Volume 93 (1, Supplement) March 2011

Journal of Plant Pathology

Formerly *Rivista di patologia vegetale* established in 1892

An International Journal of the Italian Society for Plant Pathology

PATHOLUX

FIRST LUXEMBOURGIAN CONFERENCE ON THE IMPACT OF PLANT PATHOGENS ON FOOD QUALITY OF AGRICULTURAL CROPS AND WINE

Sponsored by

Fonds National de la Recherche Luxembourg

EDIZIONI ETS, Pisa, Italy

Poste Italiane S.p.A. - Spedizione in Abbonamento Postale - D.L. 353/2003 (conv. in L. 27/02/2004 n° 46) art. 1, comma 1 - DCB AREA CENTRO 1/Pisa.

ISSN 1125-4653

The **Società Italiana di Patologia Vegetale, SIPaV, (Italian Society for Plant Pathology)** was established in 1992 following the dissolution of the Italian Society for Crop Protection (SIF) and the Italian Phytopathological Association (AFI). Its main aims are to promote research into different branches of plant pathology, to disseminate knowledge about plant diseases and their aetiological agents and to promote cooperation among experts working in the field of plant pathology, and partnership in fundamental and applied reasearch. The Society organizes meetings, gathers and distributes information about plant diseases, and maintains cooperation with other national and international scientific organizations and with national and local administrative authorities on problems involving plant health management.

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EDITORIAL PREFACE

This special issue contains abstracts and full papers from the first Conference on the impact of plant pathogens on food quality of agricultural crops and wine (Patholux) held at Remich, Luxembourg, on November 22 and 23, 2010. The Conference was organized by senior scientists working in plant pathology and food safety in Luxembourg at the 'Centre de Recherche Public-Gabriel Lippmann'. The first day of the Conference was dedicated to the pathogens of agricultural crops, the second day to the pathogens affecting vines and wine quality.

Contributions to the agricultural section comprised the quantification of the damage inflicted by pathogens, the mechanisms they use to overcome plant defence response, the development and use of forecast models, factors affecting mycotoxin production, and molecular tools enabling us to understand and prevent quality losses.

Contributions to the grape/wine section focused on the infection mechanisms of fungi, the use of forecast models for integrated disease management, fungicide resistance phenomena as well as on the negative effects of fungal metabolites on wine quality.

The scientific and organizing committees of the Conference reviewed abstracts and manuscripts of the present special issue.

We are grateful for the insightful results gained during this conference, partly presented in this special issue, and would especially like to express our gratitude to all authors for their valuable contributions that had a determining role for the success of the Conference.

We further wish to thank Olivier Marquis, Solène Chavel-Schenk, Elisabeth Clot, Alexandra Dobrowolski, Boris Untereiner and Servane Contal for organizational support and the 'Fonds National de la Recherche Luxembourg' for financial support.

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IMPACT OF PLANT PATHOGENS ON FOOD QUALITY OF AGRICULTURAL CROPS AND WINE

DAY 1

FUNGICIDE SENSITIVITY OF *SEPTORIA TRITICI* FIELD ISOLATES IS AFFECTED BY AN INTERACTION BETWEEN FUNGICIDAL MODE OF ACTION AND TIME

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SUMMARY

Sensitivity of *Septoria tritici* towards the fungicides trifloxystrobin, folpet, chlorothalonil, propiconazole, prochloraz, tebuconazole, epoxiconazole, and prothioconazole was tested in a bioassay using strains isolated in 1999, 2004, or 2008 in the high-disease-pressure and high-fungicide-input area between the North and Baltic Seas. The percentage of sprays containing at least one demethylation inhibitor (DMI, azoles) was never lower than 60% at any given point of time in the region where the fungal strains were sampled. Quinone outer binding site inhibitor (QoI) use was almost negligible in 1999, increased to about 80% in 2002, and subsequently decreased to 4% in 2008. The bioassay accurately detected the total loss of sensitivity of *S. tritici* towards trifloxystrobin as a representative of the QoIs that was previously reported. DMI concentrations inhibiting fungal growth by 50% (EC_{50}s) decreased on the average by 48±17% between 1999 and 2004, and increased by 132±42% between 2004 and 2008 compared to 1999. EC_{50}s of the multi-site inhibitors folpet and chlorothalonil increased by 128±25% between 1999 and 2004, and decreased by 40±11% between 2004 and 2008, but changes over time were non-significant for the multi site inhibitors. The period of QoI use approximately coincided with a small but consistent decrease of EC_{50}s for all DMIs tested and with a small but consistent increase of EC_{50}s for the multi site inhibitors.

Key words: Demethylation inhibitors, fungicide resistance, *Mycosphaerella graminicola*, multi site inhibitors, quinone outer binding site inhibitors, *Triticum aestivum*.

INTRODUCTION

Septoria tritici Rob. ex Desm. [teleomorph: *Mycosphaerella graminicola* (Fuckel) Schroeter] is a fungal pathogen of cereals and the primary cause of leaf blotch in wheat, particularly in humid regions (Chungu *et al*., 2001). Yield losses caused by *S. tritici* were reported to range from 30 to 50% in western Europe (Royle *et al*., 1986; Burke and Dunne, 2006).

Control methods such as tillage, crop rotation, late sowing dates, or growing resistant cultivars are either almost ineffective or contribute only marginally to the solution of the problem caused by the disease (Thomas *et al*., 1989; Gladders *et al*., 2001). Commercially available cultivars with acceptable agronomic performance do not have adequate resistance (Palmer and Skinner, 2002). Therefore, current control strategies rely mainly on fungicides with applications focusing on the putative time of infection (Henze *et al*., 2007). Due to the lack of alternatives, fungicides were used rather frequently, with 2-4 applications per season in northern Germany (Klink, 1997; Busse, 2001), giving rise to selection of fungicide-resistant *S. tritici* strains. The degree of resistance depends upon the underlying mechanisms of action.

Qo inhibitors (QoIs, strobilurins), such as trifloxystrobin, bind to the Qo site of the cytochrome bc1 complex and thereby inhibit electron transport in the mitochondrial respiration of fungi (Gisi *et al*., 2002). Even if a QoI inhibits mitochondrial respiration, fungi may survive by producing energy via alternative oxidase (Wood and Hollomon, 2003). However, significantly less energy is provided by alternative oxidase compared to the regular mitochondrial pathway (Wood and Hollomon, 2003). A single-point mutation at position 143 from glycine to alanine confer resistance to Qo inhibitors in many fungi, including *S. tritici* (Torriani *et al*., 2009). Trifloxystrobin was introduced in 1998 (Russell, 2005).

Azoles such as epoxiconazole, propiconazole, prothioconazole, or tebuconazole and imidazoles such as prochloraz belong to the group of demethylation inhibitors (DMIs). Their mode of action is the inhibition of the demethylation at the 14-α carbon of lanosterol or 24-methylene dihydrolanosterol. The latter two compounds are substrates for the cytochrome P450-dependent 14-α demethylase in the biosynthesis of fungal sterols (Gisi *et al*., 2000). The gene coding for the 14-α demethylase was termed CYP51 or ERG11, and several point mutations were associated with a gradual loss of

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sensitivity of fungi towards DMIs (Leroux *et al*., 2007). Furthermore, experimental evidence suggests that ABC (ATP-Binding Casette)-transporters located in the outer membrane of fungal cells are involved in fungicide resistance of *S. tritici* by exporting the toxic compounds (Stergiopoulos *et al*., 2003). DMIs were first introduced in the 1970s (prochloraz 1977, propiconzole 1979, tebuconazole 1986, epoxiconazole 1990, and prothioconazole 2002; Russell, 2005).

In contrast to DMIs and QoIs, folpet and chlorothalonil are non-systemic fungicides that are not taken up by the plant in significant amounts (Tomlin, 2000). They belong to the group of multi-site inhibitors, which interfere with various steps of fungal metabolism (Tomlin, 2000). Folpet and chlorothalonil were introduced in 1952 and 1964, respectively (Russell, 2005).

Northern Germany is a region with high yield/cropping potential with an average yield of about 9 tons wheat per ha (Stratmann *et al*., 2004). For comparison, wheat yield was reported to be about 3 tons per ha for the USA (OSU, 2007). The high levels of yield result from fertile soil and sufficient rain on the one hand but also from high inputs of fertilizers, pesticides, and labor on the other hand. However, regular precipitation favors the development of fungal populations in the field and results in high disease pressure in most years (Ceynowa *et al*., 1993; Verreet *et al*., 2000). Since fungicide resistance probably develops in regions with high application frequencies and low doses, fungicide resistance monitoring and resistance management is particularly important in these regions (Staub, 1991). Since the breakdown of QoI efficacy, control of *S. tritici* depends almost solely on azoles. Resistance management by alternating effective fungicides with different modes of action is therefore extraordinarily difficult with this pathogen.

