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Emerging foliar and soil-borne pathogens of leafy vegetable crops: a possible threat to Europe

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Abstract

Italy is one of the leading countries in the production and consumption of ready-to-eat salads. This has led to a steady increase in the surfaces used for the growth of leafy vegetables, under intensive systems, over the last decade. The recent evolutions of the diseases that affect lettuce, wild and cultivated rocket, lamb's lettuce, spinach and basil are here reported. *Plecosphaerella cucumerina,* on wild rocket, endive and lamb's lettuce, *Fusarium equiseti* on wild and cultivated rocket and lettuce, *Myrothecium verrucaria* on spinach and wild rocket, *Myrothecium roridum* on lamb's lettuce, *Allophoma tropica* on lettuce and *Alternaria* sp. on basil and rocket, are among the new foliar pathogens and are described hereafter. Among the soil-borne pathogens, *Pythium aphanidermatum*, *P. irregulare*, *Pythium* Cluster B2a have frequently been isolated on spinach and swiss chard, lamb's lettuce and lettuce, while *Fusarium oxysporum* f. sp. *lactucae*, which causes lettuce wilt, is gradually spreading to new countries. Some of the new pathogens have been found to be seed transmitted and typical of tropical areas, and are thus favoured by the rises in temperature that currently characterize the climate change scenario. The globalisation of markets, climate change and intensive cultivation are among the factors that are responsible for the proliferation and spread of some of those new pathogens that are 'aliens' to Italian production systems.

Introduction

Over the last few decades, significant changes have occurred in agriculture, as well as in society's and policy-makers' attitudes, and as a consequence, in disease management strategies. Agriculture continues to change in response to society's needs (Stoate *et al.*, 2009). The promotion of improved health lifestyles has led to an increased demand for fresh products in many industrialized countries. Agricultural intensification is particularly noticeable in crops such as leafy vegetables (lettuce, rocket, spinach, lamb's lettuce, basil…), and is linked to the increase in the industrial production of fresh cut and ready-to-eat processed salad, with retail being the main distribution channel. Fresh-cut products are fruit or vegetables that have been trimmed, peeled and/or cut into a fully usable product, which is subsequently packaged to offer consumers high nutrition, convenience and flavour (IFPA 2001). The European fresh-cut industry has undergone exponential growth since the early 1980s. Italy, together with Great Britain, is the leader in Europe for fresh, ready-to-eat salads, producing 90 000 tonnes in 2013, with a turnover of € 770 million (Nomisma, 2015). Italy, with a 21% increase in the consumption of fresh-cut salads from 2008 to 2013, has become the leading European country as far as annual *pro capita* consumption is concerned, with an average of 1.6 kg of fresh-cut salads consumed per person per year (Nomisma, 2015).

Leafy vegetable crops all share some specific features, such as high value, continuous intensification and innovation in their production systems, the presence of a high number of varieties, delocalization of seed production in southern countries, susceptibility to a number of pathogens and limitations in the use of chemicals for their control (Gullino *et al.*, 2014 a). Among the consequences of the dynamism and specialization of such crops, together with the lack of adequate crop rotation and the globalization of the seed market, the appearance of many new pathogens that cause severe field losses is one of the most critical.

The phytosanitary situation of leafy vegetables has constantly been monitored in different Italian and European production areas over the past 15 years. This review will focus on the most important foliar and soil-borne diseases 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 present an update of recent investigations on this topic (Garibaldi & Gullino, 2010; Garibaldi *et al.*, 2014 a; Gullino *et al.*, 2014 b; Gullino *et al.*, 2017 c) (Table 1). Control measures, based on the current scientific knowledge, have been reported.

Foliar diseases

Leaf spot caused by *Plectosphaerella cucumerin*a

A foliar disease caused by *Plectosphaerella cucumerin*a has recently been observed in Italy on wild rocket, endive (Gullino *et al.*, 2014 a) and lamb's lettuce (Carrieri *et al.*, 2014) (Table 1), with consequent severe losses. The affected leaves show spots that are usually surrounded by a yellow–grey halo, and which are predominantly located on the leaf surface, rib and petiole. Damaged tissues rot quickly after packaging, but also during transit and commercialization (Figures 1 and 2). The fast south-north spread of the disease that occurred in Italy on wild rocket in 2012 has been explained by the capability of the pathogen to infect seeds (Tables 1 and 2) (Gullino *et al.*, 2014 a).

