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REVIEW ARTICLE

Evolution of new *Plasmopara halstedii* races under the selection pressure with resistant sunflower plants: A review

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Summary Downy mildew in sunflower is a disease caused by *Plasmopara halstedii*, an oomycete with high virulence, aggressiveness and a great potential in developing new races. Understanding the pathogenic characteristics underlying virulence and aggressiveness processes by which *P. halstedii* rapidly evolves is essential to developing effective long-term control measures for the disease. In this review, new data are presented concerning all traits for the two components of pathogenicity (the evolution of virulence and aggressiveness) under different categories of resistance selection pressure, the relationships among several morphological, genetic and pathogenic traits and the intervention between pathogenic variation and durable resistance in sunflower. By combining the data presented in this study, comprehension of the complex interaction between the pathogen and its host plant could be achieved.

Additional keywords: aggressiveness, *Pl* genes, quantitative resistance, virulence

Introduction

Plant oomycete pathogens cause a vast array of destructive diseases of plants important to agriculture, forestry, ornamental and recreational plantings, and natural ecosystems. The most destructive pathogens occur within the class Peronosporomycetidae, in the orders Peronosporales which include *Phytophthora* species and downy mildews (Tourvieille de Labrouhe *et al.,* 2000; Viranyi and Spring, 2011). The biotrophic oomycete *Plasmopara halstedii* (Farl.) Berl. & de Toni is an invasive species where sunflower (*Helianthus annuus* L.) is grown, which can survive up to 10 years in the soil in the form of oospores. Downy mildew is a common sunflower disease responsible for significant yield loss and can be controlled by fungicides and cultivation of resistant hybrids. Infection of up to 90% of sunflower plants with typical symptoms of systemic infection has been reported in the fields and yield losses were estimated to be up to 50% (Tourvieille de Labrouhe *et al.,* 2000; Viranyi and Spring, 2011).

Plasmopara halstedii displays a gene-for-gene interaction with its host plant and shows physiological races (pathotypes) capable of infecting a variable range of sunflower genotypes. The nomenclature of these races is based on the reaction of a series of differential lines (Tourvieille de Labrouhe *et al.,* 2000). Disease resistance in sunflowers to *P. halstedii* can be classified into one of two categories. The first is qualitative resistance which is conferred by the major *Pl* genes and tends to produce a disease-free plant (Tourvieille de Labrouhe *et al.,* 2000). Qualitative resistance is generally unstable, because *Pl* resistance genes are quickly overcome by compatible races in the pathogen population, and too short-lived to be considered durable. The life period of *Pl* gene seems to be very short from its important use on a large scale. In sunflower,

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major genes giving race specific resistance to downy mildew have been mapped since 1995. So far, there appear to be two main clusters, one on LG8 (and a second on LG13 (Radwan *et al*., 2002). Until the present day a number of *Pl* genes have been reported (*Pl1-15*, *Plv*, *Plw*, *Plx-z*, *Mw*, *Mx*, *Plarg, PlHA-R4*), and position of 11 genes has been determined on the SSR genetic map. It is usually the case that new resistance genes provide resistance to earlier races of the pathogen. The second is quantitative resistance which is controlled by minor genes (QTLs) and tends to impact the rate of disease development (rate reducing) rather than producing a disease-free plant (Tourvieille de Labrouhe *et al.,* 2008). Quantitative resistance is generally more stable and equally active against many races of pathogen. The three major QTLs were located on LG 1, 9 and 17, and explained 54.9% of the total phenotypic variance. Vear *et al*. (2008) reported two QTL located on LG 10 and LG 8, and suggested microsatellite markers ORS613 and ORS389 for their detection.

The interaction of avirulence genes of *P. halstedii* and sunflower genotypes carrying *Pl* resistance genes led to the appearance of new virulent isolates. Indeed, it has been possible to identify up to 35 races, with different virulence patterns (Gulya, 2007). *Plasmopara halstedii* is characterized by a high level of evolutionary potential expressed by high virulence, aggressiveness and a great potential in developing new races. Variation of virulence in *P. halstedii* has been the object of several studies (Tourvieille de Labrouhe *et al.,* 2000, 2010; Sakr, 2011b, 2012c) because several cases of major monogenic resistance have been overcome rapidly by the parasite. Downy mildew races have been observed by Sakr (2009, 2011a,b,c, 2012a,c, 2013) and Sakr *et al*. (2009) to show considerable differences in aggressiveness, which affects host response. The most aggressive races often produce symptoms which cannot be compared with those produced by the less aggressive ones. In this study, we use terms according to the definitions of Van der Plank (1968). Virulence is used to indicate the qualitative component of pathogenicity that is expressed vertically and expressed as specific disease-causing abilities. Aggressiveness is used to indicate the quantitative component of pathogenicity that is expressed horizontally irrespective of plant cultivars or species and expressed as non-specific disease-causing abilities. Pathogenicity is used as a general term indicating an ability to cause disease symptoms. To explain the two components of pathogenicity, the most destructive diseases of the cultivated potato caused by *Phytophthora infestans* could be elaborated. There are two identified populations: old populations (e.g. races US1, US6 and US7) and new ones (e.g. races US8 and US11). Miller *et al.* (1997) found that new races were more virulent and aggressive than old ones.

In this review, data concerning pathogenic variation in sunflower downy mildew populations are briefly presented, since knowledge of pathogenicity in *P. halstedii* would help to understand the dynamics of pathogen populations that use their virulence and aggressiveness to better improve adaptation to their environment. Moreover, the following questions are addressed: What are the characteristics for the two components of pathogenicity: virulence and aggressiveness? How does the pathogen evolve its pathogenicity under resistance selection pressure? What are the relationships among several morphological, genetic and pathogenic traits? Is there an intervention between pathogenic variation and durable resistance in sunflower, at least from the point of view of a plant pathologist and epidemiologist? Such studies are needed because sunflower downy mildew was controlled until mid 2000 by using vertical resistance which led to the appearance of new *P. halstedii* races (Tourvieille de Labrouhe *et al.,* 2000; Viranyi and Spring, 2011).

Plasmopara halstedii race evolution in sunflower cultivated zones and molecular characterization of the pathogen

This species is widespread in all sunflower-growing countries with the exception

of Australia. *Plasmopara halstedii* is native to North America where it was first identified in 1922 and later reported in France in 1966 (Tourvieille de Labrouhe *et al.,* 2000), when the sunflower crop was developed and the pathogen was probably introduced via infected seeds during agricultural trade (loos *et al.,* 2007). The major resistance gene played a very important role in the development of sunflower acreages from 1974. Particularly in Europe and USA all modern varieties carry some major resistance genes. This resistance allowed the demonstration of physiological specialization of the pathogen, into two races, race 100 in Europe, and race 300 in North America. Race 710 was introduced into European sunflower cultivated zones from the USA during the 1980s (Tourvieille de Labrouhe *et al.,* 2000; Delmotte *et al.,* 2008; Ahmed *et al.,* 2012). After recombination events between races from the USA with races from Europe occured, a number of new races emerged in different parts of the world. Until the present day, 14 races of downy mildew have been identified in Europe (Delmotte *et al.,* 2008; Ahmed *et al.,* 2012) and 35 worldwide (Gulya, 2007).

In France, the only race known to occur was 100 until 1988 when race 710 was discovered. Further races able to overcome the *Pl* loci were discovered over the following years and were found in distinct geographical regions with 300, 304, 307, 314, 334, 700, 703, 704, 707, 714, 717, 730 and 770 (Table 1). Race 100 was ubiquitous albeit rarely found due to the use of resistant host cultivars. Six of them (304, 307, 314, 334, 704 and 714) have never been documented outside of France even though sunflower hybrids using the *Pl6–Pl7* genes are grown in other European countries (Gulya, 2007). This evolution of *P. halstedii* races seems to be linked with a quasi-exclusive use of *Pl6* gene since 1990. This gene was overcome by the parasite races 704 and 714. Tourvieille de Labrouhe *et al*. (2010) showed that the *Pl6* cluster on LG8 could be split, with about 0.5 % recombination, giving lines resistant to races 330(US), 703, 710 and 730 but susceptible to race 100 and all the other `3xx` races including 300. Moreover, the late use of another gene *Pl5* led to the apparition of the new race 334 (Delmotte *et al.,* 2008; Tourvieille de Labrouhe *et al.,* 2010; Ahmed *et al.,*

Table 1. Virulence of 15 *Plasmopara halstedii* races on sunflower differential lines

Race	Differential lines								
	D1 Ha-304[a]	D2 Rha-265[a]	D3 Rha-274[a]	D4 PMI3[b]	D5 PM-17[a]	D6 803-1[c]	D7 HAR-4[a]	D8 QHP1[b]	D9 Ha-335[a]
100	S	R	R	R	R	R	R	R	R
300	S	S	R	R	R	R	R	R	R
304	S	S	R	R	R	R	R	R	S
307	S	S	R	R	R	R	S	S	S
314	S	S	R	S	R	R	R	R	S
334	S	S	R	S	S	R	R	R	S
700	S	S	S	R	R	R	R	R	R
710	S	S	S	S	R	R	R	R	R
703	S	S	S	R	R	R	S	S	R
704	S	S	S	R	R	R	R	R	S
707	S	S	S	R	R	R	S	S	S
714	S	S	S	S	R	R	R	R	S
717	S	S	S	S	R	R	S	S	S
730	S	S	S	S	S	R	R	R	R
770	S	S	S	S	S	S	R	R	R

[a] USDA genotypes (USA), [b] INRA genotypes (France), [c] IFVC genotypes (Yugoslavia), * S: susceptible, R: resistant (Tourvieille de Labrouhe *et al.,* 2000).