It was the objective of the present study to determine the recent trend in sensitivity of *S. tritici* toward selected systemic azoles, a strobilurin and non-systemic contact fungicides in a region with high yield and frequent use of fungicides.

MATERIALS AND METHODS

Fungal strains. Wheat plants (susceptible cv. Ritmo) with typical *Septoria tritici* necroses were sampled at growth stages 21-32 (Zadoks *et al*., 1974) in Northern Germany (federal state Schleswig-Holstein, area between 53.70 and 54.38°N latitude and 8.83 and 10.88°E longitude) in 1999, 2004, and 2008. Plants were stored at -20°C until further analysis. After defrosting, leaves were inspected for *Septoria tritici* pycnidia under a binocular microscope and leaf pieces (approximately 2 cm in length) with pycnidia were incubated in Petri dishes on water agar (1.5% [w/w]) for 24 h at room temperature. Pycnidia discharged spore slime during the incubation period and the spores were diluted with sterilized deionized water and transferred to plates containing malt yeast extract agar (MYA, yeast extract, glucose, malt extract, each component at 4 g l^{-1} plus 15 g l^{-1} agar). Colonies originating from a single spore were transferred to new MYA plates and the spores produced on that plate were washed off using 10% [w/v] sterile skim milk and stored at -70°C until further use. Spores used in the bioassays described below always originated directly from the stock solution stored at -70°C. Fungal strains transferred from plate to plate quickly ceased spore production and grew as mycelium which was less suitable for our bioassay due to its heterogeneity in liquid media compared to the spores. Spores from the stock solution were transferred to MYA plates and the liquid was distributed over the medium surface using a sterile triangle-shaped metal spreader rod. After 4 days at room temperature, propagation of the spores resulted in spore clusters that were washed off using sterile deionized water and the spore concentrations of the solutions were determined using a Fuchs Rosenthal haemocytometer and a light microscope. The optical density of the same solutions was measured (Fluorescence, absorbance and luminescence reader, model Genios, Tecan Group, Switzerland) in the absorbance mode at 595 nm with a shaking period of 5 sec prior to measurements. Solutions for bioassays were diluted to approximately 500,000 spores per ml by measuring the optical density of a subsample of the spore suspension using the relationship depicted in Fig. 1. The number of isolates available for our study were 7, 2, and 7 in 1999, 2004, and 2008, respectively.

Bioassays. Fungicides were either purchased from Sigma-Aldrich, Switzerland (trifloxystrobin), Fein-

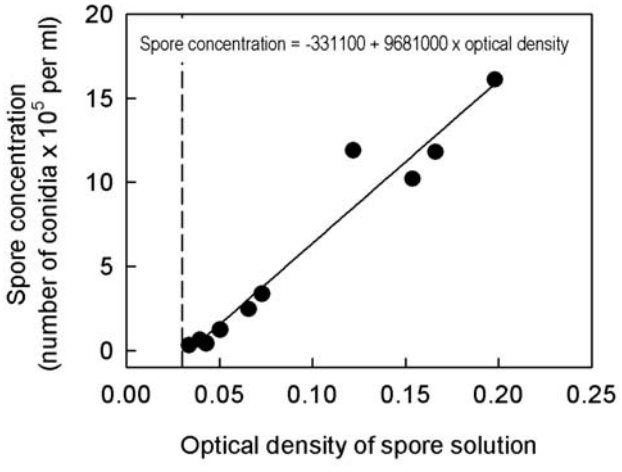

Fig. 1. Relationship between *Septoria tritici* spore concentration in deionized water and optical density [measured in absorbance (dimensionless) mode at a wavelength of 595 nm, with 200 µl in 96 microtiterplate wells]. The vertical dashed line indicates the optical density of an empty plate.

chemie Schwebda GmbH, Germany (folpet, chlorothalonil, propiconazole, prochloraz, tebuconazole, epoxiconazole), or Bayer CropScience, Germany (prothioconazole). The chemical purity was >93%. Fungicides were dissolved in ethanol except for epoxiconazole, where 10% [v/v] ethylacetate were added. The average fungicide stock solution concentration was 2.5 mg ml^{-1}. Fungicides were transferred into the wells of sterile, clear, flat bottom 96 microtiter plates resulting in final concentrations of 50, 25, 5, 2.5, 0.5, 0.05, 0.005, 0.0005, and 0% of the stock solution. Solvents were allowed to evaporate overnight. Finally, 100 µl of spore suspension (treatment) or 100 µl water (control) and 100 µl of medium (MYA + 200% glucose without agar)

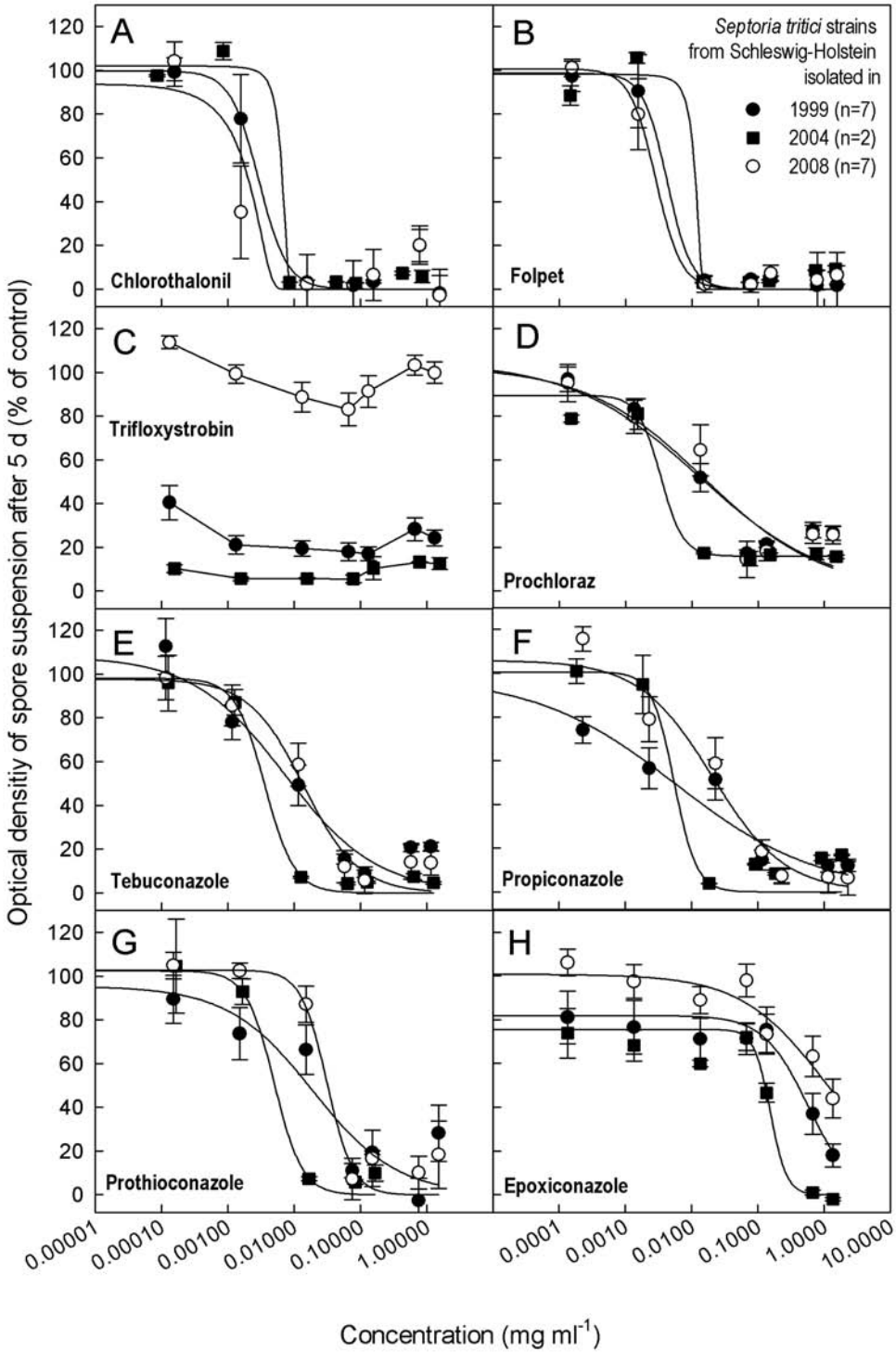

Fig. 2. Relationships between optical density (measured in absorbance mode at 595 nm) and fungicide concentration for *Septoria tritici* strains isolated in 1999, 2004, or 2008. All data are expressed as percentage of the untreated control. Data are presented as median ± SE.

were transferred into each well. All experiments were conducted with four replicates. The gap between microtiter plates and lids was sealed with parafilm and plates were incubated on an orbital shaker at 120 rpm, 22°C, and an 8-h photoperiod for 5 days. Subsequently, the optical density of the spore suspensions and the respective control liquids was determined using the absorbance reader mentioned above.