This filamentous Ascomycete is cosmopolitan in distribution, and it has been reported on many hosts (Palm *et al.*, 1995; Carlucci *et al.*, 2012; Pétriacq *et al.*, 2016; Farr & Rossman, 2017). This species can change from hemibiotrophic to necrotrophic (Pétriacq *et al.*, 2016), thus causing a wide range of symptoms, including crown and root rot, as well as leaf necrosis, on a large number of hosts such as melon, watermelon, squash, courgette, cucumber, sunflower, white lupin, parsley (Carlucci *et al.*, 2012), and banana (Farr & Rossman, 2017). It was first observed in Italy on basil and tomato, where it causes basal rot (Matta, 1978). *P. cucumerina* is a nematophagous fungus that had previously been considered as a potential biological control agent (Atkins *et al.*, 2003).

Its ability to survive as a saprotroph in soil (Domsch & Gams, 1972), its polyphagous nature and its capability to contaminate seeds (Table 2) makes its management difficult. Among chemicals tested against *Plectosphaerella* leaf spot on wild rocket, phosphite-based products, acibenzolar-S-methyl, mandipropamid and azoxystrobin, have provided the most consistent, although not complete control (Gilardi *et al.*, 2015 a). Also copper hydroxide and terpenic alcohols and *Streptomyces griseoviridis*, that are products permitted in organic farming, provided a positive effect in reducing the leaf spot by *P. cucumerina* on rocket (Gilardi *et al.*, 2015 a), while, in other studies, *Trichoderma asperellum* (strain T34) and different *Streptomyces* species (*S. cyanoviridis*, *S. murinus* and *S. griseoplanus*) have been found to be effective against *Plectosporium tabacinum* on white lupin (Youssef *et al.*, 2001) and cucumber (Segarra *et al.*, 2013). An integrated approach is necessary, considering the difficulty of reaching complete control of this pathogen with single control measures.

Leaf spot caused by *Alternaria* species

The species in the genus *Alternaria* represent a potential threat to leafy vegetables in several production areas in Italy, as well as in other countries. *A. alternata* was first observed on sweet basil in 2010 (Gullino *et al.*, 2014 a). The pathogen causes brown-black lesions, which, on older leaves, are surrounded by a yellow halo, causing the progressive defoliation of the plants, and, on rare occasions, to the death of plants (Figure 3). The disease was first observed on the leaves of plants grown in soilless systems, as well as in soil in Piedmont (Northern Italy) on the most extensively grown variety for fresh consumption and pesto production (Table 1). The pathogen has been isolated from several commercial seed lots (Table 2), with a high level of contamination (from 7.3% and 2.6% of non-disinfected and disinfected seeds), thus suggesting that seeds may be important in its dissemination in different cultivation areas (Gullino *et al.*, 2014 a). The *Alternaria* leaf spot of basil had previously been reported in California, Florida, Pakistan, Japan and Israel (Farr & Rossman 2017; Taba *et al.*, 2009; Kenigsbuch *et al.*, 2010). In the same year, *Alternaria japonica* was isolated from a number of samples taken throughout the growing season over different years from wild and cultivated rocket plants in Italy (Figure 4) (Gullino *et al.*, 2014 a). The same pathogen has recently affected the growth and quality of hydroponically grown cultivated rocket in California (Tidwell *et al.*, 2014). Rocket plants can be affected in all the growth stages, and the typical symptoms include black necrotic lesions surrounded by chlorotic areas on seedlings, leaves and stems (Gullino *et al.*, 2014 a). Seeds may be important in disseminating the pathogen because the external contamination of wild and cultivated rocket seeds has been observed (Table 2) (Gilardi *et al.,* 2015 b). Although *A. japonica* has been found to be the most prevalent species among the *Alternaria* isolates from wild and cultivated rocket plants and seeds, pathogenic isolates of *A. tenuissima*, *A. brassicicola* and *A. arborescens* have also been identified, thus suggesting that these *Alternaria* species are also a potential threat to rocket (Siciliano *et al.,* 2017 c). Moreover, almost all the isolates collected from rocket plants and seeds were able to produce Alternaria toxins (tenuazonic acid, alternariol, alternariol monomethyl ether, altenuene and tentoxin) *in vitro* (Sicicliano *et al.,* 2017 c). This pathogen may be a threat to wild and cultivated rocket production, because, once introduced into the field, it can persist on plant debris or through chlamydospores, and it can also spread by means of airborne conidia (Simmons, 2007). The management of *Alternaria* spp. requires a combination of agricultural management practices (i.e. the adoption of healthy seeds and propagation material, use of resistant cultivars whenever possible, use of appropriate crop rotation, removal and destruction of infested plant material), and multiple fungicide sprays. Fungicides registered for use against *Alternaria* spp. include sterol demethylation inhibitors (DMIs), quinine outside inhibitors (QoI), dicarboximides and carboxamide (Rimmer *et al*., 2007). A limited availability of chemicals for use on 'minor crops' might increase the risk of development of resistance.

