POPULATION STUDIES ON *PLASMOPARA HALSTEDII*: HOST SPECIFICITY AND FUNGICIDE TOLERANCE

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Summary

Plasmopara halstedii (PH) goes through its sexual cycle regularly allowing the fungus to vary. In fact, new variants of PH including those with new virulence characters and metalaxyl-tolerant strains have been described in different countries. The aim of this review article was to demonstrate how diverse the Hungarian PH population could be in respect of host specificity (pathogenicity, virulence, agressiveness) and metalaxyl tolerance, as compared to other European and overseas countries.

Using classical approaches we described some of the above mentioned variants, originating either from cultivated sunflower or from other composites. In addition, attempts were made to use biochemical/molecular methods for further characterization of such variants.

Host specificity of PH has been found to be wider than was expected, *Ambrosia artemisifolia* having been considered as a natural host in Hungary. Significant differences have been found in aggressiveness between PH isolates belonging to different pathotypes and they did compete with each other in mixed inoculations. Fungicide sensitivity tests completed so far did not verify any metalaxyl tolerance from the field. Single sporing permitted us to select, by chance, of clones with distinct virulence formula and these became the subject of biochemical and molecular characterization. These experiments are still in progress.

Introduction

Plasmopara halstedii (Farlow) Berlese et de Toni (Oomycota, Peronosporales) (PH) is a fungal pathogen causing the downy mildew disease of sunflower worldwide. PH goes through its sexual cycle regularly, allowing the fungus to vary by means of sexual recombination. In fact, with the introduction of selecting pressures, like R-gene mediated resistance in sunflowers or the regular use of the fungicide metalaxyl, an increasing number of new variants of PH have been evolved. To date, at least 17 pathotypes (pathogenic races) each with distinct virulence character have been identified (Gulya et al., 1996; Penaud, 1998; Molinero-Ruiz et al., 1998), and numerous metalaxyl-tolerant strains have been described (Lafon et al., 1996; Albourie et al., 1998; Gulya et al., 1999; Molinero-Ruiz et al., 1999).

Although there has been a contradiction in the literature concerning host specificity of PH, it seems likely that the present day fungal population existing in most countries has been evolved in several steps: it started as a complex biological species living on a wide range of composites and became a serious pathogen confined to cultivated sunflower. The fungus, however, appeared to retain, in part, its original pathogenicity to members of the Asteraceae (Virányi, 1984, 1998).

During the past decade, PH underwent a dramatic change in its virulence and by now at least 17 different pathotypes, each with a distinct virulence character have been evolved (Gulya et al., 1996; Molinero-Ruiz et al., 1998; Tourvieille, 1999). The global distribution of these pathogenic forms is shown in Table 1. As pathogenic diversity within PH populations worldwide appeared to increase, efforts have been made to standardize the identification and designation system. As a result of a meeting held in Fargo in 1998, a standard set of sunflower differentials (Gulya et al., 1998), as well as a new designation of virulence phenotypes (Gulya (1995) have been accepted for use by any sunflower pathologist.

In comparing PH isolates belonging to different pathotypes, Gulya and Masirevic (1988) found there were isolates exhibiting substantially more aggressive character as they caused more infection and stunted seedlings to a greater degree than did isolates of different virulent phenotype. Such a difference among field isolates of PH have also been found in Hungary even at a time when virulence within the fungal population was considered to be uniform (Virányi, 1984).

It was in 1979, that APRON, a seed dressing formulation of metalaxyl, developed by Ciba Geigy, had been introduced in Hungary as a seed treatment against the early infection by PH. Subsequent years revealed that this compound was highly effective to protect sunflower seedlings from the disease and the fungicide has been registered in a series of European countries and also in America. Unlike other oomycete pathogens, PH retained its sensitivity to metalaxyl until recently, and this was probably due to the application method. In 1996, however, Lafon et al. (1996) reported of reduced sensitivity to this compound from some regions in France and something similar happened in the USA where the majority of field isolates tested were tolerant to APRON seed treatment (Gulya et al., 1999). Very recently, Molinero-Ruiz et al. (1999) in Spain also noted the appearance of such metalaxyl-tolerant PH variants.

Table 1.
Distribution of *Plasmopara halstedii* virulence phenotypes identified to date on a global scale

COUNTRY	VIRULENCE FORMULA														
	100	300	310	313	330	332	700	701	703	710	713	730	732	733	770	Others
EUROPE																
Bulgaria	X											X				
France	X	X					X	X	X	X			X	X		X
Germany	X											X			X	
Hungary	X	X			X		X			X		X				
Italy	X	X					X									
Romania	X	X			X											
Spain	X	X	X	X	X		X		X		X	X				X
former U.S.S.R.	X	X														
Yugoslavia	X						X					X				
AMERICA																
Argentina							X									X
Brazil		X														
Canada	X	X					X					X			X	X
U.S.A.	X	X			X		X			X		X			X	X
ASIA																
China	X	X					X									
India	X															
AFRICA																
Morocco	X				X	X	X			X		X	X			
R. South Africa	X	X					X						X			
Zimbabwe												X				

Note: Virulence formulas 100, 300, 700, 730, 770, 710, and 330 are correspond to the old pathotype designations 1, 2, 3, 4, 5, 8, and 9, respectively.