Statistics. Optical densities were corrected for the absorbance of the microtiter plates, the medium, and the intrinsic colour of the fungicide. Data were expressed relative to the optical density of the untreated control. Medians and standard errors (SE) of the data originating from the strains isolated in the same year or for each individual isolate were calculated and plotted against fungicide concentrations. Fungicide concentrations reducing the optical density of the spore suspension by 50% (EC_{50}) were estimated using the sigmoid regression models of software package Sigmaplot 10.0 (Systat Software GmbH, Germany). Changes in EC_{50} over time were compared by Kruskall Wallis tests at $P < 0.05$ (2-sided). Effects of the interaction between time and fungicidal mode of action on EC_{50} were calculated by two factorial analyses of variance (ANOVA). Since EC_{50}s were characterized by outliers, data were log-transformed prior to ANOVA to obtain approximate Gaussian distributions. ANOVAs were carried out using the statistical software package SPSS 16 (SPSS Inc., USA).

RESULTS

Relationships between optical density of spore solutions and fungicide concentration could be described using sigmoid regression models with acceptable precision (Fig. 2). Coefficients of determination ranged from 0.88 to 0.99.

Effects of fungicides and year of fungal strain isolation as well as the interaction fungicide × year on EC_{50} values were significant at $P < 0.001$ (Table 1). Since it is quite obvious that the total breakdown of trifloxystrobin efficacy accounts for the largest difference in EC_{50} values, analyses were carried out for each mode of action group separately (Table 1).

The EC_{50} for chlorothalonil increased between 1999 and 2004 from 0.0029 mg ml^{-1} to 0.0061 mg ml^{-1}, then decreased to 0.0015 mg ml^{-1} in 2008 (Fig. 2A). The EC_{50} for folpet increased between 1999 and 2004 from 0.0043 mg ml^{-1} to 0.0105 mg ml^{-1} and then decreased again to 0.0029 mg ml^{-1} in 2008 (Fig. 2B). The fungal sensitivity towards folpet and chlorothalonil decreased between 1999 and 2004 (average EC_{50} increase by 128±25%), and increased until 2008 by 40±11% (compared to 1999, Fig. 3). However, differences between EC_{50} for the multi-site inhibitors chlorothalonil and folpet were not significant, irrespective of year (Table 1).

The EC_{50} for trifloxystrobin was <0.00013 mg ml^{-1} in 1999 and 2004, >0.4 mg ml^{-1} in 2006 (N. Scheider, personal communication) and >1.3 mg ml^{-1} in 2008. (Figs. 2C, 3A).

Effects of fungicide and year were significant at $P < 0.001$ and $P = 0.001$, respectively when azoles were compared (Table 1). The interaction between azole and year was non-significant at $P = 0.948$, indicating that azoles expressed a similar change in sensitivity over time (Fig. 3). Sensitivity of *S. tritici* towards triazoles in-

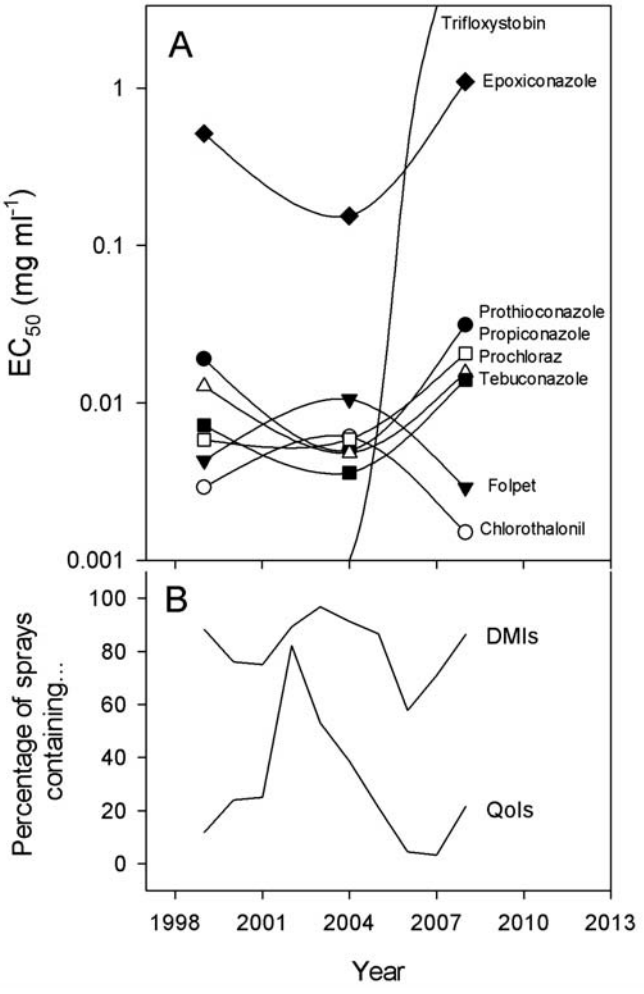

Fig. 3. A. Changes in fungicide sensitivity of *Septoria tritici* strains isolated in Northern Germany between 1999 and 2008. Sensitivity is expressed as the fungicide concentration suppressing fungal growth by 50% (EC_{50}). Note the logarithmic scaling of the y-axis. Data points represent medians. The number of isolates was n=7 in 1999, n=2 in 2004, and n=7 in 2008. The EC_{50} for trifloxystrobin was < 0.00013 mg ml^{-1} in 1999 and 2004, >0.4 mg m^{-1} in 2006 (N. Scheider, personal communication) and >1.3 mg ml^{-1} in 2008. B. Changes in DMI and QoI use in Northern Germany between 1999 and 2008. Data were extracted from the annual reports of plant pathology unit of the administrative bodies for rural regions in Husum, Kiel and Lübeck (ALR 1999-2008).

Table 1. Analyses of variance (ANOVAs) for the effects of fungicides and year of fungal strain isolation and the interaction between fungicides and years on EC_{50} values. EC_{50} values were log transformed prior to ANOVAs to limit the impact of outliers.

Fungicides included in analysis	Source	Type III sum of squares	df	Mean square	F	*P*
All	Fungicide (F)	44.73	7	6.39	24.28	<0.001
	Year (Y)	14.20	2	7.10	26.98	<0.001
	F × Y	57.81	14	4.13	15.69	<0.001
Azoles	Fungicide (F)	31.26	4	7.81	29.02	<0.001
	Year (Y)	3.98	2	1.99	7.38	0.001
	F × Y	0.73	8	0.09	0.34	0.948
Chlorothalonil,	Fungicide (F)	0.08	1	0.08	0.22	0.645
Folpet	Year (Y)	1.93	2	0.97	2.51	0.100
	F × Y	0.15	2	0.07	0.19	0.826

creased between 1999 and 2004, as indicated by an average decrease of EC_{50} values by 48±17%. Subsequently, sensitivity decreased, as indicated by an average increase in EC_{50} values by 132±42% (Fig. 3A). Prochloraz followed the same pattern as triazoles, but the decrease of EC_{50} values was somewhat higher between 1999 and 2004 (63 %) and the increase between 2004 and 2008 lower (22%). The EC_{50} for prochloraz decreased between 1999 and 2004 from 0.0128 mg ml^{-1} to 0.0048 mg ml^{-1}, then increased again to 0.0155 mg ml^{-1} in 2008 (Fig. 2D). The EC_{50} for tebuconazole decreased between 1999 and 2004 from 0.0072 mg ml^{-1} to 0.0036 mg ml^{-1} to increase again to 0.0140 mg ml^{-1} in 2008 (Fig. 2E). The EC_{50} for propiconazole was constant between 1999 and 2004 at 0.0058 mg ml^{-1}, then increased to 0.0205 mg ml^{-1} in 2008 (Fig. 2F). The EC_{50} for prothioconazole decreased between 1999 and 2004 from 0.0190 mg ml^{-1} to 0.0049 mg ml^{-1} to increase again to 0.0313 mg ml^{-1} in 2008 (Fig. 2G). The EC_{50} for epoxiconazole decreased between 1999 and 2004 from 0.5140 mg ml^{-1} to 0.1543 mg ml^{-1} and increased again to 1.0994 mg ml^{-1} in 2008 (Fig. 2H).