Leaf spot caused by *Fusarium equiseti*

Fusarium equiseti could represent a serious threat for many Mediterranean crops. First found in Northern Italy on cultivated rocket in 2010 (Gullino *et al.,* 2014 a), it was later reported on wild rocket (Garibaldi *et al.,* 2015 b) and lettuce (Garibaldi *et al.,* 2016 e) in Northern and Southern Italy (Table 1): in all these cases, the symptoms consisted of leaf spots (Figures 5 and 6). Yield losses of about 10% have been observed at temperatures of between 15 and 30°C and under high relative humidity. Since *F. equiseti* is a member of the *Fusarium incarnatum-equiseti* species complex (FIESC), phylogenetic analysis, based on multilocus DNA sequencing, has been used widely for its identification (O' Donnell *et al.,* 2012).

F. equiseti is a well-known seed-borne pathogen that is found on many host plants (Farr & Rossman, 2017). The pathogen has been found as an external contaminant of *Diplotaxis* spp. seeds (Gilardi *et al.,* 2017 c) (Table 2). Once introduced in the field, its survival is favoured because this pathogen is able to survive in soil or crop debris. Such a feature makes it adapt easily to different cropping systems, in particular to those of a very intensive nature. Moreover, this species generally occurs in tropical and subtropical regions on a wide range of plants (cotton, lentils, sugar beet, cumin, potatoes, cowpeas, pines, ginseng, asparagus…) on which it causes a variety of symptoms (Bottalico 1988; Goswami *et al.,* 2008; Farr & Rossman, 2017) and spreads rapidly in warm and humid environments. Lettuce and rocket are very susceptible to *F. equiseti* at temperatures ranging from 25 to 35°C, and 1 to 3 hours of high relative humidity are sufficient to damage the yield quality of such crops in the presence of the pathogen; while longer periods under high relative humidity (6-12 hours) are necessary at lower temperatures of 15 to 20°C to cause significant losses (Garibaldi *et al.,* 2016 a). Along with the broad host range, the conditions that are favourable for the pathogen make disease management very difficult. Managing the environment by avoiding high temperatures and keeping the relative humidity to a minimum recommended level are among the preventative measures that have been suggested (Garibaldi *et al.*, 2016 a). Since it is not always possible to control the environmental parameters, growers often have to rely on chemical control. However, the chemical management of this pathogen is still under investigation. Fludioxonil, benomyl, thiophanate-methyl, propiconazole and fludioxonil are able to reduce the *in vitro* mycelial growth of *F. equiseti* taken from ginseng (Punja *et al.,* 2008). The ability of *F. equiseti* to produce diverse mycotoxins, including trichothecenes, leads to further health risks (Bosch & Mirocha, 1992; Bottalico, 1988; Bottalico & Perrone, 2002; Goswami *et al.*, 2008).