2012). Consequently, the *Pl* gene life period seems to be very short from the important use of *Pl* gene on a large cultivated zone. The development of sunflower resistant cultivars that retain the characteristics required for the oil crop is a lengthy process and the rate of new *P. halstedii* virulence emergence is more rapid than the rate that newly resistant hosts can be bred. Host and race evolution in downy mildew is therefore an issue of key agricultural and commercial importance.

In the last decade, advanced tools of biotechnology have enabled discernment of groups of *P. halstedii* on the molecular level and led to the shift from a morphological to a phylogenetic species concept (Spring and Thines, 2004). Indeed, early studies of *P. halstedii* typically found low levels of genetic diversity using RAPD markers (Komjati *et al.,* 2004), ISSR (Intelmann and Spring, 2002) and ITS sequences (Spring and Zipper, 2006). A study of 77 samples from twelve different countries of six virulence races using 21 RAPD primers found low levels of differentiation within and between samples grouped by race and country (Roeckel-Drevet *et al.,* 2003). With molecular markers based on partial sequence of the nuclear ITS regions, Spring *et al*. (2006) detected polymorphism between profiles of races 100, 310 and 330, as well as between groups of populations representing races 700, 701, 703, 710 and 730. Giress *et al*. (2007) found high genetic variability between the isolates from France and Russia using SNP markers. Also, Delmotte *et al*. (2008) identified three genetically differentiated groups of isolates organized around the first three races described in France: 100, 710 and 703. Recently, Ahmed *et al*. (2012) suggested that multiple introductions have aided in the establishment of *P. halstedii* in France, and noted that recombination facilitated by these introductions is driving the emergence of new and endemic races in response to host resistance. As with the neutral markers these SNPs separated races 100 and 304 from races 703 and 710 (As-sadi *et al.,* 2011). Interestingly, new insights from molecular phylogenetics often verify a narrow species concept that was proposed by Gäumann in 1918 (Voglmayr and Constantinescu, 2008).

Variation of aggressiveness in *Plasmopara halstedii*

Decomposing the pathogen cycle into elementary life traits allows the precise identification and quantification of differences among pathogen or host phenotypes, but relating these life traits to the pathogen fitness under field conditions or to the epidemic development rate is complicated. However, to characterize aggressiveness of sunflower downy mildew populations, sunflower inbred lines showing different levels of quantitative resistance and carrying no *Pl* gene should be used (Tourvieille de Labrouhe *et al.,* 2008). Four aggressiveness criteria were established for *P. halstedii* races: percentage infection, latent period, sporulation density and reduction of hypocotyl length (dwarfing). Here, the four criteria are presented in brief: infection was considered as successful when the seedlings showed sporulation of the pathogen on the shoot surface. Latent period was defined as the number of days of incubation necessary to obtain the pathogen sporulating on 80% of the plants. Sporulation density was defined as the number of zoosporangia of the pathogen produced on a cotyledon. Reduction of hypocotyl length (dwarfing) corresponded to the distance from the stem base to cotyledon insertion and was measured after 13 days of infection on diseased plants showing sporulation of the pathogen on the shoot.

Differences in disease development (percentage of infection) could be due to differences in the rate of maturation of zoosporangia or in the capacity to penetrate healthy host tissue (Delanoe, 1972). Latent period corresponds to the time interval between infection and appearance of symptoms on infected plants. The number of spores produced by a diseased plant will determine the quantity of inoculum that can infect neighbouring plants. But they are also indicative of the interaction between the host plant and the parasite, since the quantity of spores produced will depend on the ag-

gressiveness of parasite and the level of host quantitative resistance. It also reflects the capacity of the fungus to invade host tissues during the incubation period. Dwarfing is a symptom characteristic of plants systemically infected by *P. halstedii* and is explained by a decrease in the concentration of growth hormone (Iodole Acetic Acid) in infected tissue (Cohen and Sackston, 1974). This decrease in size can be observed at a very early stage. Also, the index of aggressiveness of the *P. halstedii* isolate was used to summarize all values for two criteria (pathogen virulence and aggressiveness) on sunflower inbred lines in one value to facilitate the comparison between the different *P. halstedii* races. The index of aggressiveness of *P. halstedii* single zoosporangium isolate was calculated as the ratio of (percentage infection × sporulation density) / (latent period × dwarfing).

Research to analyze aggressiveness has been carried out in several sunflower downy mildew races. Differences in aggressiveness of *P. halstedii* races are indicated when pathogen isolates vary in the amount of damage they cause on sunflower plants. Sakr *et al.* (2009) used two aggressiveness criteria, latent period and sporulation density, to differentiate between two races 100 and 710, and it was found that race 100 was more aggressive than race 710. Moreover, Sakr (2009, 2011a,b,c, 2012c, 2013) included four criteria to distinguish among seven pathogen races. It was found that pathotypes 100, 300, 304 and 314 were characterized with shorter latent period and higher sporulation density than pathotypes 710, 704 and 714 (Figure 1). Also, it was noted that all pathotypes showed high percentage infection values and caused a large reduction in seedling size except for pathotype 314 involved in dwarfing (Figure 1). Then, Sakr (2009, 2011a,b,c, 2012c, 2013) concluded that races 100 and 3xx were more aggressive than races 7xx (Figure 1). Regarding virulence and aggressiveness reaction of pathogen races to sunflower inbred lines carrying several levels of resistance, Sakr (2009, 2011a,b,c, 2012c, 2013) revealed two groups. One group including the more aggressive isolates of races 100 and 3xx, and another group including less aggressive isolates of races 7xx. It is possible that variability between *P. halstedii* races is due to the origin of pathogen. Additional virulence genes may be found in *P. halstedii* races as observed for another oomycete *Phytophthora infestans* (Montarry *et al*., 2010).

Evolution of two components of pathogenicity (virulence and aggressiveness) in *Plasmopara halstedii* under resistance selection pressure

Selective effect on pathogenicity due to host resistance is an important aspect of plant-pathogen interactions, which can be divided into two parts: virulence and aggressiveness. In plant-pathogen interactions, the selection pressure exercised by qualitative resistance on parasitic populations might lead to the appearance of new virulent isolates. On the other hand, the wide usage of host plant varieties presenting high levels of quantitative resistance led to increases in aggressiveness (Stukenbrock and McDonald, 2008). Cowger and Mundt (2002) showed that wheat cultivars with good quantitative resistance selected more aggressive isolates of *Mycosphaerella graminicola*. However, this is not always true, Sullivan *et al.* (2005) reported that tobacco cultivars with high levels of quantitative resistance did not select for more aggressive isolates of *Phytophthora parasitica* var. *nicotiana*e. Also, Flier *et al.* (2007) showed that, following large-scale introduction of more resistant potato varieties in organic production systems in Europe, there was no shift towards increased levels of aggressiveness of *Phytophthora infestans* populations.

