

REVIEW - FOCUS ISSUE ON PLANT HEALTH SUSTAINING MEDITERRANEAN ECOSYSTEMS

Emerging pathogens as a consequence of globalization and climate change: leafy vegetables as a case study

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Summary. In the recent years (2010–2016), leafy vegetables have been found to be hosts for many new diseases. The causal agents of these diseases had often been known on other crops, but have appeared for the first time throughout the world on several cultivated vegetable hosts such as: *Lactuca sativa*, *Diplotaxis* spp., *Eruca sativa*, *Valerianella oleroria*, *Ocimum basilicum* and *Spinacia oleracea*. This review considers pathogens such as *Alternaria* spp. on basil and rocket, *Fusarium equiseti* on lettuce, rocket and radish, and *Myrothecium verrucaria* and *M. roridum* on lamb's lettuce, spinach and wild rocket. All these pathogens are seed-transmitted, a biological characteristic that raises the risk of worldwide dissemination. The role of climate change and globalization on the appearance of such diseases, and potential tools for their management under a climate change scenario, is discussed using leafy vegetables as a case study.

Key words: lettuce, rocket, basil, spinach, foliar diseases, seed-borne pathogens.

Introduction

Globalization, by facilitating the movement of plants and plant material across borders, helps the rapid spread of plant pathogens between and across continents (Anderson *et al.*, 2004; MacLeod *et al.*, 2010). The arrival of a pathogen in a new environment can disrupt the biological equilibrium and be responsible for the start of severe plant diseases epidemics.

Climate change, generated by human activities, leading to increases in greenhouse gases, and resulting increases in temperature and shifts in rainfall patterns, interacts with globalization to influence development and spread of plant pathogens, together with other multiple indirect effects on processes that affect plant stands, cultivated fields, and crop production (Anderson *et al.*, 2004; Chakraborty and Newton, 2011; Savary *et al.*, 2017).

Globalization of seed markets is one of the main causes of the recent and rapid spread of plant pathogens to new hosts. Moreover, some of the newly introduced pathogens that are typical of warm areas are spreading easily in temperate regions, due to the increases in temperature.

This review discusses the possible roles of interacting globalization and climate change on the appearance and spread of pathogens that affect the aerial parts of leafy vegetables.

Leafy vegetables for ready-to-eat market sectors: the context

The demand for, and consumption of, fresh products is increasing in industrialized countries, with very rapid growth since the early 1980s (Rabobank, 2010), in part because they are considered a component of healthy lifestyles. Italy, together with the United Kingdom, is the leader in Europe in the production of fresh, ready-to-eat salads, (90,000 tonnes in

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2013). Italy has become the leading European country for consumption of fresh-cut salads (1.6 kg per person per year) (Nomisma, 2015).

The dynamism and innovation of this sector have led to intensification of crop production in many areas. Leafy vegetables allow multiple, short-duration production cycles, and share several common features, including: high value of the harvested crops, with many new species and varieties, continuous intensification and innovation of the production systems, seed production in third-party countries, the absence of crop rotation, susceptibility to a range of pathogens and restrictions in the use of chemicals for their management (Gullino *et al.*, 2014a; 2014b). Other aspects of the leafy vegetable sectors include the rapid turnover of varieties and the introduction of new crop species to adapt to market demands. The likely benefits that can be obtained from growing new crop species, such as wild rocket, can also lead to the risk that these newly introduced crops will be accompanied by new pathogens. Therefore, many of the new pathogens that cause severe crop losses (Savary *et al.*, 2012) constitute one of the sector's most critical features. The recent spread of new pathogens is clearly linked to the fact that, being seed-borne, their diffusion is favoured by market globalization and/or by the effect of global warming on plants and their hosts. This review focuses on *Alternaria* spp., *Fusarium equiseti* and *Myrothecium* spp., which have recently been observed on lettuce (*Lactuca sativa* L.), wild (*Diplotaxis* spp.) and cultivated (*Eruca sativa* Mill.) rocket, lamb's lettuce (*Valerianella olitoria* L.), basil (*Ocimum basilicum* L.) and spinach (*Spinacia oleracea* L.), and will update recent reviews on this topic (Gullino *et al.*, 2014a; Gilardi *et al.*, 2018).