Leaf spot caused by *Myrothecium* species

The number of first reports regarding *Myrothecium verrucaria* and *M. roridum* on new hosts has increased in recent years. *Myrothecium roridum* has been found on lamb’s lettuce (Garibaldi *et al.*, 2016 b), while *M. verrucaria* has been isolated from spinach (Garibaldi *et al.,* 2016 c) and wild rocket (Garibaldi *et al.,* 2016 d). These *Myrothecium* species were both first observed in Italy in 2015 under plastic tunnels (Table 1). The symptoms caused by both species are similar: circular, sunken, grey-brown spots, with a well-defined border, that develop on affected leaves. Creamy to black sporodochia appear on the leaf surface, in concentric rings, under high relative humidity (Figures 7-9).

Myrothecium spp. are soil inhabiting fungi, and are the causal agents of leaf spots and stem rot on several different plant hosts, such as cotton, tomato, cacao, coffee, potato, soybean, cucurbits and corn, as well as on many ornamentals (Farr & Rossman, 2017). There is no evidence of host specificity for these species, and this also makes the use of resistant cultivars difficult (Chase, 1983; Yang & Jong, 1995; Fish *et al.*, 2012). However, moderately level of resistance to Myrothecium leaf spot caused by *M. roridum* in some cucurbit crops and ornamental plants is reported (Norman *et al.*, 2003; Fish *et al.*, 2012). The management of Myrothecium leaf spot is complicated, because this pathogen is seed-transmitted in many cases (Nguyen *et al.*, 1973; Belisario *et al.*, 1999; Bharath *et al.*, 2006). Moreover, no information is available for the control of Myrothecium leaf spot on leafy vegetables by chemicals means, and further investigations are needed.

Myrotechium leaf spot may become important in the future considering the forecasted climate change scenario (Siciliano *et al*., 2017 b). Another characteristic that makes this genus a serious threat is linked to its capability to produce macrocyclic trichothecenes, which are cytotoxic compounds. The biosynthesis of verrucarin A mycotoxin has been found to increase significantly at 35 °C, with an average concentration of 19.5 µg/g compared to 7.1 µg/g at temperature of 10 °C, while at CO_2 concentration of 800 ppm, roridin E biosynthesis increased from 28.4 µg/g at 22-26°C to 49.6 µg/g at 26-30 °C, thus indicating a possible positive correlation between climate change and macrocyclic trichothecene production (Siciliano *et al.*, 2017 b).

Leaf spot caused by *Phoma* species

Species from the *Phoma* genus are found worldwide, and these pathogens have recently received increased attention because they can cause extensive losses (Chen *et al.*, 2015; Farr & Rossman, 2017). The *Phoma* genus is also well known because it includes species of quarantine concern (Aveskamp *et al.*, 2008). *Phoma valerianellae* and *Pleospora betae* have repeatedly been observed on lamb's lettuce and on swiss chard, respectively, on many farms in Italy in spring and autumn crops under plastic tunnels (Gullino *et al.*, 2014 a) (Table 1), thus severely reducing the quality of the plants. The symptoms appear on young plants as blackening and necrosis of the cotyledons, stem and roots, while red-brown necrosis may be observed on older plants on the lower leaves and on the stem base eventually causing plant collapse. Both these causal agents are seed-borne, and their introduction into the field can be due to the use of infected seeds (Neergaard, 1979; Nathaniels, 1985) (Table 2).

Allophoma tropica (syn. *Phoma tropica*) has recently been observed in Northern Italy on lettuce (Gullino *et al.*, 2014 a) (Table 1). Although the economic importance of this disease is still limited, it has been observed that this pathogen increases in frequency in years characterized by relatively low temperatures in spring and summer. The pathogen is more aggressive between 20 to 25°C (Gullino *et al.*, 2017 b), that is, at a temperature that is also *optimum* for its growth *in vitro* (Schneider & Boerema, 1975). *A. tropica* was first associated with leaf spots and stem lesions on different ornamental plants in the Netherlands and Germany (Schneider & Boerema, 1975), and has so far been detected on several hosts (Farr & Rossman, 2017). Growers and agricultural advisory services need to be particularly careful when attempting to identify *A. tropica*, because the leaf spot it causes on lettuce (Figure 10) can be confused with the grey mould caused by *Botryotinia fuckeliana*.