Concerning the influence of *Pl* genes selection pressure on virulence of sunflower downy mildew, the interaction of *Avr* avirulence genes of *P. halstedii* and sunflower genotypes carrying *Pl* effective genes led to the emergence of new virulent pathogen isolates in the field. *Plasmopara halstedii* monitoring revealed that between 1987 and 2008 there was a rapid increase in the number of

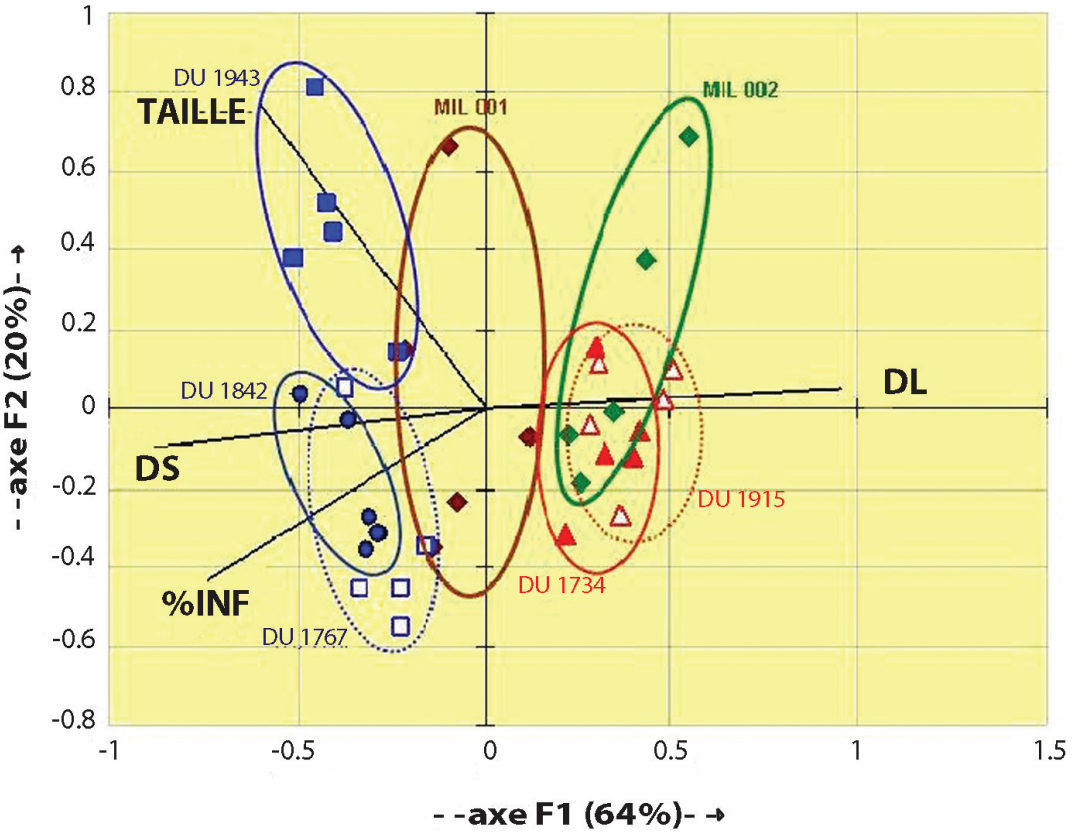

Figure 1. Plot of the principal component analysis for aggressiveness criteria (%INF=percentage infection, DL=latent period, DS=sporulation density, TAILLE= dwarfing) on sunflower inbred line carrying a high level of quantitative resistance for seven Plasmopara halstedii races (MIL001=race100, DU1842=race300, DU1767=race304, DU1943=race314, MIL002=race710, DU1734=race704, DU1915=race714)

A single representative of each race was included in the Principal Component Analysis (PCA) based on data from Sakr (2013). F1 x F2 plot explains more than 80% of the total variation for the four measured criteria of aggressiveness. The PCA revealed strong differentiation in the spread of the data, with 64% of the observed variation on the first axis (latent period and sporulation density criteria) and 20% on the second axis (percentage infection and dwarfing criteria). When plotted, these data formed three distinct groups. Group 1 consisted of races 300, 304 and 314. Group 2 comprised of samples belonging to race 100. Group 3 was made up of races 710, 704 and 717. The first axis clearly separated the three groups, and the second axis separated race 314 from the two races 300 and 304 in the first group.

virulent races ranging from 1 to15. In 2001 and in 2003 three new races were encountered, within the same year of cultivation. Recombination between races takes place and probably contributes to the emergence of new pathotypes in sunflower downy mildew. This is in agreement with other Oomycetes such as *Phytophthora ramorum* (Goss *et al.*, 2009) and *Phytophthora infestans* (Miller *et al.*, 1997) where sexual reproduction occurs depending on geographical location and environmental conditions. It was previously unknown whether field populations of *P. halstedii* hybridise. For example, the admixed samples of race 707 first documented in 2004 in France, are probably the product of a hybrid between races 703 and 304 resulting in a new race able to overcome resistance in sunflower differential lines D7, D8 and D9 as shown in Table 1. Novel pathogen types arising from interspecific hybridization in natural populations are becoming increasingly documented due to molecular studies (Joly *et al.*, 2006). Although we were

able to demonstrate how new virulent races may have emerged, it was not possible to ascertain from these data at what frequency such events occur, or if crosses between certain races are more likely than others.

New endemic races could also arise through clonal evolution, a ubiquitous process in the evolution of many pathogens. Evolution of virulence by accumulation of mutations has been previously described for rusts such *Puccinia striiformis* where 20 different pathotypes were found to have arisen from a single introduction followed by a stepwise mutation process (Steele *et al.,* 2002). Mutation is the ultimate source of genetic variation, directly leading to changes in the DNA sequence of an individual gene and thus creating new alleles in populations. The mutation accumulation suggests that the evolutionary effect of adverse events declines following the age at which an organism is initially capable of reproduction (McDonald and Linde, 2002). Furthermore, recent evidence for race emergence in *P. halstedii* was recently demonstrated by infecting an experimental plot with races 100 and 710. After 5 years six other races were observed (300, 304, 314, 700, 704, 714) that had not been present at the start of the study (Tourvieille de Labrouhe *et al.,* 2010). Although there is no sufficient data to conclude that this mechanism has led to race emergence in situ, it appears to be a plausible explanation given that these genotypes have not arisen by recombination. Furthermore, in the absence of selection pressure, Sakr *et al.* (2011) reported that *P. halstedii* did not evolve its virulence. This rapid evolution of virulence in *P. halstedii* was revealed in France exerted by the intensive *Pl* genes resistance selection pressure; fifteen different races of this pathogen have now been characterized, nine of which emerged in the last ten years (Delmotte *et al.,* 2008). Concerning the influence of *Pl* genes selection pressure on aggressiveness of *P. halstedii*, Sakr *et al.* (2011) suggested that the method of *Pl* gene management affects aggressiveness of pathogen populations because it determines the number of susceptible plants harbored by the parasite.

Concerning the influence of quantitative resistance of aggressiveness of *P. halstedii*, few studies were carried out. Indeed, Sakr (2012a) analyzed the quantitative component of pathogenicity for *P. halstedii* isolates of the race 710; it was found that the index of aggressiveness varied significantly among the isolates. The differences between the more aggressive and less aggressive isolates of sunflower downy mildew is likely to be matter of extreme differences evident in quantitative resistance in cultivated sunflower cultivars. It seems that the quantitative resistance selection pressure in sunflower cultures could vary in aggressiveness in *P. halstedii* isolates of the race 710 as observed for *Venturia inaequalis* (Parisi *et al.,* 2004). The selection pressure by both: qualitative and quantitative resistance in sunflower plants may modify the two components of pathogenicity in *P. halstedii*.