***Alternaria* spp.**

Several species of *Alternaria* are threats to several leafy vegetables in many production areas. Sweet basil has been found to be a new host for *Alternaria* sp. in California, Florida, Pakistan, Japan, and Israel (Taba *et al.*, 2009; Kenigsbuch *et al.*, 2010; Farr and Rossman, 2017). Since 2010, *Alternaria alternata* has been shown to cause a new leaf spot disease on sweet basil plants grown in soil-less systems in Italy, as well as on the most popular variety grown for fresh consumption and pesto production in soil in Piedmont (North West Italy) (Gullino *et al.*, 2014a). This pathogen has been isolated at high incidence levels (from 7.3% on non-

disinfected seeds and 2.6% on disinfected seeds) respectively) from several commercial seed lots. These proportions of infected seeds suggest an important role of seeds for dissemination of *A. alternata*.

Alternaria japonica has consistently been isolated from wild and cultivated rocket plants in Italy (Gullino *et al.*, 2014a), and has been shown to affect the growth and quality of hydroponically grown cultivated rocket in California (Tidwell *et al.*, 2014). Wild and cultivated rocket seeds may be responsible for disseminating the pathogen, because of their external contamination (Gilardi *et al.*, 2015). Pathogenic isolates of *A. tenuissima*, *A. brassicicola* and *A. arborescens* have also been identified on wild and cultivated rocket plants and seeds, which suggests that these *Alternaria* species are also potential threats to rocket, although *A. japonica* has been found to be the most prevalent species (Siciliano *et al.*, 2017a). *Alternaria* spp., when introduced into the field, can survive on plant debris, and they have shown high potential to cause severe losses on many leafy vegetables grown in succession. *Alternaria* spp. are also potential producers of mycotoxins, such as tenuazonic acid, alternariol, alternariol monomethyl ether, altenuene, and tentoxin, and as a result should be considered a threat to humans. Tenuazoic acid has been found to be the most frequently produced mycotoxin by *Alternaria* spp. under increased temperature and carbon dioxide (Siciliano *et al.*, 2017a). Since significant variability in aggressiveness of *Alternaria* spp. from brassica plants has been documented for different brassica hosts (Rotem, 1994; Saharan *et al.*, 2016), temperatures from 22 to 26°C and 800 ppm of carbon dioxide generally cause increases in leaf spot severity on rocket, cauliflower and cabbage (Siciliano *et al.*, 2017a). An opposite trend of mycotoxin production to that of virulence has been observed on rocket and cauliflower grown under these conditions (Siciliano *et al.*, 2017a). However, *Alternaria* toxins have been produced from 14 to 26°C, with differences according to, the host plants and pathogen strain (Siciliano *et al.*, 2017a).

Fusarium equiseti

Fusarium equiseti is a well-recognized seed-borne pathogen that generally occurs in tropical and subtropical regions on a wide range of plants (including cotton, lentil, sugar beet, cumin, potato, cowpea, pine, ginseng, and asparagus), on which it causes a variety of symptoms (Farr and Rossman, 2017). In 2010, culti-

vated and wild rocket and lettuce were found as new hosts of *F. equiseti* in northern Italy, and the pathogen was later observed on intensively grown leafy vegetables in northern and southern Italy (Gilardi *et al.*, 2018). The same pathogen has recently been reported on radish (*Raphanus sativus* L.) seedlings in a nursery in Piedmont (Garibaldi *et al.*, 2017), where it caused yield losses of about 10% at temperatures of between 15 and 30°C and under high relative humidity. Lettuce and rocket are particularly susceptible to *F. equiseti* at temperatures from 25 to 35°C: at such temperatures, short periods of high relative humidity are sufficient to damage the yield and quality of these crops (Garibaldi *et al.*, 2016).

Fusarium equiseti has been found as an external seed contaminant on *Diplotaxis* spp. (Gilardi *et al.*, 2017b), which suggests that seeds may be important in its spread throughout several production areas. Once introduced into the field, this pathogen survives as a saprotroph in soil or crop debris, and is very adaptable to different cropping systems, in particular to intensive vegetable production.

The broad host range of *F. equiseti*, together with its ability to produce a diversity of mycotoxins, including trichothecenes, causes further health risks (Bottalico, 1988; Bosch and Mirocha, 1992; Bottalico and Perrone, 2002; Desjardins, 2006). The severity of leaf spot caused by *F. equiseti* on rocket and radish was found to increase for 850 ppm of carbon dioxide at temperatures of between 22 to 30°C, providing support to the hypothesis that the recent spread of this pathogen to some new hosts is probably linked to climate change, as a result of increased temperatures and carbon dioxide values (Gullino *et al.*, 2017a).