Since controlling the environmental conditions does not always provide adequate disease suppression of *Phoma* spp., fungicides are also needed. Mancozeb, fludioxonil, iprodione, pyraclostrobin and boscalid, which are known to be effective against Botrytis grey mould, resulted also effective against *A. tropica*. Moreover, the efficacy shown by copper-based products is of special interest, particularly for crops grown under organic farming conditions (Pintore *et al.*, 2017).

Downy mildew of basil

Basil downy mildew which is caused by *Peronospora belbahrii* (Belbahri *et al.*, 2005; Thines *et al.*, 2009), is one of the most economically important basil diseases, and has caused significant yield losses in several countries in Europe, (Switzerland, Italy, France and Belgium), in the United States, where it has been reported in several States since 2009, in Argentina, Israel (Cohen *et al.*, 2013) and China (Farr & Rossman, 2017). The transmission of this pathogen via infected seeds is generally recognised as the main means of survival of *P. belbahrii* from season to season, because the pathogen rarely produces oospores (Cohen *et al.*, 2013; Djalali *et al.*, 2012; Gullino *et al.*, 2014 a; Wyenandt *et al.*, 2015; Elad *et al.*, 2016). Moreover, it has been shown that basil seeds are a potential source of mefenoxam-resistant inoculum for *P. belbahrii* (Pintore *et al.*, 2016).

Because no cultivars of marketable interest are known to be resistant or tolerant to downy mildew (Ben-Naim *et al.*, 2015), the control of downy mildew is mainly based on the application of fungicides, such as for examples mefenoxam, potassium phosphite, mandipropamid, fluopicolid, mancozeb and azoxystrobin (Gilardi *et al.*, 2013; 2015 c; Wyenandt *et al.*, 2015). Furthermore, the resistance inducer, acibenzolar-S-methyl, significantly reduces the disease (Mersha *et al.*, 2013; Gilardi *et al.*, 2013). The use of chemicals in the field is complicated by the continuous nature of the harvest, and by the high risk of development of pathogen strains resistant to the fungicides through specific modes of action. For instance, the field resistance of *P. belbahrii* to phenylamides was first observed in Israel in 2013 (Cohen *et al.*, 2013), and later in Italy (Garibaldi *et al.*, 2016). An integrated approach to basil downy mildew control is suggested in which the microclimate environmental conditions are managed. This is because infection, sporulation and the duration of the latent period (from infection to sporulation) of *P. belbahrii* is dependent to a great extent on the occurrence of high levels of humidity (>85%), on temperature and light regime. For instance, spore germination and infection occur at temperatures from 5 to 28.5°C, sporulation occurs at 10 to 26°C (optimum of 18°C), and the shortest latent period is 5 days at 25°C under continuous light (Garibaldi *et al.*, 2007; Cohen *et al.*, 2013; Cohen *et al.*, 2017). Nocturnal illumination and fanning, a daytime solar heating of 35-45°C for 6-9 hours and for 3 consecutive days, increasing the temperature (26 to 31°C) in the root zone of basil using passive heat treatment, are among the practices suggested to prevent or suppress the disease under controlled environmental conditions (Cohen *et al.*, 2013; 2016; Cohen & Rubin, 2015; Elad *et al.*, 2016).

Soil-borne pathogens

Among the soil-borne diseases, damping off, caused by *Pythium ultimum,* has been observed with increasing frequency in Italy on lettuce, wild rocket and lamb's lettuce at temperatures of between 15 and 25 °C. The presence of new species of *Pythium* in Italy, such as *P. aphanidermatum* on spinach (Garibaldi *et al.*, 2015 c) and on swiss chard, *P. irregulare* on lamb's lettuce (Garibaldi *et al.*, 2015 a) and *Pythium* Cluster B2a on lettuce (*P. dissotocum*, *P. coloratum*, *P. diclinum*, *P.* cf. *dictyosporum*, *P. lutarium*, *P.* sp. 'Group F' and *P.* sp. *tumidum*) (Table 1), is particularly critical (Garibaldi *et al.*, 2017) at warm temperatures (Figures 11 -12).