Relationships among the morphological, genetic and pathogenic traits in *P. halstedii*

Different attempts were made to correlate pathogenic variability including virulence and aggressiveness traits with morphological and genetic levels. However, from an epidemiological perspective, it is important to know whether variability at the pathogenic level is just neutral variability or whether it is related to other phenotypic traits. In plant pathogens, some studies have revealed the relationship between the two components of pathogenicity: virulence and aggressiveness. Sullivan *et al.* (2005) found a negative relationship between virulence and aggressiveness for *Phytophthora parasitica* var. *nicotianae* on tobacco and a positive case for *Phakospora pachyrhizi* on soybean (Bonde *et al.,* 2006). For sunflower downy mildew, the relationship between virulence and aggressiveness was found to be positive or negative and two hypotheses were made to explain them (Sakr, 2011c). First, it seems that virulence and aggressiveness are independent, and the coincidence makes this relation

positive and negative. Second, there is an effect of additional virulence genes in *P. halstedii* races for variation of aggressiveness as observed for other pathogens (Brown and Tellier, 2011). Knowledge of virulence cost, trade-off between virulence and aggressiveness, would help to understand the dynamics of sunflower downy mildew populations that use their pathogenicity to better improve adaptation to their environment. Indeed, Van der Plank (1968) defined the cost of virulence as reduction in pathogen fitness induced by a mutation from an avirulence state to virulence, and the changes in pathogen aggressiveness resulting directly from the loss of avirulence gene function. Indeed, Sakr (2012c) found that the 100 and 3xx avirulent races had a virulence cost measured by differences in aggressiveness (from 58.3 to 78.2%, depicts aggressiveness from the virulent to a virulent state) compared to 7xx virulent races carrying unnecessary virulence gene. However, accumulation of virulence genes results in reduced aggressiveness as observed for *Puccinia graminis* f. sp. *avenae* (Leonard 1969), *Bipolaris maydis* (Leonard 1977), *Puccinia graminis* f.sp. *tritici* (Grant and Archer 1983) and for *Erysiphe graminis* f. sp. *hordei* (Grant and Archer 1983). Usually in a gene for gene interaction, virulence cost in new races comes from loss (and not accumulation) or mutation of avr genes that might contribute to virulence (Stahl and Bishop 2000). The trade-off between virulence and aggressiveness probably has considerable consequences for *P. halstedii* evolution, because races that accumulate a large number of virulence genes might never be the most aggressive on sunflower genotypes. Moreover, it seems that negative relationship between the two components of pathogenicity may play an important role in generating local adaptation in the pathosystem of *P. halstedii* and sunflower by impeding the emergence and evolution of races that are both highly aggressive and capable of multiplying on all sunflower genotypes.

Regarding the correlation between pathogenicity traits and genetic variability, Sakr (2011a, 2013) found no correlation between molecular genotypes and pathogenicity traits. The low degree of association between traits of virulence and aggressiveness and the molecular variation is expedited considering the high degree of variation in pathogenicity of *P. halstedii*. However, the lack of matching between pathogenicity traits and groups based on molecular markers was not surprising. Indeed, Montarry *et al*. (2006) did not find a clear correlation between phenotypes and genotypes based on molecular markers for *Phytophthora infestans***.** Pathogenicity is known to evolve through mutation without highly altering molecular fingerprints (Goodwin, 1997). Because most molecular markers used for fingerprinting are selectively neutral, they can be used to assess evolutionary forces other than selection (such as gene flow or genetic drift). Concerning the correlation between pathogenicity traits and morphological characteristics in sunflower downy mildew races, Sakr (2013) found no correlation between morphological genotypes and pathogenicity traits. Sakr (2013) found that the proportion of zoosporangia of different forms and their sizes and the morphology of sporangiophores do not appear to be useable to differentiate the virulent characteristics for *P. halstedii* isolates. The results also showed that zoosporangia and sporangiophores morphology did not distinguish the aggressiveness groups. Regarding the correlation between morphological traits and genetic variability, Sakr (2013) found no correlation between molecular genotypes and morphological traits.

Durable resistance in sunflower against *Plasmopara halstedii*

Regarding the intervention between pathogenic variation in *P. halstedii* and durability of resistance in sunflower plants, new data (Sakr, 2009, 2011a,b,c, 2012a,b,c, 2013) showed that large variation in pathogenicity has been detected within and between *P. halstedii* races. Such variation could arise due to the agroecological environment, host diversity, cultural practices, and sexual recombination. According to Sakr (2009,

2011a,b,c, 2012a,b,c, 2013), important difference in pathogenicity affects sunflower plant's response by showing an importance for *Pl* gene to differentiate among pathogen isolates for virulence (Tourvieille de Labrouhe *et al.,* 2010; Sakr, 2011b; Franchel *et al.,* 2013) and by masking the impact of genetic factors underlying QTL for aggressiveness (Vear *et al.,* 2008; Vincourt *et al.,* 2012).

Results obtained in recent studies (Sakr 2009, 2011a,b,c, 2012a,b,c, 2013) could be used in a strategy that helps to improve durability of sunflower resistance to downy mildew (Vincourt *et al*., 2012; Franchel *et al*., 2013). Results presented by Sakr (2009, 2011a,b,c, 2012a,b,c, 2013) showed that virulent groups had different levels of aggressiveness. Understanding principal causes which explain the intervention between virulence and durability of sunflower plants is crucial. Qualitative *Pl* resistance genes in sunflower plants were the main keys for the emergence of new and endemic *P. halstedii* races in response to recombination facilitated by pathogen introductions as noted recently by Ahmed *et al*. (2012). Indeed, the potential of *P. halstedii* to evolve new races has also been enhanced by the introduction of several genetically differentiated genotypes: repeated introductions of *P. halstedii* isolates, combined with the selective pressure exerted by host resistance genes, may have greatly accelerated the breakdown of qualitative resistance (Ahmed *et al.,* 2012). Exploring aggressiveness variation of sunflower downy mildew populations may provide significant knowledge about host resistance mechanisms and the potential breakdown of resistance. Because quantitative resistance is likely to play a prominent role in future breeding programs, it might be important to assess resistance against diverse *P. halstedii* populations, including challenging breeding material with *P. halstedii* isolates collected from wild *Helianthus* species, because they are more aggressive than those from other host species and may include diverse mechanisms with the potential to break the qualitative sunflower resistance. Moreover, Sakr (2011b) reported that dynamic of evolution in *P. halstedii* isolates showing new virulence and carrying different levels of aggressiveness is correlated with qualitative and quantitative selection pressure.

Recently, Sakr (2012b) proposed a mixture model of sunflower inbred lines carrying the two types of resistance (qualitative and quantitative). This sunflower model may enhance durable resistance against *P. halstedii*. This strategy supposes that pathogenicity of *P. halstedii* would slowly and difficultly develop on sunflower genotypes carrying qualitative and quantitative resistance. Consequently, it would limit fungal capacity to reproduce and disperse among the plants of a mixture.

Although *P. halstedii* has an evolutionary capacity to produce new virulent races under the selection pressure of *Pl* genes (Gulya, 2007), it seems that the ability of pathogen to develop its pathogenicity may be limited by the presence of the two types of resistances in our mixture model. The sunflower inbred lines carrying effective *Pl* genes could prevent the dispersion of more aggressive pathotypes in *P. halstedii*. In addition, the sunflower inbred lines showing different levels of quantitative resistance could limit the reproduction and dispersion of virulent pathotypes in sunflower downy mildew. Super races present the most difficult hindrance for the cultivation of sunflower on a large surface. Indeed, the agricultural system containing the two types of resistance in a given environment may provide a satisfactory control for the development of the parasite. Also, this model may reduce costs of sunflower production by improving the best conditions that limit the reproduction of the pathogen.

Conclusions

Even though a big step has been made towards understanding the complex interaction of *P. halstedii* with sunflower, as well as the mechanisms of pathogenicity evolution, a number of questions still remaine

unanswered. Virulence is a driving force in host-pathogen co-evolution since it enables pathogen to overcome qualitative *R* resistance genes. Aggressiveness enables the pathogen to develop within the host plant (Van der Plank, 1968). Indeed, the parameters measuring changes on the level of virulence and aggressiveness (the two components of pathogenicity) could be used to evaluate the level of co-evolution of *P. halstedii* with its sunflower host.

Furthermore, since it appears that *P. halstedii* evolves new races through both mutation and hybridization, this species may provide a useful model for further research into the genetics of pathogen evolution. These issues are not only important for informing disease management policy, but also they address key questions in the genetics of adaptation. Recently, few molecular studies have been performed in the *P. halstedii – H. annuus* system to explain resistance from the plant's side and virulence from the pathogen's side (Bouzidi *et al*., 2007; As-Sadi *et al*., 2011). Enriching the genomic resources available for exploring the interaction between *H. annuus* and *P. halstedii* is crucial for research on *P. halstedii*, especially with respect to discovering the effectors involved in its pathogenicity. Finally, the pathosystem of *P. halstedii* and *H. annuus* will continue to evolve virulence in the pathogen unless it was reengineered to make it less conductive to pathogen evolution (Sakr, 2011b) in accordance with Stukenbrock and McDonald's (2008) hypothesis.