***Myrothecium* spp.**

Myrothecium spp. are fungi that live in the soil, and are the causal agents of leaf spots and stem rot on approx. 20 different plant species, including cotton, tomato, cacao, coffee, potato, soybean, cucurbits and maize, and many ornamentals (Farr and Rossman, 2017). There is no evidence of host specificity for these fungi (Chase, 1983; Yang and Jong, 1995; Fish *et al.*, 2012). *Myrothecium* spp. were first observed on lamb's lettuce, spinach and wild rocket grown under plastic houses in Italy in 2015. *Myrothecium roridum* has been found on lamb's lettuce, while *M. verrucaria* has been isolated from spinach and wild rocket (Gilardi *et al.*, 2018). A characteristic that makes this ge-

nus a serious threat to the productivity of spinach and wild rocket is its capacity for producing macrocyclic trichothecenes, which are dangerous cytotoxic compounds. *Myrothecium* spp. may be seed-transmitted in many cases (Nguyen *et al.*, 1973; Belisario *et al.*, 1999; Bharath *et al.*, 2006). As these pathogens are generally associated with warm environments and short wetting periods, they may become important in the future considering anticipated climate changes. Rises in temperature, as well as in carbon dioxide concentrations, have shown significant effects under phytotron conditions, causing increased *Myrothecium* leaf spot on spinach (Siciliano *et al.*, 2017b). The biosynthesis of the trichothecene verrucaric acid has been found to increase at 35°C, while that of roridin E has increased for a carbon dioxide concentration of 800 ppm. This indicates positive correlation between climate change and macrocyclic trichothecene production (Siciliano *et al.*, 2017b). Leaf spot on spinach, caused by *M. verrucaria*, was of increased severity at elevated temperatures and carbon dioxide concentrations. Verrucaric acid biosynthesis increased at the highest tested temperature (35°C), while roridin E was influenced by the rise in carbon dioxide (Siciliano *et al.*, 2017b).

In the case of leaf spot caused by *M. roridum* on cultivated rocket (*E. sativa*), disease severity was assessed under eight different temperature and carbon dioxide concentration combinations (from 14–18°C to 26–30°C and with 400–450 or 800–850 ppm of carbon dioxide). Severity increased at elevated temperatures at both carbon dioxide levels. Verrucaric acid and roridin E mycotoxins were produced under all the tested temperatures under high carbon dioxide conditions. The maximum production of verrucaric acid occurred at 14–18°C, and the maximum roridin E production was detected at 26–30°C, with 800–850 ppm of carbon dioxide for both toxins (Bosio *et al.*, 2017).

Factors that favour emergence of new pathogens

Environmental conditions, and thus global warming, influence the interactions between plants, pests and pathogens, and climate change could affect the range and impacts of pests and pathogens (Van Maanen and Xu, 2003; Lake and Wade, 2009; Luck *et al.*, 2011).

Observations of changing pest and pathogen distributions during the twentieth century suggest that expansion of agricultural production, the shifts in

cropping practices (Savary *et al.*, 2011), and trade have been the most important reasons for the dissemination of these pathogens. There is some evidence, however, of latitudinal bias in range shifts that indicates a global warming signal (Bebber, 2015). Global travel and the trade of agricultural seeds and products have moved crops and pathogens away from their original environments. The above-described examples of leafy vegetables show that many of the pathogens that cause severe losses can be seed-transmitted. Their transmission, through infected or contaminated seeds, guarantees their spread, and even low levels of seed contamination can lead to the rapid emergence of new diseases in different and distant geographic areas (Gitaitis and Walcott, 2007; Munkvold, 2009; Gullino *et al.*, 2014a). Good diagnostic tools and the use of appropriate seed treatments are the best preventive measures in many cases (Munkvold, 2009; Gullino *et al.*, 2014b; Thomas *et al.*, 2017). The International Seed Testing Association, the International Seed Health Initiative for Vegetable Crops and the National Seed Health System are organizations that publish standardized seed health testing methods. Specificity, sensitivity, reproducibility and cost-effectiveness are required for the selection and application of a reliable seed testing method (OEPP/EPPO, 2010). The consequences of false positive or false negative diagnostic results can jeopardize the seed trade or the phytosanitary safety of a country.