DISCUSSION

Resistance of microbial populations towards antibiotics usually increases with time until an antibiotic application is abandoned. Afterwards, the level of resistance will remain high, if resistant individuals do not suffer from a fitness penalty compared to non-resistant individuals. If the mechanism of resistance is, for instance, energy dependant, resistant individuals will waste energy for maintaining a resistance that is not needed any longer. In the latter case, the level of resistance will decrease again because non-resistant individuals use energy more efficiently.

Trifloxystrobin/QoIs. The bioassay used in the present study detected the total loss of sensitivity of *S. tritici* towards strobilurins that was reported previously by other authors in other regions. Gisi *et al*. (2002) reported that among thousands of tested *Mycosphaerella graminicola* isolates, no resistant isolates were found until 2001. QoI resistant strains were first reported from UK in 2002 (Fraaije *et al*., 2005). More recent data suggest that QoI resistance in *M. graminicola* emerged at least four times independently in European populations and that spread of resistant isolates occurred preferentially in west-to-east direction (Torriani *et al*., 2009). Our results suggest that the spread of strobilurin resistant isolates or a mutation occurred between 2004 and 2006 in northern Germany.

In 2002, about 80% of the fungicide sprays contained QoIs in a wheat disease monitoring program in northern Germany (Fig. 3B). After the breakdown of QoI efficacy, QoI use decreased to about 4% in 2006. Even though strobilurin use was abandoned almost completely, sensitivity did not recover until 2008, indicating that the QoI resistance is not coupled to a significant fitness penalty in *S. tritici* (Fraaije *et al*., 2003).

Azoles/DMIs. In the Fusarium head blight pathogen *Gibberella zeae* (anamorph *F. graminearum*), triazole sensitivity slowly decreased with time between 1999 and 2004 at an approximately constant rate (Klix *et al*., 2007). Analysis of amino acid sequences deduced from DMI resistant *F. graminearum* strains suggested that mechanisms other than mutations of CYP51A and CYP51B were responsible for resistance (Yin *et al*.,

2009). In contrast, triazole sensitivity of *S. tritici* fluctuated (Table 1, Fig. 3A), suggesting that the factors affecting sensitivity development over time were different between *G. zeae* and *S. tritici*. *G. zeae* causes a monocyclic disease that is targeted by one spray per season at maximum, whereas *S. tritici* elicits a polycyclic disease targeted by 1 to 3 sprays per season. Hence, effects of fungicide regimes on sensitivity are likely to become obvious in a shorter period of time in *S. tritici* than in *G. zeae*. Furthermore, mycotoxin levels associated with Fusarium head blight pathogens were hardly reduced by QoIs (Chala *et al*., 2003), and, therefore, azoles were preferentially applied against *Fusarium* species, whereas QoIs were largely avoided, resulting in a moderate but permanent selective pressure of DMIs on *G. zeae* with hardly an interference by QoIs.

Leroux *et al*. (2007) reported that some mutations in the CYP51 gene were associated with changes in sensitivity of European *S. tritici* strains towards DMIs. Data by Brunner *et al*. (2008) suggest that CYP51 mutations conferring partial resistance towards azoles probably arose in Denmark or UK and then spread towards eastern Europe. Furthermore, no CYP51 mutations conferring partial resistance towards azoles have been found in non-European populations so far (Brunner *et al*., 2008). Stammler *et al*. (2008) screened 615 strains sampled in 2007 throughout Europe for their sensitivity towards epoxiconazole. They found no consistent relationship between CYP51 haplotypes and sensitivity. In our study, less isolates from the same region were tested, but over a longer period of time, allowing us to study the effects of temporal factors on fungicide sensitivity, largely unbiased by spatial factors. A general interpretation of our results focusing on the effect of the composition of fungicide sprays on sensitivity over time is given below.

General interpretation. Changes of fungicide sensitivity over time were characteristic and very consistent for each group of fungicides having the same mode of action (Fig. 3). In 1999, DMIs were used on a routine basis against *S. tritici* in northern Germany (Fig. 3B). In subsequent years, QoIs use increased dramatically (Fig. 3B), such that the selective pressure on *S. tritici* was not exerted by DMIs alone. Despite approximately constant use of DMIs at high frequency (Fig. 3B), EC_{50} values for DMIs slightly decreased during the period of frequent QoI use until 2004.

First *S. tritici* isolates resistant towards strobilurins were found in UK in 2002 (Fraaije *et al*., 2005) resulting in a decrease of QoI use for *S. tritici* control in subsequent years. Hence, the fraction of selective pressure applied by DMIs increased again and is reflected by an increase of EC_{50} for azoles until 2008. Interestingly, prothioconazole followed the sensitivity pattern of azoles, even though it was not yet introduced to the market in 1999. Hence, at least a limited degree of cross resistance between (tri)azoles seems to exist in *S. tritici*. Given the continuous use of DMIs at high frequency and earlier reports on mutations of the DMI target gene CYP51 (Leroux *et al*., 2007), resistance phenomena observed in our study were surprisingly small, raising the question whether CYP51 is the only DMI target in *S. tritici*. Fungicides having a different mode of action such as folpet or chlorothalonil resulted in a different pattern of sensitivity development over time. Generally, development of resistance towards multi site inhibitors is considered to be more unlikely than development towards single site inhibitors, because several toxic mechanisms have to be circumvented by the target organism at the same time with multi site inhibitors, whereas only one mutation can confer resistance to single site inhibitors (Deising *et al*., 2008). Even though the changes in sensitivity were non-significant for the multi site inhibitors folpet and chlorothalonil in our study, the consistency of EC_{50} changes over time suggests that *S. tritici* populations have the potential to adapt also to multi site inhibitors on a long term perspective. Furthermore, EC_{50}s for multi site inhibitors slightly increased during the period of QoI use and decreased again afterwards. This phenomenon might be interpreted as evidence for a limited degree of cross resistance between trifloxystrobin and the multi site inhibitors, raising the question whether the multi site inhibitors used here may – at least partly – affect mitochondrial respiration, as trifloxystobin does.

The low number of strains available for 2004 from the particular region of interest was rather unsatisfying. However, the main effect (interaction between time and fungicidal mode of action) of this paper did not depend on the data from 2004. In fact, the effect of time × fungicide on $\log(EC_{50})$ (same analysis as given in Table 1) was significant at $P<0.001$ after exclusion of the data from 2004. Therefore, we decided not to exclude the data from the analysis and presentation.

Some of our EC_{50} values differ considerably from EC_{50}s reported earlier (see for instance Stammler *et al*., 2008). We allowed solvents to evaporate before starting the bioassay and used no additives or formulated ready-for-use fungicide products. Thus, fungicidal effects of solvents, additives, and effects of interactions between additives and fungicides such as improved uptake into the target organism can be ruled out here. This may have resulted in markedly higher EC_{50}s for some compounds in our study.

ACKNOWLEDGEMENTS

We thank Bettina Bastian for excellent technical assistance and Matias Pasquali for critical comments on an early version of the manuscript.

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TIMELY FUNGICIDE APPLICATION: A STRATEGY TO MINIMIZE FUSARIUM HEAD BLIGHT AND ASSOCIATED MYCOTOXIN PRODUCTION IN WINTER WHEAT

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SUMMARY

Re-emergence of Fusarium head blight (FHB) on wheat should be taken into account in the global management of cropped fields, especially with respect to fungicide application schemes, due to harmful toxin production. The aim of this study was to assess, in three experimental fields representative of the various topoclimatological zones of Luxembourg, the impact of timing of fungicide spray application on the prevalence and severity of FHB, the concentration of mycotoxins, and *Fusarium* strain pattern in winter wheat. It was found that fungicide treatments and the time of application had a significant impact on the amount of deoxynivalenol (DON) detected (P=0.027, ANOVA). In our experimental design, the application of fungicides at 3 different times increased the amount of DON in winter wheat compared to two and single applications. The importance of the timing of fungicide application is discussed in relation to limiting toxin contamination in the field.