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Εμφάνιση και εξέλιξη νέων παθογόνων φυλών του Ωομύκητα *Plasmopara halstedii* υπό την πίεση επιλογής σε ανθεκτικές ποικιλίες ηλίανθου

Nachaat Sakr

Περίληψη Ο περονόσπορος του ηλίανθου είναι μια ασθένεια της οποίας το παθογόνο αίτιο, ο Ωομύκητας *Plasmopara halstedii*, παρουσιάζει υψηλή μολυσματική ικανότητα και επιθετικότητα, όσο και ικανότητα να διαφοροποιεί νέες παθογόνες φυλές. Η κατανόηση των παθογόνων παραγόντων που καθορίζουν τους μηχανισμούς μολυσματικότητας και επιθετικότητας, μέσω των οποίων το συγκεκριμένο παθογόνο εξελίσσεται ραγδαία, είναι απαραίτητη για την ανάπτυξη μακροχρόνιων και αποτελεσματικών μέτρων αντιμετώπισης. Σε αυτή την ανασκόπηση παρουσιάζεται και αναλύεται de novo το σύνολο των χαρακτηριστικών που σχετίζονται με τις δύο συνιστώσες της παθογένειας (μολυσματικότητα και επιθετικότητα) υπό διαφορετικές κατηγορίες πίεσης επιλογής για ανθεκτικότητα. Επίσης συσχετίζονται μεταξύ τους τα διαφορετικά μορφολογικά, γενετικά και παθογόνα χαρακτηριστικά καθώς και η

διαφοροποίηση στη παθογένεια με την ανθεκτικότητα του ηλίανθου. Το σύνολο των δεδομένων και ο συνδυασμός τους παρατίθενται σε αυτή τη μελέτη με σκοπό την κατανόηση των πολύπλοκων αλληλεπιδράσεων μεταξύ του παθογόνου μύκητα και του φυτού-ξενιστή του.

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SHORT COMMUNICATION

Presence of *Culex tritaeniorhynchus* (Diptera: Culicidae) in rice fields of Western Greece

I.Ch. Lytra[1] and N.G. Emmanouel[1]

Summary The presence of the mosquito *Culex tritaeniorhynchus* was recorded in large numbers in rice in Western Greece for 6 consecutive years (2008-2013). As only few specimens were previously collected in Attica in 2003 and the mosquito is a potential vector of pathogens which cause human diseases, the importance of this species for the emergence of these diseases in Europe is discussed.

The mosquito species *Culex tritaeniorhynchus* Giles is part of the *Culex vishnui* subgroup, which also includes *Culex pseudovishnui* Colless and *Cx. vishnui* Theobald (Toma *et al*., 2000). It is widely distributed throughout the Oriental region extending into the Middle East, the Mediterranean and Afrotropical region, China, Russia, Japan, Korea, Micronesia and Indonesia (Lee *et al*., 1989). It has also been recorded in Angola, Cameroon, Central African Republic, Egypt, Gabon, Gambia, Ghana, India, Iran, Iraq, Israel, Jordan, Kenya, Lebanon, Maldives Islands, Mozambique, Nigeria, Saudi Arabia, Senegal, Sri Lanka, Syria, Tanzania, Togo, Turkey, Turkmenistan (Walter Reed Biosystematics Unit). In Europe, *Cx. tritaeniorhynchus* has been reported in Albania (Danielová and Adhami, 1960; Adhami, 1987; Samanidou and Harbach, 2003) and it was first recorded in Greece in 2003 from samples which were taken from a coastal marsh in the area of Marathon, Prefecture of Attica (Fig. 1) (Samanidou and Harbach, 2003).

This is the first record of *Cx. tritaeniorhynchus* in agricultural land in Greece and the second reference in the country. Moreover, it is the first time that large numbers of this mosquito species are recorded in Greece for 6 consecutive years (2008-2013) as the previous report concerns only a few specimens (Samanidou and Harbach, 2003).

Specimens of *Cx. tritaeniorhynchus* were obtained through samplings which were conducted at an organic irrigated rice field for a research study on the mosquito fauna throughout the growing season. The size of the sampling field was approximately 8 ha. The rice field was located in a rural area close to the Delta of Acheloos river (38°20'20"N, 21°15'06"E) in the Prefecture of Aitoloakarnania, Western Greece, where a total of 1,500 ha of rice fields exist (Fig. 1). The samplings were performed every 10 days during the rice cultivation period (between June and September) in the years 2009, 2010 and 2011, whereas they were conducted monthly during the same period in 2008, 2012 and 2013.

Twenty samples of larvae and pupae were taken using a standard larval dipper (350 ml, 13 cm diameter) with an elongated handle (BioQuip, Rancho Dominguez, CA). The samples were transferred to the laboratory in the Agricultural University of Athens, where mosquito larvae and pupae were counted and reared to adults in a rearing room at 25-26°C. Adults were collected every day, killed in killing boxes using ethyl acetate and pinned on paper points. The mosquitoes were then identified to species by the authors using taxonomic keys (DuBose

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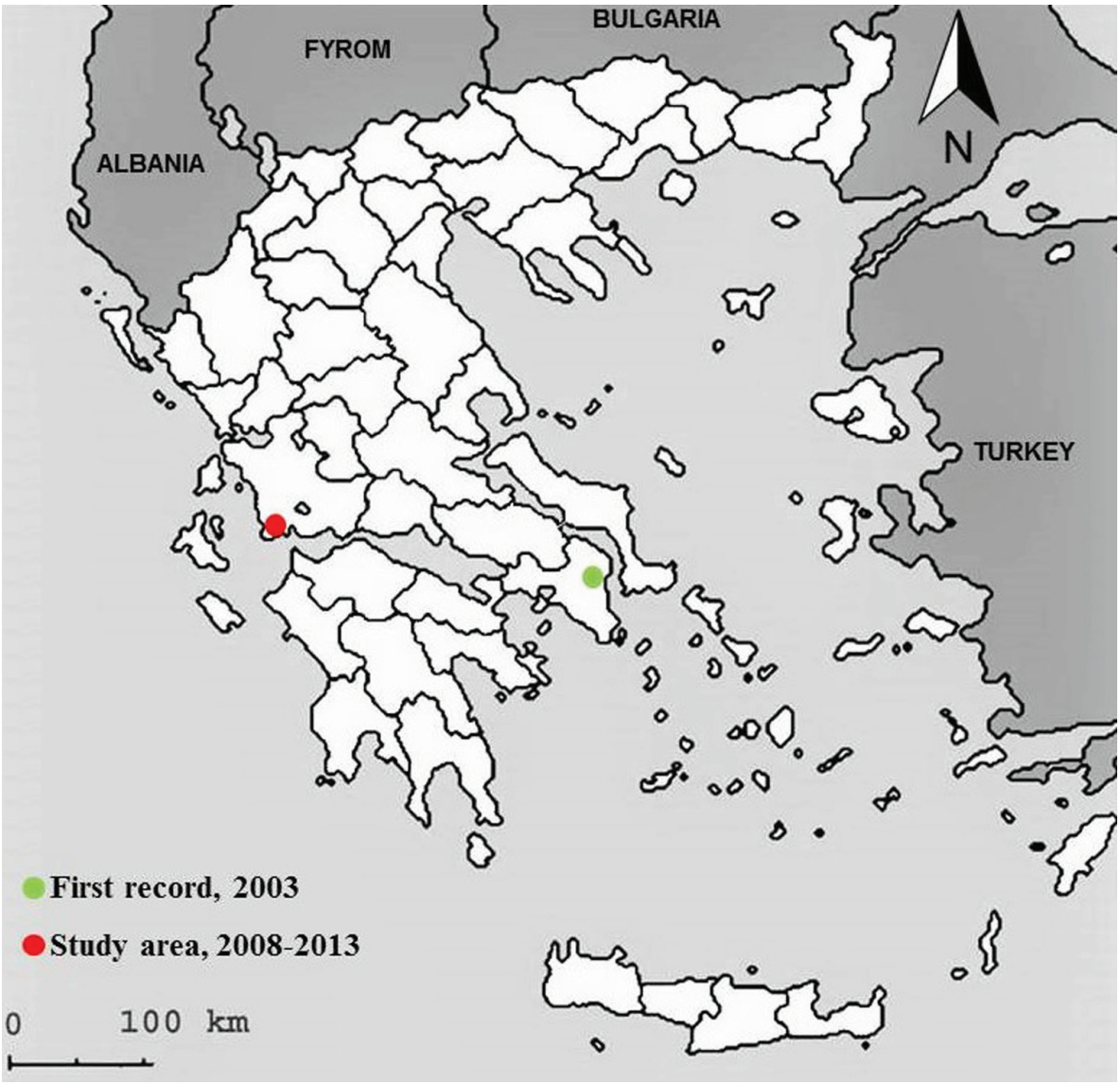

Figure 1. Areas where *Culex tritaeniorhynchus* has been recorded in Greece: Marathon, Prefecture of Attica in 2003 (first report); Delta of Acheloos River, Prefecture of Aitoloakarnania from 2008 to 2013.

and Curtin, 1965; Harbach, 1985; Samanidou and Harbach, 2003; Becker *et al*., 2010).