Moreover, *Fusarium oxysporum* f. sp. *lactucae* has also increasingly been observed in lettuce cultivation areas (Matheron & Gullino, 2012), and has recently spread to the Netherlands (Gilardi *et al.*, 2017a) and France (Gilardi *et al.*, 2017 d). Like other soil-borne pathogens, Fusarium wilt is difficult to manage with a single approach and/or with a single product (Katan, 2017). Moreover, different measures are needed for different cropping systems (Matheron & Gullino, 2012; Gordon & Koike 2015). Among chemical measures, available soil disinfestation is becoming very difficult due to the loss of registered fumigants. In addition, chemical control may be limited by the observation that *Pythium* spp. can develop resistance to common fungicides as regularly updated in the FRAC Resistance Survey List (www.frac.info). Various non-chemical strategies, including sanitation, cultural practices, the use of resistant cultivars and biological control agents are being intensively investigated (Katan, 2017). For instance, the use of resistant cultivars represents the most effective choice to control lettuce Fusarium wilt (Matheron & Gullino, 2012; Gordon & Koike 2015). Nevertheless, a careful monitoring of the race situation in the field is also necessary for an efficient use of genetic control approaches. However, resistant cultivars are generally not available for most leafy vegetables against *Pythium* spp..

Factors favouring the emergence of new pathogens

There is much evidence concerning the causes of the development and spread of new diseases, including the globalisation of the seed and planting material markets, the effect of climate change, and changes in cultural and management practices. Global travel and the trade of agricultural seeds and products have moved crops and pathogens away from their original environments. The examples described for leafy vegetables suggest that most of the fungal pathogens that cause severe losses are seed-borne (Gullino *et al.,* 2014 a). Moreover, very low levels of contamination can lead to the rapid emergence of new diseases in different geographic areas. In this respect, the downy mildew of basil and Fusarium wilt of lettuce represent two very interesting case studies.

Considering the losses caused by these emerging pathogens (Table 1), the first preventative strategy that should be considered by seed producers and farmers is the use of healthy seeds. Stock seeds should be produced in the areas associated with the lowest disease risk, and the seeds subjected to standardised seed health analysis (Munkvold, 2009). The availability of good diagnostic tools and an early pathogen detection represent the best preventative measures in many cases (Thomas *et al.*, 2017), as has been observed for the case of *P. cucumerina* on wild rocket (Gilardi *et al.,* 2016 b) as well as for *formae speciales* and races of *Fusarium oxysporum* from seeds, plants and soil samples (Pasquali *et al.,* 2007; Mbofung & Pryor, 2010; Gilardi *et al.*, 2017 a).

In order to further reduce the risk from seed-borne pathogens, it is recommended that stock seeds should undergo precautionary decontamination treatments. Chemical seed treatments have successfully been applied to vegetable seeds and are in commercial use for a wide range of crops against different seed-borne pathogens (Munkvold, 2009; Koch & Roberts, 2014). Heat treatments with hot water, aerated steam or dry heat can be very effective, but they need to be optimised due to the differences in temperature and time required for different target pathogens and crops (Nega *et al.*, 2003; Koch & Roberts, 2014). Although the current non-chemical seed treatments, including the use of resistance inducers, antagonistic microorganisms, as well as fungal and plant extracts are presently being investigated intensively for seeds produced under organic farming rules, there are only a few examples of commercial use (Koch & Roberts, 2014; Gullino *et al.*, 2014 b). For instance, several options for non-chemical control of *Alternaria brassicicola* on Brassica seeds resulted comparable in efficacy to the chemical standard (Amein *et al.,* 2011). Moreover, the inclusion of non-chemical seed treatments such as dry heat air (65°C for 10 min), resistance inducers and thyme oil seed treatments, in basil downy mildew management programmes, merits further investigation (Gilardi *et al.,* 2015 c; Lopez *et al.,* 2016).

