastidiosa in almond tree (Mallorca, Spain)

Botryosphaeriaceae spp. in avocado tree (Málaga, Spain)

Ceratitis capitata in peach fruit (Madrid, Spain)

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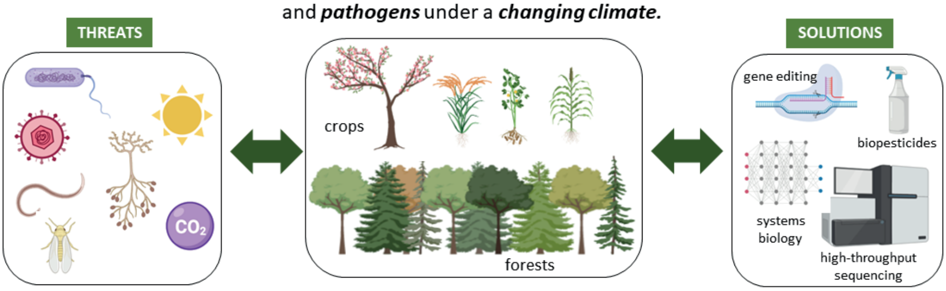
SUMMARY FOR THE GENERAL PUBLIC

PLANT HEALTH – Resistance to pests and diseases

Plant health is of global importance for sustainable agriculture, food security and environmental protection. To satisfy a growing demand for food, global agricultural production must increase by 70% by 2050.

Crops and *forests* are threatened by ***pests*** and ***pathogens*** under a ***changing climate.***



There is a need to develop ***new knowledge*** and ***technologies*** for a more efficient control of pests and diseases.

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