Culex tritaeniorhynchus was permanently present in large numbers in the samples, ranging from 125 to 198 individuals per sampling (adults derived after the immature rearing). *Culex tritaeniorhynchus* is recorded for the first time in Aitoloakarnania, Western Greece. Although there is a severe nuisance from mosquitoes in this area, available data for the mosquito species that breed in these rice fields are lucking and no relevant control programs have been conducted for decades.

These findings are important because *Cx. tritaeniorhynchus* is a potential vector of pathogens that cause human diseases. It is the primary vector of Japanese encephalitis (JE) in southern Asia and has also been found infected with Dengue, Rift Valley fever, Sindbis, Getah and Tembusu viruses, and microfilariae of both *Brugia malayi* and *Wuchereria bancrofti*, in many areas of eastern and southeastern Asia (Lacey and Lacey, 1990). The females of *Cx. tritaeniorhynchus* feed primarily on domestic animals such as cattle and pigs, but will bite man in their absence (Bram, 1967). They mainly bite outdoors between sunset and midnight, but may enter in cattle sheds and dwellings and

bite man during any time of the night (Gutsevich *et al*., 1974; Sirivanakarn, 1976). The larvae of *Cx. tritaeniorhynchus* can be found in various temporary and permanent ground water habitats that are sunlit and contain vegetation such as ground pools, streams, swamps, shallow marshes, irrigation ditches, rice fields, and animal hoof prints (Bram, 1967; Harbach, 1988).

Taking these data into account, the rural area close to the Delta of Acheloos river with almost 7,000 residents, large surface area of rice fields and farms of cows and pigs could be under a potential threat of JE emergence. JE is the leading cause of viral encephalitis in South East Asia, being endemic in India, China, and Japan and all of South East Asia (Das, 2013). It is largely restricted to rural settings (Self *et al*., 1973; Solomon *et al*., 2000) and the incidence of pigs and marsh birds is crucial in the etiology of JE, as the virus is carried by birds and amplified by pigs (Broom *et al*., 2003). The management of paddy water strongly influences the transmission of JE (Keiser *et al*., 2005). The establishment of Japanese encephalitis virus (JEV) in new ecosystems outside of its current range is difficult (van den Hurk *et al*., 2009). However, with the spread of JEV into the Indian subcontinent, other destinations served by frequent routes of commerce or passenger air travel (Africa and Europe) also could be at risk (Weaver and Reisen, 2010).

The present record indicates also the urgent need of further investigation about the presence of *Cx. tritaeniorhynchus* in other regions of Greece. The risk for the introduction and installation in Greece and Europe of the diseases that this species can transmit should be taken into consideration by the local authorities in order to establish effective control programs against mosquitoes.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Παρουσία του είδους *Culex tritaeniorhynchus* (Diptera: Culicidae) σε ορυζώνες της Ελλάδας

Ι.Χ. Λύτρα και Ν.Γ. Εμμανουήλ

Περίληψη Το είδος *Culex tritaeniorhynchus* καταγράφηκε σε υψηλές πληθυσμιακές πυκνότητες σε ορυζώνα στη δυτική Ελλάδα για 6 συνεχόμενα έτη (2008-2013). Δεδομένου ότι ελάχιστα άτομα του είδους αυτού είχαν στο παρελθόν συλλεγεί στην Αττική (2003) και ότι το κουνούπι αυτό αποτελεί δυνητικό φορέα παθογόνων για τον άνθρωπο, αξιολογείται η σημασία του είδους για την πιθανή εμφάνιση των ασθενειών αυτών στην Ευρώπη.

Hellenic Plant Protection Journal **7:** 15-18, 2014

Hellenic Plant Protection Journal **7:** 19-23, 2014

SHORT COMMUNICATION

First record of *Glycaspis brimblecombei* Moore, 1964 (Hemiptera: Psyllidae) in Greece

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Summary The psyllid *Glycaspis brimblecombei* has been recorded for the first time in Greece. Infested eucalyptus leaves were found in Attica region, Aitoloakarnania and Chania during the summer months of 2013.

Additional keywords: distribution, first record, *Glycaspis brimblecombei*, Hemiptera, redgum lerp psyllid

Psyllids are tiny sap-sucking insects (1-10 mm) of the superfamily Psylloidea, resembling with small cicadas. There are about 3,850 species that have been described worldwide (Li, 2011), where most develop in woody dicotyledons (Burckhardt, 1994; Hodkinson, 1988). One of the most dangerous pests for a variety of eucalyptus species in different regions of the world is the redgum lerp psyllid *Glycaspis brimblecombei* (Hemiptera: Psyllidae). It is native to Australia (Moore, 1964) and introduced into the USA in 1998 (Brennan *et al*., 1999; Gill, 1998), where the recent years has shown invasive behaviour and spread across several countries (de Queiroz *et al.,* 2013). In Palaearctic region it was detected in Portugal in 2007 (Valente and Hodkinson, 2009), Spain in 2008 (Hurtado and Reina, 2008) and in Italy in 2010 (Laudonia and Garonna, 2010). It has been projected that there is a great potential to colonize in new countries, especially in latitude between 20° and 40° in both hemispheres (de Queiroz *et al.,* 2013).

This work reports the presence of *Glycaspis brimblecombei* in Greece. It was found to infest Eucalyptus trees in Attica region (Aigina, Vary, Varymbombi, Laurio, Marousi, Metamorfosi, Neo Irakleio, Peristeri, Kifisia) in June 2013, and in Aitoloakarnania and Chania in July and August 2013, respectively (Figure 1). The identification was done by the first author following the key developed by Laudonia and Garonna (2010). The National Plant Protection Organisation of the Ministry of Rural Development and Food was notified immediately for the presence of the pest in Greece.

Adults of *G. brimblecombei* are 2.5-4mm long, winged, highly mobile, light green to brownish colour with yellow and orange patches (Figure 2). They can easily distinguish from other species from the very long genal processes and the dorsally flat thorax (Laudonia and Garonna, 2010). Detailed morphological description of redgum lerp psyllid has been published by Moore (1964) and Halbert *et al.* (2001).

Eggs are orange-yellowish, stalked ovoid and laid loose by female in new leaves (Figure 3). Larvae are reddish bronze colour with darker wing pads which have bright white spots. Characteristic of its appearance is that larvae are settled and protected from sugary, crystalline white conical cover called lerps, with wax and honeydew excretions resembling with armoured scale insects (Halbert *et al*., 2001) (Figure 4). Larvae continue to feed and grow under protective