Key words: *Fusarium spp.*, deoxynivalenol, cereals, chemical treatments

INTRODUCTION

Fungi of the genus *Fusarium* are of great economic significance due to their widespread occurrence and high pathogenicity to all crop species grown throughout the world. Fusarium head blight (FHB), mainly caused by *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, and *F. poae* can be devastating, with an overall decrease in yield reaching 70%. In addition, toxic secondary metabolites produced by *Fusarium* species can be present in contaminated FHB-affected grain (Bullerman and Bianchini, 2007; Dexter *et al.*, 1997; Pirgozliev *et al.*, 2003). The most common among them are trichotecenes, mostly of type B, which include deoxynivalenol (DON), nivalenol (NIV)) and zearalenone (ZON). The above substances exhibit very strong phytotoxic and zootoxic effects (Gutleb *et al.*, 2002; Rotter *et al.*, 1996; Minervini *et al.*, 2004).

FHB has been increasing in incidence and severity in recent years, due to the implementation of simplified crop rotation (in particular with respect to wheat and maize production) and the lack of effective fungicide control for *Fusarium*, thus resulting in the development of resistant strains and the absence of crop varieties resistant to the disease.

Fungicide control of FHB has proved non-constant, and conflicting evidence exists regarding the effect of mycotoxin accumulation in grains contaminated by *Fusarium* spp. (Pirgozliev *et al.*, 2002). Previous studies from Luxembourg showed that type B trichothecene contamination frequently occurrs in winter wheat (Giraud *et al.*, 2010) and could be predicted by genetic chemotyping (Pasquali *et al.*, 2010).

The aim of the present study was to assess the impact of the time of fungicide spray application on the concentration of mycotoxins in winter wheat, i.e. in the grains at harvest time. Efficacy of fungicide treatments as measured by FHB prevalence and the occurrence of *Fusarium* mycotoxins were assessed in three experimental fields. In addition, changes in *Fusarium* population composition were investigated by morphological and molecular methods.

MATERIAL AND METHODS

Three fungicide treatments were tested in each of three experimental sites (Burmerange, Reuler and Christnach), representative of the three topoclimatological zones of Luxembourg (south, north and center, respectively).

Treatments were assigned to experimental units, using a randomized complete block split-plot design with four replications for each sub-plot (each site was composed of 16 experimental units). Each sub-plot was 12 m^2 in size and wheat was harvested in early to mid-July at the southern site (Burmerange) and at the beginning of August at the northern site (Reuler), with a cereal

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Table 1. Fungicide treatment and spray application carried out in 2007 in the three experimental sites of Luxembourg. Growing stages were defined according to the BBCH scale (BASF, Bayer, Ciba-Geigy and Hoechst, Landshare *et al.*, 1991).

Experimental code	Stages of fungicide application	Fungicide treatment	Burmerange		Christnach		Reuler	
			Growth stage	Date	Growth stage	Date	Growth stage	Date
T 0	control	no fungicide application						
T 1	EC 59	1.6 l/ha Input pro set + 1l/ha Bravo	62	23/05/07	57	23/05/07	65	05/06/07
T 2	EC31	0.75l/ha Opus team + 1l/ha Bravo	31+	17/04/07	31	17/04/07	30+	18/04/07
	EC59	1.6 l/ha Input pro set + 1 l/ha Bravo	62	23/05/07	57	23/05/07	65	05/06/07
T 3	EC31	0.7l/ha Stereo + 1 l/ha Bravo	31+	17/04/07	31	17/04/07	30+	18/04/07
	EC37	1.6 l/ha Input pro set + 1 l/ha Bravo	37	03/05/2007	37	03/05/07	37	10/05/07
	EC59	0.75l/ha Opus team + 1 l/ha Bravo	62	23/05/2007	57	23/05/07	65	05/06/07

plot combine harvester. Patterns of fungicide treatment were associated with wheat growth stages, and the products used were commercially available (Table 1).

The products Input pro set, Bravo, Opus team, and Stereo contained the active ingredients prothioconazole (250g/l) and spiroxamine (500g/l); chlorothalonil (500g/l); epoxiconazole (84g/l) and fenpropimorphe (250g/)l; cyprodinil (250g/l) and propiconazole (62.5g/l), respectively. Fungicide treatments were not only oriented toward FHB but were aimed also at controlling other leaf and ear diseases.

Prevalence (percentage of infected spikes) and severity (percentage of infected kernels per spike) of the disease, species determination, DON, NIV and ZON quantifications were assessed according to Giraud *et al.* (2010) and Pasquali *et al.* (2010).

Statistical analyses were carried out using SPSS 16.0 (Chicago, USA). Normality of data was tested with Q-Q plots and Kolmogorov-Smirnoff tests, equality of variance by Box-plots and Levene's test. For further analyses, data were transformed by the root mean square operation. Comparison of prevalence and severity between different areas was carried out in a linear mixed model using prevalence, severity, and DON concentration as dependent variables, and location and field site as independent variables. In a second model, differences between the timing of spray application and *Fusarium* strain composition were assessed for the various locations, with the species *Fusarium* as the dependent variable and treatment and place as the observed variable. Post-hoc tests (Tukey's) were carried out given significant results following the Fisher-F test. *P*-values below 0.05 (2-sided) were considered as significantly different. For correlations between previous crops and FHB, Spearman correlation coefficients were obtained. Unless otherwise stated, all values represent mean±SD (standard deviation).

RESULTS AND DISCUSSION

FHB prevalence was significantly different between locations, considering all treatments, Christnach (center) showed the highest prevalence, followed by Reuler (north) and Burmerange (south, P<0.001, Fisher-F-Test). (Table 2).

Severity (Table 3) was likewise significantly different between the locations: considering all treatments. Severity was significantly higher in Burmerange (south) compared with both Reuler and Christnach (P<0.001).

Toxin analyses revealed that 100% of the investigated experimental fields were contaminated by DON, with a range of 261-1,588 µg/kg. All three sites were significantly different from one another (Table 4), with Christnach (center) showing the highest contamination, followed by Burmerange (south) and Reuler (north).

Chemical treatments and timing of spray application had a significant impact on the amount of DON detect-

Table 2. Prevalence (% infected wheat spikes) in the 3 experimental sites for 2007. The data are based on the mean of 16 assessments for each condition (4 observations for one replication, 4 replications for one condition).

Experimental code	Time of spray application	Prevalence (mean ± SD)		
		Reuler	Christnach	Burmerange
T 0	Control	10.6±4.4	24.6±21.8	0.9±0.9
T 1	59	8.2±3.4	16.4±17.3	1.1±0.8
T 2	31 + 59	5.9±2.6	9.5±8.3	0.5±0.6
T 3	31+37+59	7.8±3.6	13.2±11.9	0.7±0.8

Table 3. Severity (% infected grains per spike) in the 3 experimental sites for 2007. The data was based on the mean of 16 assessments for each condition (4 observations for one replication, 4 replications for one condition).

Experimental code	Time of spray application	Severity (mean ± SD)		
		Reuler	Christnach	Burmerange
T 0	Control	21.6±10.8	20.4±13.8	31.3±36.4
T 1	59	21.4±10.1	18.3±11.3	40.3±34.8
T 2	31 + 59	18.8±9.0	11.2±8.4	27.1±40.3
T 3	31+37+59	22.1±10.9	15.4±11.0	28.2±36.4

Table 4. DON content determined in wheat samples collected from the 3 sites. Data based on means of 3 independent wheat sample analyses. Level of quantification (LOQ) was 76µg/kg.

Experimental code	Location	DON (µg/kg dry weight)					
		Reuler		Christnach		Burmerange	
	Time of spray application	Average	Range	Average	Range	Average	Range
T 0	Control	261	(218-299)	1588	(933-2657)	783	(615-968)
T 1	59	136	(65-265)	808	(425-1134)	614	(479-853)
T 2	31 + 59	283	(92-565)	1465	(818-1919)	693	(493-1074)
T 3	31+37+59	398	(167-871)	934	(432-1244)	857	(571-1253)

ed (P=0.027, ANOVA). The single treatment (EC59) showed a trend toward lower DON concentrations compared with the untreated plots (control, P=0.096), and differed significantly from the three applications (P<0.05). NIV was only detected in one location, Christnach (Table 5). The highest concentration was detected in the plots where three applications were employed. ZON was not detected.

Results of the morphological and molecular identification showed that the most common species isolated from diseased winter wheat spikes collected in the three experimental sites (Fig. 1) were *F. avenaceum* (41.3%) *F. culmorum* (37.9%), *F. graminearum* (16.4%) and *F. poae* (4.4%). *F. avenaceum* is known for its ability to produce moniliformine while the other species detected are known to be potential trichotecenes producers (e.g. DON and NIV).