The pathotype identification *in planta* is rather handsome, space and time consuming, and the result may sometimes be uncertain if plant response to infection is not sufficiently uniform. This prompted researchers to find new and more precise methods of pathotype characterization by using biochemical or molecular methods. So far, just a few laboratories afforded to use such techniques in PH research. Borovkov and coworkers in the USA were the pioneers who first attempted to discriminate PH pathotypes by DNA probes (Borovkova et al., 1992). Years later a French group, using the RAPD technique, did not found genetic variability between pathotypes, although some amplified DNA fragments were specific to particular pathotypes (Roeckel-Drevet et al., 1997). However, the PCR appeared to be suitable for detecting PH in seed samples (Tourvieille et al., 1998), as was the case with another French group by using monoclonal antibodies for the detection of PH in sunflower seed (Bouterige et al., 1998).

Materials and Methods

PH isolates used in our experiments were collected from diseased sunflower plants in various regions of Hungary or from an other composite, *Ambrosia artemisifolia* that showed mildew-like symptoms. Maintainance, preservation, and inoculation of the isolates were made using classical methods, pathogenicity, and/or virulence tests followed the method described by Gulya et al., (1998), single spore isolations were made according to Bogár and Virányi (1999), and the metalaxyl-tolerance tests by using different methodologies (Oros and Virányi, 1987; Mouzeyar et al., 1995; Albourie et al., 1998; Gulya et al., 1999).

Results and Discussion

Host range. Microscopical observations of individuals of *Ambrosia artemisifolia*, collected in South of Hungary in 1998, revealed the co-existence of both PH and *Albugo tragopogonis* in the same leaf tissue. Furthermore, inoculations with washed-off sporangial suspensions were successful in susceptible sunflower (cv. GK-70) even some of the detached leaves had been preserved at -25^{o}C for a year prior to use (Walcz et al., 2000). To our best knowledge, this was the second record of the natural occurrence of PH on a wild composite, with positive re-inoculation onto cultivated sunflower. Very recently, Gulya (personal communication) found PH on mars-elder (*Iva xanthifolia*) in North America and the fungus from this natural host was pathogenic to sunflower as well. All these findings are in contrast to Novotel'nova's concept according to which the downy mildew pathogen of sunflower, called *Plasmopara helianthi*, is confined to *Helianthus annuus* and a few annual species of this genus (Novotel'nova, 1966).

The co-evolution of sunflower and PH provided the opportunity to discover resistance genes (Pl) in wild sunflower that could be transferred into cultivated sunflower. The frequency of Pl genes in the wild species is high, especially in the annual species (Seiler, 1998). These findings, however, contradict previous host range studies where four annual (*H. annuus, H. argophyllus, H. debilis, H. petiolaris*) and two perennial species (*H.divaricatus, H. grosseserratus*) appeared to be susceptible in WSI-inoculation tests (Virányi, 1984). The explanation of this contradiction would be that the virulence character of inoculum used at that time was not known, most probably being pathotype 1 (virulence formula 100).

Virulence. In Hungary, five different pathotypes (100, 700, 730, 710, 330) have been identified (Virányi and Gulya, 1995). Field surveys in the last two years could not detect new virulence phenotypes, and the majority of new isolations tested had a virulence formula of 700, with much less samples being either 730 or 710.
When the *Ambrosia* isolates of PH were inoculated on to a standard set of differentials (Gulya et al., 1998), their virulence formula was found to be 730 (Walcz et al., 2000).

Aggressiveness. In glasshouse experiments there were significant differences found among PH isolates for their pathogenic ability, either belonging to one or different virulence phenotypes (Kormány and Virányi, 1997). Even the reduced plant growth, a typical symptom of downy mildewed sunflower could differentiate between isolates with various aggressiveness. In mixed inoculations, disease severity not necessarily followed the ratio of the two partners, the more aggressive isolate retaining its relative dominance even being in minority. It is possible therefore that PH isolates diferring in aggressiveness can compete with each other in nature but this ability appears not to be confined to a particular virulence character as was concluded by Gulya and Masirevic (1988) in similar experiments.

Sensitivity to metalaxyl. Although reduced sensitivity to metalaxyl (tolerance or resistance to this compound) has already been described in several oomycete fungi soon after the introduction of this chemical into praxis, PH retained its sensitivity until recently, except that such tolerant strains could be detected under laboratory conditions (Oros and Virányi, 1984). First in France (Lafon et al., 1996), then in the USA (Gulya et al., 1999) PH field isolates tolerant to metalaxyl have been found and something similar happened also in Spain (Molinero-Ruiz et al., 1999). In Hungary, however, no reduced sensitivity could be detected, although the number of samples tested so far does not allow saying with certainty this phenomenon is lacking in our coutry. Tolerance to metalaxyl, and to other phenylamides is a big challenge for the sunflower industry prompting researchers in many countries to look for other, not necessarily fungicidal, means of control. Preliminary experiments on non-fungicidal control of PH have been made in several laboratories, where the use of potential biological antagonists (Sackston et al., 1992) or the treatment of sunflower with a plant activator (Almási and Virányi, unpublished) were the subject of investigations. Apart from these, the phenomenon of induced resistance could also have some potential in downy mildew control.

Molecular characterization. In order to characterize more precisely the various pathogenic forms of PH, and to be able to detect the pathogen from symptomless plant parts (e.g. seed), modern biochemical and molecular techniques started to be introduced in sunflower pathological laboratories. As for the most promising ones, methodologies based on DNA polymorphism have been initiated to use in the USA (Borovkova et al., 1992), in France (Roeckel-Drevet et al., 1997), and similar attempts have recently been made in Germany, Hungary and Spain (the latter three still unpublished). In addition, experiments using serological methods, like Mabs, provided positive results indicating such techniques might serve as inspection for the presence of PH in seed lots (Bouterige et al., 1998).

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