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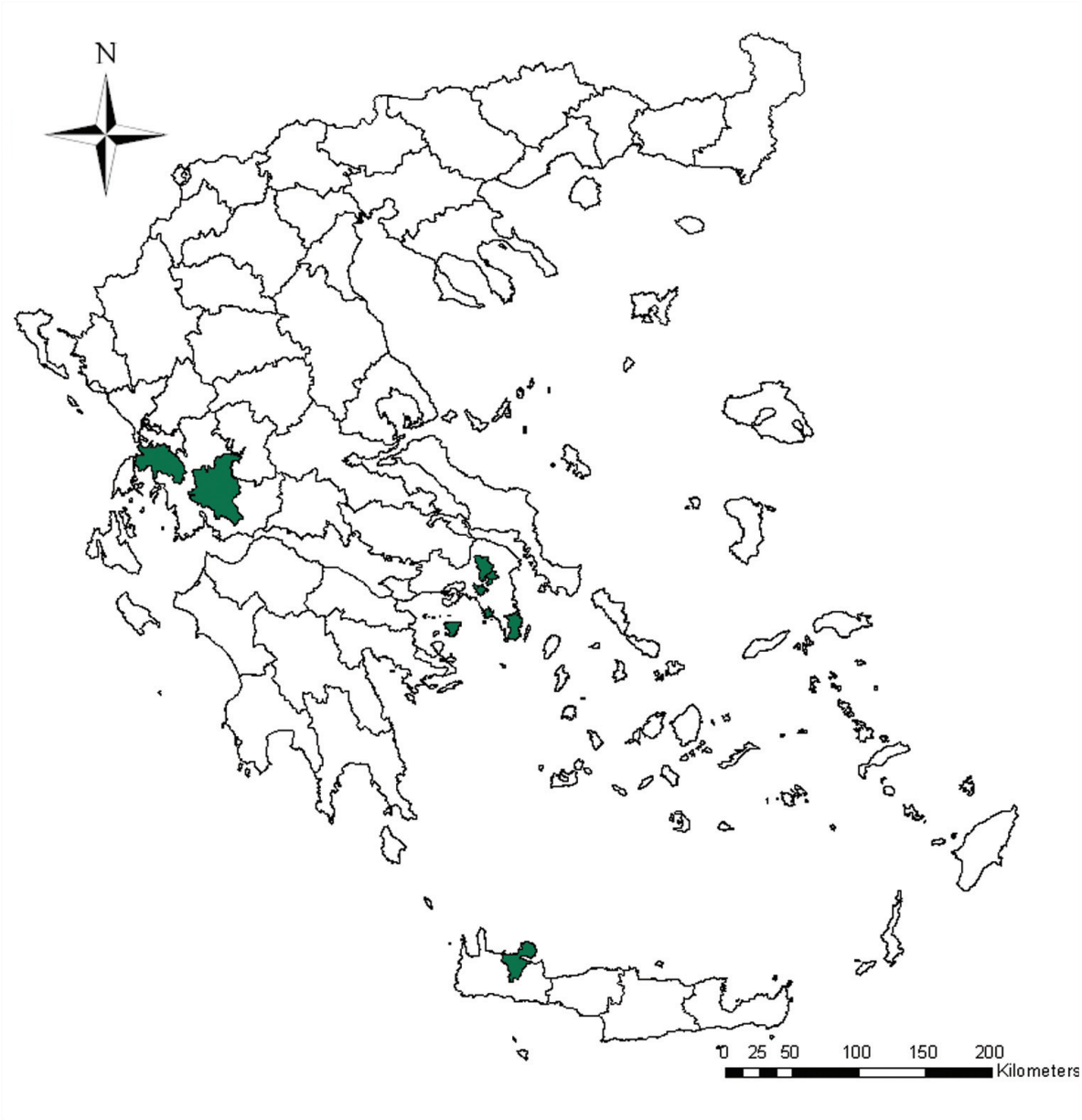

Figure 1. Occurrence of *G. brimblecombei* in Greece.

Figure 2. *Glycaspis brimblecombei* adult.

cover. When larval development completes, adults emerge and start infesting and feeding in new host plants.

The main damage that it causes is tree weakening by suction of sap, where in high population density larvae produce large amounts of waxy secretions and honeydew, and the resulting sooty mould reduce photosynthesis, induce premature leaf drop, growth reduce, branches and shoots dieback and occasionally tree death (Brennan *et al*., 1999; Paine *et al*., 2006). Moreover, in urban areas honeydew secretions cause

Figure 3. Eggs and lerps of *G. brimblecombei.*

Figure 4. *Eucalyptus* sp. leaves infested by *G. brimblecombei.*

considerable nuisance (Paine *et al*., 2006). Laudonia and Garonna (2010) observed 2-4 generations per year in Australia, but there are not available data for life history in Europe.

Glycaspis brimblecombei develops on various *Eucalyptus* species including *E. blakelyi* Maiden, *E. brassiana* Blake, *E. bridgesiana* Baker, *E. camaldulensis* Dehnh., *E. camphora* Baker, *E. dealbata* Cunn. ex Schauer, *E. mannifera* ssp. *maculosa* Baker, *E. nitens* Deane and Maiden, *E. teriticornis* Smith, , *E. lehmannii* (Schauer) Benth., *E. diversicolor* Muell, *E. globulus* Labill, *E. sideroxylon* Cunn ex Woollis, *E. rudis* Endl., *E. cinerea* Muell, *E. cladocalyx* Muell, *E. ficifolia* Muell, *E. grandis* Hill ex Maiden, *E. paniculata* Smith, *E. platypus* Hook, *E. polyanthemos* Schauer, *E. pulverulenta* Sims, *E. robusta* Smith, *E. saligna* Smith, *E. viminalis* Labill, *E. leucoxylon* Meull, *E. macrandra* Muell ex Benth and *E. nicholii* Maiden and Blakely (Brennan *et al*., 1999; Brennan *et al*. 2001; Hollis 2004; Percy *et al*., 2012). From the above *Eucalyptus* species, *E. camaldulensis*, *E. rudis* and *E. teriticornis* are moderate to highly susceptible with heavy defoliation (Brennan *et al*., 2001) and is considered preferable to avoid their planting.

Economic impact of redgum lerp psyllid may be more serious than other eucalyptus psyllids. *Glycaspis brimblecombei* has a wide host range compared with other eucalyptus psyllids and has impact to nurseries, ornamental and forestry plantations. Life cycle completes in immature and mature leaves and an infestation of this pest can defoliate the host. Continuing defoliation causes stress to the trees and makes them more susceptible to other pathogens and insect infestations (Landsberg, 1990).

A sustainable IPM programme should be performed for sufficient control, as *G. brimblecombei* spreads quickly (Santana and Burckhardt, 2007). Many generalist predators have been recorded to feed on this psyllid, such as coccinellid beetles, lacewings (Erbilgin *et al*., 2004), syrphids, chrysopids and anthocorids. The parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) has been reported as highly efficient and has been introduced in the USA from Australia in an effort of classical biological con-

trol (Daane *et al*., 2005; Dahlsten *et al*., 2005; Huerta *et al*., 2011; Sime *et al*., 2004).

Cultural practices and tree health can also affect the psyllid population and the extent of damage. With tolerant varieties, infrequent irrigation and nitrogen fertilization, tree stress can be reduced and avoid damage (Paine *et al*., 2006). Systemic insecticides provide control sometimes, but generally, their effectiveness in the U.S.A. is considered inappreciable and foliage sprays are not recommended as natural enemies can be harmed (Paine *et al*., 2006).

P.S.: After finalizing the manuscript for publication and uploading, a paper about the occurrence of *Glycaspis brimblecombei* in Greece was published (Tsagkarakis *et al.,* 2013).

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Glycaspis brimblecombei* Moore, 1964 (Hemiptera: Psyllidae) στην Ελλάδα

Π.Γ. Μυλωνάς και Γ.Κ. Παρτσινέβελος

Περίληψη Στην παρούσα εργασία αναφέρεται για πρώτη φορά στην Ελλάδα η παρουσία της ψύλλας του ευκαλύπτου *Glycaspis brimblecombei* (Hemiptera: Psyllidae), σοβαρού εχθρού πολλών ειδών ευκαλύπτων σε διάφορες περιοχές του κόσμου. Το *G. brimblecombei* το οποίο κατάγεται από την Αυστραλία, εισήχθη στις ΗΠΑ το 1998 και από τότε εξαπλώθηκε σε αρκετές χώρες του κόσμου συμπεριλαμβανομένων και χωρών της Μεσογείου. Στην Ελλάδα η παρουσία του διαπιστώθηκε στους Νομούς Αιτωλοακαρνανίας, Χανίων και Αττικής. Ειδικότερα στον Ν. Αττικής διαπιστώθηκε στους δήμους Αίγινας, Βάρης, Αχαρνών, Κηφισιάς, Λαυρεωτικής, Αμαρουσίου, Μεταμορφώσεως και Περιστερίου.

Hellenic Plant Protection Journal **7:** 19-23, 2014

Hellenic Plant Protection Journal **7:** 25-29, 2014

SHORT COMMUNICATION

Occurrence of European field pansy (*Viola arvensis*) in Orestiada, Greece

C.A. Damalas[1], S.D. Koutroubas[1] and S. Fotiadis[1]

Summary The European field pansy (*Viola arvensis* Murray), an annual broadleaf weed, occurs with increasing frequency over the last few years in arable land of the Farm of Democritus University of Thrace in Orestiada. Naturally-occurring weed populations were observed in densities from 5 to 20 plants per m^2 mostly in irregular patches, in the margins (edges) of a winter wheat field and at points of the field with low wheat density, in minor spring-sown legumes such as lentils, faba beans, lupine, and winged vetchling, and in parts of the fields without crop. The occurrence of the species in the area is probably associated with the absence of chemical weed control (no use of herbicides) coupled with the increased nitrogen availability in the soil, which favors the overall productivity of plants (i.e. biomass accumulation, seed production and seed dispersal) in subsequent generations. In this report, basic morphological traits at different stages of the life cycle of the species are presented.