The distribution of *Fusarium* species varied strongly from location to location. *F. avenaceum* was the predominant isolated species in Reuler (68.9%) while *F. culmorum* was the strain with the highest incidence in Christnach and in Burmerange (45.4% and 48.3%, respectively). *F. graminearum* was significantly more prevalent (%) in Christnach compared with other locations (P<0.001). In terms of treatment, the control resulted in the highest prevalence of *F. graminearum*

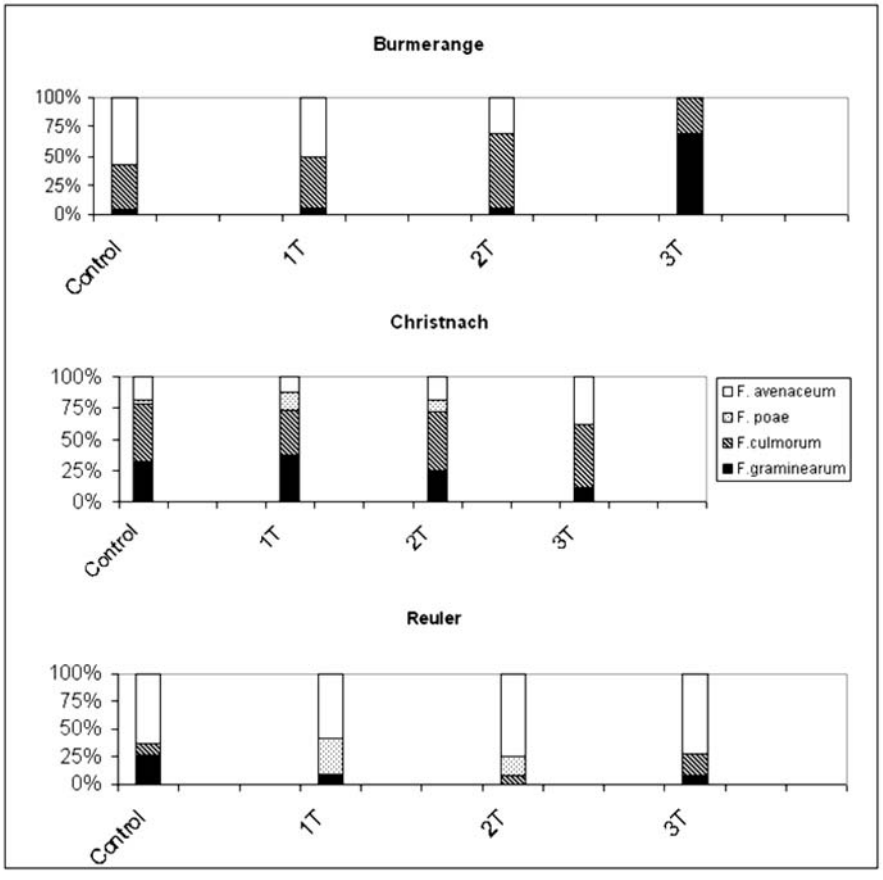

Fig. 1. Percentage of species found in each site and parcel according to the 4 l treatments (control, 1T for a single treatment at GS 59, 2T for 2 treatments at GS 31 and GS 59 and 3T for 3 treatments at GS 31, GS 37 and GS 59).

Table 5. NIV content determined in wheat samples collected from the Christnach site. Data based on means of 6 analyses. Level of quantification or LOQ was 76µg/kg (determined for for DON and estimated for NIV) in Luxembourg. Even though a trend was found for differences between various treatments effecting NIV (P=0.061), this did not reach significance.

Experimental code	Time of spray application	NIV (µg/kg)	
		Average	Range
T 0	Control	409	(237-569)
T 1	59	308	(194-490)
T 2	31 + 59	279	(126-421)
T 3	31+37+59	445	(351-559)

(F<0.001) compared with all other groups, which differed not significantly. *F. culmorum* was more prevalent in Burmerange, compared to Christnach and Reuler, with all three places being significantly different from one another (P<0.05). Treatment 2 resulted in highest prevalence of *F. culmorum*, significantly different from all other treatments, including the control (Fig. 2), with the sequence 2, control, 1, 3. *F. poae* was most prevalent in Christnach followed by Burmerange followed by Reuler, with all three locations being significantly different from one another (P<0.005). Treatment 2 was associated with the highest prevalence of this species, followed by treatment 1, control and 3, with only the latter two being non-significantly different from one another, while all others were (P<0.005).

Changes in the composition of the *Fusarium* population were observed according to the number of fungicide applications sprayed in the experimental sites. In Burmerange, three applications significantly increased the percentage of *F. graminearum,* while this type of treatment seemed to favor the *F. culmorum* population in Christnach. In theses two locations, the three treatments increased the proportion of fungi with the ability to produce trichothecenes. In the north (Reuler), the situation was different for the treatment applied at three stages increased significantly the proportion of isolated *F. avenaceum*, which does not produce trichotecene mycotoxins.

In our experimental design, one surprising result was the negative impact of three applications on the amount of DON in winter wheat. A similar result was observed in the case of NIV contamination, although it did not reach significance. A general possible explanation is that the repeated, multiple treatments increase the pathogen's stress, resulting in a higher toxigenic response (Reverberi *et al.*, 2010) as recently observed in the laboratory (Audenaert *et al.*, 2010).

In conclusion, the results have shown that multiple treatments at several growth stages could result in increased infection by *Fusarium* species, resulting also in increased DON production. However, specific influences due to different region, such as climate, gave variable results with respect to the impact of fungicide application and the effect of *Fusarium* strain population. It is apparent that management strategies based on fungicide application should take into account also the effect that chemical treatments may have on toxin induction by *Fusarium* species.

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ESTIMATES ON THE IMPACT OF DISEASES AND DISEASE CONTROL IN ARABLE CROPS*. E.C. Oerke. *Institute of Crop Research and Resource Conservation - Phytomedicine, Rheinische Friedrich-Wilhelms-Universität Bonn, Nussallee 9, 53315 Bonn, Germany. E-mail: ec-oerke@uni-bonn.de*

Crop losses due to pathogens and other harmful organisms can be substantial and may be prevented or reduced by various control options, e.g. host plant resistance, physical, biological, and chemical control. For the period 2002-04, the potential losses due to pathogens, i.e. fungi, chromista, bacteria and viruses in worldwide production of 11 crops (barley, cotton, maize, oilseed rape, peanut, potato, rice, soybean, sugar beet, tomato, and wheat) was estimated at 15.8 and 3.8% of attainable production, respectively, compared to 34% and 18% for weeds and animal pests. The role of pathogens in production varies with crop, geographic region, and the intensity of production. The efficacy of disease control, i.e. the proportion of loss potential prevented by direct control methods varied from 71% in high-input crop production in north-west Europe to <10% in low-input systems. It heavily relies on the use of synthetic chemicals and reached an overall average of 33%, considerably lower than the efficacy of weed control (75%) and animal pest control (40%). Actual losses to pathogens ranged from 7.2% in cotton to 17.7% in peanut, and from 5.2% in north-west Europe to 18.4% in west Africa, respectively. Minor crop losses may be economically acceptable. An increase in crop productivity needed to meet the rising demand of global population, however, is only reasonable with an adjustment of disease control intensity, because increases in attainable yields often are associated with higher susceptibility to pathogens and higher yield losses.

* *Invited keynote lecture*

POWDERY MILDEW INDUCED TRANSCRIPTION FACTORS HVWRKY1 AND –2 MEDIATE COMPATIBILITY AND REPRESS THE DEFENSE RELATED GENE HVGER4C IN BARLEY. D. Liu, G. Langen and K.H. Kogel. *Institute of Phytopathology and Applied Zoology, Justus-Liebig-University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germay. E-mail: karl-heinz.kogel@agrar.uni-giessen.de*

Biotrophic pathogens have to suppress different layers of plant defense responses for successful infection. To accomplish this, pathogen effector molecules manipulate host immunity. In barley, the expression of *HvWRKY1* and *HvWRKY2* is strongly induced during the early stage of infection by powdery mildew fungus (*Blumeria graminis* f.sp *hordei, Bgh*). Transient over-expression of *HvWRKY1* and *HvWRKY2* via particle bombardment enhanced susceptibility of epidermal leaf cells to *Bgh*, whereas artificial microRNA-based silencing of both genes reduced the *Bgh* penetration rate. These data indicate their role as susceptibility factors for powdery mildew. *HvGER4c*, a member of the germin-like GER4 gene cluster, exhibits high transcript abundance in powdery mildew infected barley leaf epidermis and contributes to plant basal defense. The promoter of *HvGER4c* contains multiple putative WRKY factor binding sites (W-boxes), thereof at least 4 positively regulate promoter activities after pathogen attack. Overexpression of *HvWRKY1* and *HvWRKY2* in barley epidermal cells represses the *Bgh* induced expression of a *HvGER4c* promoter: reporter construct. This demonstrates a mechanism, how the *HvGER4c* expression is negatively regulated by the transcriptional repressors *HvWRKY1/2*, which might be target genes activated by powdery mildew effectors to suppress host defence.