Given the predicted increase in global air temperatures and CO_2 concentrations, downy mildew on basil, Alternaria leaf spot on rocket and Phoma leaf spot on swiss chard and lettuce, Myrothecium leaf spot on spinach, *Fusarium equiseti* leaf spot on wild rocket and lettuce are expected to become more problematic due to a good adaptation capacity under different environmental conditions, but also under a climate change scenario (Gilardi *et al.,* 2016 a; Gilardi *et al.,* 2017 b; Gullino *et al.,* 2017 b; Siciliano *et al.,* 2017 a,b, c). Moreover, the increase in temperatures predicted for the future could also induce an increase in the incidence of diseases caused by plant pathogenic Fusarium species, probably through plant-mediated effects (Chitarra *et al.,* 2015; Ferrocino *et al.,* 2013). In particular, climate change is likely to have direct influence on the spatial and temporal dispersal of propagules, on the rapidity of disease development, and on the pathogen survival or indirectly affect both the host and the pathogen by changing, for example, the frequency of suitable infection conditions (West *et al.,* 2012). Moreover, climate change is expected to affect strategies for disease control; for instance, it can reduce host resistance because some resistance genes are temperature sensitive and may indirectly affect the efficacy of applied agrochemicals under slightly warmer temperatures (Coakley *et al.*, 1999; Garret *et al.*, 2006; West *et al.*, 2012; Gilardi *et al.*, 2017 b).

Conclusions

The risks posed by epidemic outbreaks of plant diseases have recently been reviewed by Zadoks (2017) who has pointed out the negative implications on agriculture, on the environment, on international trade, as well as the social and political consequences. This perspective emphasizes the important need to understand the disease dynamics of new pathogens to improve effective disease management measures (Gamliel, 2008; Gullino *et al.,* 2017c; Mumford *et al.*, 2017).

For instance, *F. equiseti* and *Myrothecium* are currently spreading quickly in Italy on different leafy vegetables that are grown intensively under monocultures or in succession. Such a feature makes these pathogens a serious threat for many Mediterranean crops, because most of the emerging leafy vegetable pathogens have shown a broad host range and they can easily adapt to many different cropping systems, and in particularly to those of a very intensive nature. Furthermore, *F. equiseti* represents a clear example of a fungus that in the past was considered a weak pathogen, but which has now become more serious and invasive.

The expected increases in climatic variability are of even more concern, as they could lead to an increase in the number of losses due to diseases and pests in a given locality, as well as to the year by year variations of their occurrence. Additionally, the ability of some of the newly introduced pathogens to produce a diversity of mycotoxins increases the health risk to consumers.

Given the substantial losses caused by the increased number of epidemic invasions that have resulted from globalisation (Gullino *et al.*, 2014 a), and by the significant impact of global climate changes (West *et al.*, 2012), intense efforts are needed to prevent diseases and to provide control measures.

The multiple mechanism by which seed-borne pathogens survive and disseminate cause both opportunities and difficulties in their disease management. Attempts to control pathogen populations are include the use of pesticides, resistance genes, crop rotations and a variety of cultural practices aimed at reducing plant infections.

As these foliar emerging pathogens of leafy vegetables are generally associated with warmer environments and short wetting periods, the management of microclimate environmental conditions, may become very important.

Using resistant cultivars for the management of soil-borne and foliar pathogens of leafy vegetables would be the most practical, effective and economical approach in both conventional and organic farming. Unfortunately, there is no information available on cultivars that are less susceptible to these new or re-emerging foliar pathogens of leafy vegetables, despite the intensive research that has been conducted on some diseases (i.e. basil downy mildew), and further investigations are necessary. Moreover, the rapid substitution of varieties to adapt to the market demand and the use of mono and oligo-genic resistances to diseases, which may favour the rise of special new forms and new families of pathogens, could complicate its practical application.

Since these measures do not always provide adequate disease suppression, fungicides are also needed, as part of Integrated Pest Management (IPM). Diversity of fungicides, concerning their chemistry and mode of action, is essential to ensure effective crop protection, to control the new threats and to manage fungicide resistance (Leadbeater & Gisi, 2009). However, it is possible, by means of epidemiological studies, to elaborate an efficient disease management programme which would allow the number of chemical treatments to be reduced (Ojiambo *et al.*, 2017).

Effort for a continuous monitoring and disease surveillance is necessary. Quick and reliable diagnostic tools, measures to produce healthy seeds and seed treatment methods need to be investigated and made available to seed companies and growers.

Adopting preventative and combined methods of disease management has become the tool of choice for the control of a wide spectrum of pests. This is essential for the productive sector of leafy vegetables, affected by several new diseases.

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Table 1 – Main foliar pathogens on leafy vegetables first reported in Italy during the past few years (adapted from Gullino *et al.*, 2014 a).