The global warming predicted for the future could also increase the incidence of diseases caused by plant pathogens. *Alternaria* leaf spot on rocket and *F. equiseti* leaf spot on lettuce and radish are expected to become more problematic, due to the foreseen increased temperatures and carbon dioxide concentrations. This could directly or indirectly affect hosts and pathogens, as well as their disease control (Garibaldi *et al.*, 2016; Gilardi *et al.*, 2017a; Gullino *et al.*, 2017a; Siciliano *et al.*, 2017a). The sudden appearance of new pathogens, such as *F. equiseti* and *Myrothecium* spp., on leafy vegetables in Italy, and their spread to different hosts, can be associated with the increase in temperatures observed in northern Italy as a consequence of climate change. Colombo *et al.*, (2007) provided evidence of temperature and precipitation variations in Italy starting from the end of the 1970s, with an increase in tropical nights ($T_{\min} > 20^{\circ}\text{C}$) and, at the same time, a decrease in frost days ($T_{\min} < 0^{\circ}\text{C}$).

Research focused on *F. equiseti* and *Myrothecium* spp. on leafy vegetables under simulated environ-

mental conditions in phytotrons has shown that these pathogens are well-adapted to different environmental conditions, and to climate change scenarios (Garibaldi *et al.*, 2016; Gullino *et al.*, 2017a).

The ability of some of the newly introduced pathogens to produce diverse mycotoxins increases their risks to human health. Climate change could also influence mycotoxin production of most of these emerging pathogens (Siciliano *et al.*, 2017a; 2017b). In general, climate change is expected to increase mycotoxin contamination of crops. However, due to the complexity of mycoflora associated with each crop and their interactions with the environment, it is difficult to draw conclusions without conducting specific studies. For instance, Magan *et al.*, (2011) reviewed the effects of climate change (water and temperature stress, interactions between elevated CO_2 with other key environmental factors) on pre- and post-harvest contamination by mycotoxins of key crops, and the potential for molecular and ecological data to predict the risk from mycotoxins occurrence on a regional scale. However, quantitative estimations of the impacts of climate change on mycotoxin occurrence have only been made very recently (Van der Fels-Klerx *et al.*, 2016).

Disease control measures in view of globalization and climate change

Globalization, due to the intensification of trade, new modes of consumption, shifts in diets, and climate change, are among the factors influencing the occurrence, frequency and severity of new plant diseases on leafy vegetables. These factors have important impacts on decision-making for the related disease management measures that should be adopted.

Considering the high probability of inadvertently using already infected seed lots, the primary preventive strategy for seed producers and farmers is the use of healthy seeds and effective seed treatments (Gullino *et al.*, 2014b). Seed treatments, including the use of heat, resistance inducers, antagonistic microorganisms, fungal or plant extracts, have been tested against seed-borne pathogens on vegetable seeds in recent years (Koch and Roberts, 2014; Gullino *et al.*, 2014b).

Potential tools for disease management under climate change conditions are needed. These include, growing crop species that are better adapted to local climatic conditions and grown in crop rotations,

changing planting and/or harvest dates of annual crops, management of overwintering pathogens in crop residues through crop rotation and other agronomic practices to reduce inoculum levels between cropping seasons, and changes in water supply and fertilizer applications to support improved plant growth and development due to carbon dioxide fertilization effects (Legreve and Duveiller, 2010; Juroszek and von Tiedemann, 2011). However, these methods may not be completely effective, and they should be combined with other measures under integrated pest management. For example, managing the environment by avoiding high temperatures and maintaining low relative humidity are among the preventative measures considered useful to manage diseases caused by *Fusarium equiseti* (Garibaldi et al., 2016).

Since it is not always possible to control the environmental parameters, growers often apply pesticide chemicals. However, fungicide applications under climate change conditions should be specific for the disease/crop pathosystem and location (Juroszek and von Tiedemann, 2011). Additional strategies are becoming essential as many pesticides are being phased out as because of evolving resistance in the target pathogens, pesticide toxicity, or registration withdrawal. Careful use of pesticides is necessary, and integrated pest management is essential to guarantee successful long-term disease control.

Conclusions

The expected increase in climatic variability is of great concern, as this could lead to increased losses due to often newly introduced diseases and pests in particular localities, as well as to the yearly climate variations. The risks posed by epidemics of new plant diseases have unpredictable negative consequences on agriculture, the environment, international trade and, in the worst cases, cause social repercussions (Gullino et al., 2017b; 2018).

This review has shown how leafy vegetables, in particular because of their intensive production systems, are relevant case studies to evaluate how climate change and trade globalization can affect the phytosanitary situations for particular economic crops. Most of the emerging leafy vegetable pathogens described here have broad host ranges. *Fusarium equiseti* and *Myrothecium* spp. Are serious threats for many Mediterranean crops. Extension services should be well trained to detect new pathogens, and

timely diagnosis and reliable diagnostic tools, along with effective seed treatment methods, need to be developed and made widely available to seed companies and growers.

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