INFLUENCE OF THE LEAF SPOT COMPLEX OF BARLEY ON YIELD QUALITY AND QUANTITY IN RELATION TO THE CLIMATICALLY AND ECONOMICALLY CHANGING SITUATION AS A BASIS FOR INTEGRATED PEST MANAGEMENT. M. Hess[1], M. Nyman[1], H. Hausladen[1], S. Weigand[2] and R. Hückelhoven[1]. *[1]Technische Universität München, Lehrstuhl für Phytopathologie, Emil-Ramann-Strasse 2, 85350 Freising, Germany. [2]Bayerische Landesanstalt für Landwirtschaft, Institut für Pflanzenschutz, Lange Pont 10, 85354 Freising, Germany. E-mail: m.hess@lrz.tum.de*

The leaf spot-complex of winter and spring barley has become a widespread presence in European barley production for several years. It is an increasing problem and has developed into a yearly phenomenon on barley crops in Bavaria. It is regarded as one of the main reasons for reduction in yield quantity and quality. The exact cause of this leaf spotting is intensively investigated, and a complex of several factors is thought to be involved, mainly climatic stress, cultivar-specific physiological reactions to the environment, and the fungal pathogen *Ramularia collo-cygni*. The aim of a joint project between the Bavarian State Research Centre for Agriculture and the Technische Universität Münnchen is to employ fundamental research on host and parasite biology, epidemiological studies and crop monitoring in cooperation with Bavarian authorities, to try to elucidate the primary cause of the spotting complex. Methods for optimum control according to integrated pest management will be developed and long established decision support systems such as the "Gerstenmodell Bayern" will be improved and updated. The results from this research will give indications on the effects of climatic change on the changing patterns of barley pathogens looking for the reason for the increasing occurrence of formerly less important diseases late in the season, including the leaf spot complex. During monitoring, *Ramularia collo-cygni* could be detected at all sites. Despite the early, presymptomatic detection with sensitive molecular methods, onset of epidemics appeared late in the season with heavy symptoms and sporulation on the top leaves, suggesting a long latency and endophytic phase of the pathogen. The production of a photodynamic toxin by the fungus and the heavy symptoms on the upper leaves, which are directly exposed to light, suggest an important role of radiation, but it remains difficult to relate environmental factors directly to the observed epidemics. Trials with different varieties and sowing dates showed a significant role of plant development and growth stage on disease development. The impact on yield in winter and spring barley was evaluated with field trials by employing several treatments, including control measures specifically targeted at the leaf spot complex. Better control of the leaf spot complex generally improved yield, showing considerable variation between trials and sites. Special focus will be given on the quality parameters, especially regarding their impact on the economically important malting barley.

SITE–SPECIFIC MONITORING FOR DISEASE FORECASTING IN WINTER WHEAT. M. El Jarroudi[1], F. Giraud[2,3], P. Delfosse[2], M. Beyer[2], L. Hoffmann[2], H. Maraite[4] and B. Tychon[1]. *[1]Université de Liège, 185 Avenue de Longwy, 6700 Arlon, Belgique. [2]Centre de Recherche Public-Gabriel Lippmann, Département Environnement et Agro-biotechnologies, 41 Rue du Brill, 4422 Belvaux, Luxembourg. [3]Present address: Staphyt/BIORIZON, Rue Magendie/Bordeaux Montesquieu, 33650 Martillac, France. [4]Unité de Phytopathologie, Université Catholique de Louvain (UCL), Croix du Sud 2/3, 1348 Louvain-la-Neuve, Belgium. E-mail: meljarroudi@ulg.ac.be*

In the Grand-Duchy of Luxembourg (GDL), winter wheat is

the most important cereal crop (14,597 ha) with an annual production of ca. 75,000 tons in 2008 (Le Portail des Statistiques du Grand Duché de Luxembourg, 2008). In the GDL, fungicide applications are an essential part of cereal crop management. Crop protection often relies on preventive fungicide applications, and small grain cereals are systemically protected with two or three foliar treatments. Environmental concerns and changes in the cost/revenue ratio for winter wheat are likely to increase the demand for more accurate identification of spraying needs. Integrated pest management requires that pesticides are only applied at particular infection stages, and when the pathogen has been correctly identified. Diseases that have become economically important are Septoria leaf blotch (SLB) caused by *Septoria tritici* Roberge in Desmaz., wheat leaf rust (WLR) caused by *Puccinia triticina* Eriks., wheat stripe rust (WSR) caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., wheat powdery mildew (WPM) caused by *Blumeria graminis* DC. f. sp. *tritici* Em. Marchal, and Fusarium head blight (FHB) mainly caused by *Fusarium graminearum* Schwabe. Control of the diseases caused by these pathogens has a high priority in minimizing yield losses. SLB is widespread in the GDL winter wheat and is considered to cause one of the most serious foliar diseases that farmers need to take into account when deciding upon fungicide application during stem elongation. The mechanistic model "PROCULTURE" has been validated to be correct in about 85% of all cases (El Jarroudi *et al.*, 2009a). The fungicide treatment recommended by the simulation model over the 2003 to 2009 period resulted in a better return of investment (80%) than the other single treatments tested, or as a double fungicide application [GS31 (growth stage, first node detectable) and GS 59 (emergence of inflorescence completed)] in the three experimental sites of Everlange, Christnach and Burmerange (El Jarroudi *et al*., 2010a; El Jarroudi *et al*., 2011a). Over the 2003-2009 period, at Reuler, located in the North, only in 2007 one treatment based on the *Septoria* risk simulation was recommended (El Jarroudi *et al*., 2010a; El Jarroudi *et al*., 2011a). A stochastic model was developed to predict the wheat leaf rust severity (El Jarroudi *et al*., 2010b; El Jarroudi *et al*., 2010c). During the 2004 to 2009 period, at four sites, the linear regression between simulated and observed values for *Puccinia triticina* was highly significant ($P<0.01$) and the coefficient of determination (R^2) explained 80 to 85% of the variability (El Jarroudi *et al*., 2010c). WPM appeared much earlier in the northern Ösling (GS 30, pseudostem erection) than in the southern Gutland (GS 39, flag leaf ligule visible (El Jarroudi *et al*., 2009b). Two major climatic factors favoured the 2003 and 2009 outbreaks, i.e. a daily mean temperature between 15 and 22°C and a relative humidity of at least 80% during May-June (El Jarroudi *et al*., 2009b). For the WSR, from 1999 to 2010, the difference between $Yr17^+$ (Yr17 resistance gene) cultivars and cultivars not possessing this gene was highly significant ($P<0.001$), with the highest severity being observed for the $Yr17^+$ cultivars (mean severity of 13%) compared to the $Yr17^-$ cultivars (0.2%) (El Jarroudi *et al*., 2011b). This new virulent pathotype appeared to be more aggressive on the $Yr17^+$ cultivars than on the $Yr17^-$ ones. A stochastic model based on Monte Carlo simulation methods was developed to determine the conditions conducive to WSR in GDL. Simulations of infection and latency data by this model are in very good agreement ($R = 0.92$, $P < 0.05$) with the observational data. Finally, a stochastic model was developed to predict FHB in the GDL. The linear regression between simulated and observed values was highly significant ($P<0.05$) and R^2 explained 75% of the variability. The model results are used in warning bulletins. The model has predicted the disease correctly between 2004 and 2010. The research group will pursue the development and fine-tuning of these models in order to provide a large service to the farmers' community in the domain of pest and disease control through environmentally friendly methods in the GDL and Belgium.

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MYCOTOXINS: THEIR OCCURRENCE AND THEIR THREATS TO HEALTH AND FOOD SAFETY*. R. Strange.

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Species of *Fusarium* and *Aspergillus* are notorious for mycotoxin production. Contamination of food for human consumption by mycotoxins often results from infection of crops in the field by these fungi as their growth and mycotoxin production may continue after harvest. Cereals such as wheat are particularly susceptible to infection by *Fusarium* species at anthesis. Anthers contain two compounds, choline and glycinebetaine, which alter the growth habit of the fungus, giving rise to longer leading hyphae at the expense of laterals, thus facilitating entry into the plant. Besides selecting cultivars of plants for low concentrations of choline and glycinebetaine, breeding plants for cleistogamy (anthers enclosed in the florets) would help eliminating or, at least, reducing infection, and subsequent mycotoxin contamination. An account of the various chemical classes of mycotoxins produced by *Fusarium* species will be given as well as their toxicity and carcinogenicity. Maize, cotton, peanuts, and tree nuts are often contaminated by aflatoxins, a group of compounds produced by *Asperillus flavus* and *A. parasiticus.* Peanuts are particularly susceptible when they are drought-stressed. A reason for this may be that one of the defence mechanisms of the plant, phytoalexin production, is severely compromised by drought. An account is also given of food safety and quality aspects of mycotoxin contamination and of the options for reducing their level in food destined for human consumption, including genetic modification.