Host	Species	Pathogen	First year of detection, region
Cultivated rocket	*Eruca vesicaria*	*Alternaria japonica*	2010, Piedmont
		Fusarium equiseti	2011, Piedmont
		Colletotrichum kahawae	2014, Piedmont
Wild rocket	*Diplotaxis tenuifolia*	*Alternaria japonica*	2010, Liguria
		Plectosphaerella cucumerina	2012, Campania
		Fusarium equiseti	2014, Campania
		Myrothecium verrucaria	2015, Lombardy
Lettuce	*Lactuca sativa*	*Allophoma tropica*	2011, Lombardy
		Fusarium equiseti	2014, Veneto
		Pythium Cluster B2a (*P.dissotocum, P. coloratum, P. diclinum, P. cf. dictyosporum, P. lutarium, P. sp. 'Group F' and P. sp. tumidum*)	2015, Lombardy
Lamb's lettuce	*Valerianella olitoria*	*Phoma valerianellae*	1966, Liguria
		Pythium irregulare	2013, Piedmont
		Plectosphaerella cucumerina	2014, Campania
		Myrothecium roridum	2015, Lombardy
Basil	*Ocimum basilicum*	*Alternaria alternata*	2010, Piedmont, Liguria
Swiss chard	*Beta vulgaris subsp. vulgaris*	*Pleospora betae*	2007, Piedmont
		Pythium aphanidermatum	2010, Piedmont
Spinach	*Spinacia oleracea*		
		Fusarium oxysporum f.sp. *spinacie*	2011 Campania
		Pythium aphanidermatum	2014 Campania
		Myrothecium verrucaria	2015 Lombardy

Table 2 Contamination of lettuce, rocket, corn salad, spinach and basil seeds by some fungal pathogens of leafy vegetable crops (adapted from Gullino *et al.*, 2014 b).

Host	Pathogen	% of contaminated seeds	Seed treatments
Lettuce	*Fusarium oxysporum* f. sp. *lactucae*	0.1	Physical, chemical, biological, thyme oil seed treatments; resistant inducers (i.e. acibenzolar-S-methyl),
Rocket	*Fusarium oxysporum*	0.1	-*
Wild rocket	*Plectosphaerella cucumerina*	0.15	-
Lamb's lettuce	*Phoma valerianellae*	0.6 – 15	Aerated steam, hot water, thyme oil
Basil	*Fusarium oxysporum* f. sp. *basilici*	0.4 (external contamination) 0.2 (embrio contamination)	Dry hot air, thyme oil; chemical, biological seed treatments
Basil	*Alternaria alternata*	0.2-15.0 (external contamination) 0.2-2.0 (embrio contamination)	-
Basil	*Peronospora belbahrii*	0.01	Dry hot air, thyme oil, resistant inducers (i.e. potassium phosphite or acibenzolar-S-methyl),
Wild and cultivated rocket	*Alternaria japonica*	0.4 (external contamination) 0.2 (embrio contamination)	-
Wild rocket	*Fusarium equiseti*	0.6% (external contamination)	-

*N.t. Data not available

Figures

Figure 1 Symptoms caused by *Plectosphaerella cucumerina* on *Diplotaxis tenuifolia*.

Figure 2 Necrotic spots caused by *Plectosphaerella cucumerina* on endive.

Figure 3 Alternaria leaf spot on basil.

Figure 4 Leaf spot caused by *Alternaria japonica* on *Eruca vesicaria*.

Figure 5 Leaf spot caused by *Fusarium equiseti* on *Diplotaxis tenuifolia*.

Figure 6 Details of necrosis caused by *Fusarium equiseti* on lettuce.

Figure 7 Leaf necrosis on lamb's lettuce caused by *Myrothecium roridum*.

Figure 8 Leaf necrosis on spinach caused by *Myrothecium verrucaria*.

Figure 9 Leaf necrosis on wild rocket caused by *Myrothecium verrucaria*.

Figure 10 Leaf necrosis on lettuce caused by *Allophoma tropica.*

Figure 11 Symptoms caused by *Pythium irregulare* on lamb's lettuce.

Figure 12 Symptoms caused by *Pythium aphanidermatum* on spinach.