Additional keywords: identification, morphology, *Viola arvensis*

The European field pansy (*Viola arvensis*) occurs with increasing frequency over the last few years in arable land (at an acreage of 15 ha) of the Farm of Democritus University of Thrace in Orestiada. Increasing densities of plants were confirmed by systematic visual observations in the area. According to the literature, this species is mentioned as an annual weed mainly of cereals and oilseed rape in northern Europe (Vanaga *et al.*, 2010; Salonen *et al.*, 2011; Andreasen and Stryhn, 2012; Hanzlik and Gerowitt, 2012), in New Zealand (Bourdôt *et al.*, 1998), and in Canada (Degenhardt *et al.*, 2005a, 2005b, 2005c). It appears that this weed is considered to be a minor threat in agroecosystems (Degenhardt *et al.*, 2005c) and a rather easy weed species to tackle, particularly with the use of herbicides, depending on the available herbicides and the crop management system (Huggenberger and Gueguen, 1987; Degenhardt *et al.*, 2005a, 2005b; Becker *et al.*, 2008; Koo and Caseley, 2008; Richardson and Zandstra, 2009). In general, identification of a weed is the first step towards finding potential practices for effective management (Dekker, 1997). However, information about the performance of this weed under Greek conditions does not exist. Thus, the aim of this study was the depiction of basic morphological traits of European field pansy to assist correct identification and also to provide basic information on its occurrence from the area of Orestiada in Greece. To our knowledge, European field pansy has been reported previously as a minor weed of wheat fields in Greece (Damanakis, 1983) and it is also mentioned as a herb in FILOTIS database, a Greek information system for the natural environment of Greece (FILOTIS DATABASE, 2014). However, further information lacks in the Greek literature.

A field survey was conducted in 2011 at the Farm of Democritus University of Thrace in Orestiada, Greece (41°30'N latitude, 26°32'E, 22 m asl), where the species was initially recorded. The soil was silty

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clay loam (Typic Xerofluvent) with organic matter 1% and pH 6.68. Because of the uneven spatial distribution of the weed plants in the previous year, an area of 1.5 ha was defined for the observations and the measurements. The area was cultivated mainly with winter wheat and spring-sown legume crops. Shortly after weed emergence in the defined area in late March, 30 solitary plants of the same growth stage were selected and their position was marked by long wooden stakes. The stakes remained until the end of the survey (i.e. until maturity of the plants in early July) to indicate easily the selected plants. Plants that emerged both inside and outside cultivated area were selected (15 plants from each area). Morphological traits at different growth stages of the life cycle of the weed were visually recorded and measured. Measurements concerned the thickness of the central stem, the length and width of the lower and the upper leaves, the length and width of the open flower, the diameter of the closed capsule at maturity, the number of seeds/capsule, and the plant height. The above morphological parameters were determined with a portable digital caliper (of measuring range 0-150 mm and measurement increments of 0.01 mm).

Identification of the weed was based on keys and illustrations provided in the book of Hanf (1983) and in the guide of Cowbrough and Smith (2009), which are often used for weed identification. According to our own observations, the cotyledons are petiolate, almost round, with entire margins, smooth and fleshy surface, and with a shallow notch (indentation) at the tip. The lower leaves are simple, petiolate (mostly with large stalks), oval, with small incisions in the leaf margins (toothed margins), and smooth with brightly shiny surface (Figure 1). The upper leaves are simple, petiolate (mostly with short stalks), narrowly lanceolate to ovate, with margins coarsely to regularly crenate-serrate, hairy veins on the leaf underside and large leafy stipules at the base of the leaf stalks.

The European field pansy is morphologically similar to and can be often confused with the species *V. bicolor* (syn. *V. rafinesquei*) and *V. tricolor*, but in *V. bicolor* the color of the flower is pale blue-violet to pale yellow in the centre, while the species *V. tricolor* has larger flowers, in which the upper pair of petals are dark blue or purple from the middle to the edges (Doohan and Monaco, 1991).

Figure 1. Young seedling of *Viola arvensis* out of crop.

Figure 2. Young seedling of *Viola arvensis* in a rosette form.

Figure 3. Young seedling of *Viola arvensis* inside wheat crop.

At low temperatures in the early growth stages, juvenile stems are acaulescent, bearing a rosette of leaves at the ground level (rosette form), which may complicate identification (Figure 2). With rising temperatures, the central stem is expanded, becoming erect, ramose, and angular (Figure 3). The flowers are bilaterally symmetrical, chasmogamous, and complete (Figure 4). They consist of a calyx, auricled at the base, with five lanceolate, acute sepals, and a corolla of five petals, which are shorter than or equal in length to the sepals. The lower petal is white, with a small, conspicuous yellow spot at the throat. The upper petals are creamy white, occasionally tinged with some pink or mauve. Flowers arise in leaf axils and are borne singly on slender pedicels. Fruits are 3-valved capsules, which dehisce upon drying at maturity to expel the seed (Figure 5). Mean values of basic morphological traits are shown in Table 1.

Natural infestations of the weed were observed in an open area of arable land (annually disturbed environment or land in temporary fallow) in densities ranging from 5 to 20 plants per m^2 mostly in irregular patches. The weed populations were found mainly in the margins (edges) of the winter wheat field and at localities of the field with low wheat density, in minor spring-sown legumes crops such as lentils, faba beans, lupine, and winged vetchling (a type of traditional Greek fava), and in parts of the field without crop. In all these localities of the defined area, there was no strong competition for light. The occurrence of the species in the area is probably associated with the absence of chemical control (no use of herbicides) coupled with the increased nitrogen availability in soil, which favors the overall productivity of plants, including seed pro-

Figure 4. Plant of *Viola arvensis* at the flowering stage.

Figure 5. Fruit (capsule) of *Viola arvensis*.

Table 1. Mean values of basic morphological traits of *Viola arvensis* (n = 30).

Trait	Mean	Min	Max
Plant height (maturity) (cm)	28.2	25.4	32.6
Thickness of main stem (mm)	2.6	2.2	3.0
Length of basal leaves (mm)	18.2	13.9	22.2
Width of basal leaves (mm)	14.0	10.6	16.0
Length of upper leaves (mm)	32.2	25.8	42.2
Width of upper leaves (mm)	7.8	5.6	10.2
Length of open flower (mm)	18.8	15.4	20.6
Width of open flower (mm)	15.2	12.2	17.6
Capsule diameter (mm)	5.1	4.9	5.2
Seeds per capsule	44.8	32	58

duction and seed dispersal in subsequent generations.

The information reported in this study concerns basic morphological traits of *Viola arvensis* as a guide for correct identification of this species. Our observations indicate that the species is rather easy to identify since there are adequate diagnostic characters in morphology, even for non-experts. Complicating factors could be the occurrence of hybrids (as reported in the literature) and the unpredictable phenological plasticity of individuals growing outside their normal habitat. Supplementary research is under way to assist in acquiring detailed knowledge on the biology of the species and in the selection of appropriate weed control practices.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Εμφάνιση του κοινού αγριοπανσέ (*Viola arvensis*) στην Ορεστιάδα

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Περίληψη Ο κοινός αγριοπανσές (*Viola arvensis* Murray), ένα ετήσιο πλατύφυλλο ζιζάνιο, εμφανίζεται με αυξανόμενη συχνότητα τα τελευταία έτη σε καλλιεργήσιμη γη στο αγρόκτημα του Δημοκριτείου Πανεπιστημίου Θράκης στην Ορεστιάδα. Φυσικοί πληθυσμοί του ζιζανίου παρατηρήθηκαν σε πυκνότητες που κυμαίνονταν από 5 έως 20 φυτά ανά m^2 συνηθέστερα σε ακανόνιστες κηλίδες, στις άκρες (περιθώρια) αγρού με καλλιέργεια μαλακού σίτου και σε σημεία του αγρού με χαμηλή πυκνότητα φυτών, σε καλλιέργειες ανοιξιάτικων ψυχανθών, όπως φακές, κουκιά, λούπινο και λαθούρι, καθώς και σε τμήματα των αγρών χωρίς καλλιέργεια. Η εμφάνιση του είδους στην περιοχή πιθανώς σχετίζεται με την απουσία χημικού ελέγχου των ζιζανίων (μη χρήση ζιζανιοκτόνων) σε συνδυασμό με την αυξημένη διαθεσιμότητα αζώτου στο έδαφος που ευνοεί τη συνολική παραγωγικότητα των φυτών (π.χ. συσσώρευση βιομάζας, παραγωγή και διάδοση σπόρου) στις επόμενες γενεές. Σε αυτή την αναφορά, παρουσιάζονται βασικά μορφολογικά χαρακτηριστικά του ζιζανίου σε διάφορα στάδια του βιολογικού κύκλου.

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