**Invited keynote lecture*

FUSARIUM HEAD BLIGHT OF BARLEY. A. Linkmeyer, M. Heß, R. Hückelhoven and H. Hausladen. *Lehrstuhl für Phytopathologie, Technische Universität Weihenstephan, Germany. E-mail: a.linkmeyer@wzw.tum.de*

Fusarium head blight (FHB) is a widespread fungal disease of cereals including wheat (*Triticum* spp.) and barley (*Hordeum vulgare*), caused by different species of the genus *Fusarium*. Apart from direct yield losses, the most serious concern is the contamination of the grain with mycotoxins. In Germany, FHB is predominantly associated with wheat, *F. graminearum* and *F. culmorum* being the most common species. In recent years, an enhanced incidence of FHB was also observed in German barley crops. *Fusarium*-infested grains are not acceptable as raw material for malting and brewing industry. We carried out monitoring studies in Bavaria, the main region for malting barley production in Germany. These studies indicated an infestation situation different from that known for wheat. A large number of different *Fusarium* species was observed all over Bavaria, including *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. langsethiae*, *F. poae*, *F. sporotrichioides* and *F. tricinctum*. These species produce a wide range of different mycotoxins such as type A trichothecenes (T2; HT2), type B trichothecenes (deoxynivalenol, DON; nivalenol), zearalenon (ZEA), moniliformin, enniatins and beauvericins. It seems that the classical DON-producing species *F. graminearum* and *F. culmorum* occur less often in barley, whereas type A trichothecene- producing species such as *F. sporotrichioides*, *F. poae* and *F. langsethiae* are more frequent. Infestation with *Fusarium* in general, and the occurrence of different species were highly dependent on environmental conditions and geographical location. This shift in the spectrum of *Fusarium* species compared to wheat does have consequences for the mycotoxins produced. Standardized techniques for toxin measurements and threshold values exist for DON and ZEA, the most prevalent mycotoxins in wheat. but not for the determination of relevant toxins in barley, especially for the type A trichothecenes T2 and HT2. Further questions addressed are the consequences of this shift in the spectrum of *Fusarium* species on pathology, epidemiology, host-parasite interaction as well as on possibilities of disease control. Inoculation experiments were carried out to investigate the impact of different *Fusarium* species on the pathology, grain infestation and mycotoxin contamination of barley. Whereas artificial inoculations with *F. culmorum* and *F. avenaceum* at anthesis resulted in strong symptom expression, inoculation with *F. sporotrichioides*, *F. langsethiae*, *F. poae* and *F. tricinctum* did not. Symptoms caused by *F. culmorum* were in agreement with high levels of fungal DNA (qPCR) and DON contamination (LC-MS/MS) in grains. In contrast, infestation with *F. sporotrichioides* was hardly detectable by visual assessments and qPCR after inoculation, but resulted in high levels of T2 and HT2 in the grains. Additionally, in competition with other species, *F. sporotrichioides* caused high mycotoxin contaminations, even though this species is described as a weak pathogen with low impact on FHB. Inoculation with a mixture of the *Fusarium* species mentioned above resulted in high levels of DON as well as of T2 and HT2. These results indicate that there are great difficulties to estimate risks of mycotoxin contaminations based on visual assessments of the growth of *Fusarium* sp. on barley grains. Further studies are needed to better understand the impact of the different species on FHB in barley.

FOOD QUALITY IMPROVEMENT BY POST HARVEST MANAGEMENT AND MOLECULAR BIOLOGY TOOLS*. V. Balmas, L. Cogotzi, S. Fiori, A. Marcello, M. Orrù, B. Scherm, F. Spanu and Q. Migheli. *Dipartimento di Protezione delle Piante - Unità di Ricerca Istituto Nazionale Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: qmigheli@uniss.it*

In 2009, the United Nations estimated the human population to reach 7,000,000,000 people in 2011. Current projections indicate that between the years 2040 and 2050, the world population is expected to range from 8 to 10.5 billion. This sudden increase over the course of the last two centuries raises serious concern about how longer an overpopulated earth will be able to sustain multiple threats to its ecosystems, such as rising levels of carbon dioxide, global warming, chemical and radioactive pollution, and the progressive erosion of biodiversity. Under these circumstances, there is a need to increase the production of high-quality food by reducing agricultural inputs, including water, pesticides and farmland areas, an accomplishment that appears particularly challenging if we consider the ongoing global environmental change. Plant pathogens are most effective self-evolving threats to food quality. Filamentous fungi are able to deteriorate fruit, vegetable and grain commodities during the post-harvest management stages. An increasing array of species, mainly belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium*, is reported to be responsible for the contamination of food and feed with mycotoxins. Crops in tropical and subtropical latitudes are more prone to contamination than those grown or stored in temperate areas, due to the presence of environmental and management conditions (temperature, humidity, improper storage) that are most suitable for infection and, hence, mycotoxin formation. With this respect, the increasing demand for organically grown food, which is commonly perceived as a healthier and safer alternative to conventional food by spoiled and hyper-nourished western consumers, calls into the question whether "organic" should be really considered as a synonym of "safe". Among the most bioactive secondary metabolites, aflatoxins, ochratoxins, fumonisins, zearalenone, patulin, and trichothecenes are now considered of worldwide public health importance. In both the USA and Europe, there are now strictly enforced limits as to the concentration of mycotoxins permitted in unprocessed plant products as well as in processed foods and animal feed. Control of mycotoxin contamination below acceptable levels can only be achieved by an integrated approach, which should be based on: (i) adoption of suitable agronomic measures and epidemiological schemes; (ii) sound application of effective fungicides (and, in some instances, insecticides); (iii) use of environmentally safe and economically sustainable biological control agents; (iv) development of sensitive, fast, reliable, and possibly cheap diagnostic/analytical methods; (v) selection of resistant varieties, which should minimize the risk of mycotoxin contamination. Thus, there is a growing need for new molecules targeted at the secondary metabolic pathways; for biological control agents able to specifically interact with mycotoxin-producing fungi; for user-friendly diagnostic methods that should be integrated into a more complex risk assessment framework; and for selection strategies aimed at retrieving resistant or tolerant plant germplasm from wild relatives. Molecular biology tools are being developed to assist the research along each one of these converging pathways, and most relevant examples will be summarised.

** Invited keynote lecture*

DETECTION AND QUANTIFICATION OF *PECTOBACTERIUM ATROSEPTICUM* DIRECTLY FROM DISEASED AND ASYMPTOMATIC TISSUES: A STUDY IN PATHOGEN TESTING USING ISOTHERMAL DNA AMPLIFICATION. D. Lee, H. Jones, A. Kretzschmar, J. Thomas

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Isothermal DNA amplification methods appear to be more robust in their ability to amplify from crude samples than PCR. For example, the methods of whole genome amplification (WGA), loop-mediated isothermal amplification (LAMP) and smart amplification processes (SMAP) can be performed directly from biological materials such as blood or plant tissues without the need for prior DNA purification. We have used LAMP to detect the presence of *Pectobacterium atrosepticum*, a causative agent of potato black leg, in symptomatic and asymptomatic tuber and stem tissues. Identification of *Pectobacterium spp.* in seed potatoes is crucial as disease in the field can lead to heavy yield losses. Currently, diagnostic testing requires peeling of tuber samples and plating of peel extracts on indicative media or DNA preparation from extracts to enable identification by PCR. Identification and quantification from peel extracts directly would be a considerable improvement in current testing methods. LAMP utilizes a combination of hairpin-creating primers and displacement properties of certain polymerases to produce a cascade reaction which generates tandemly reiterating copies of the target sequence, and is both fast and specific. LAMP primers were designed with the aid of Optigene software to amplify specifically *P. atrosepticum* genomic DNA. Reactions were performed using Optigene's Genie Thermal Controller, which permits real-time data acquisition using fluorescent DNA intercalating dyes such SyBr Green or Eva Green and facilitates product identification by annealing temperature analysis, providing confirmation of target. *P. atrosepticum* was detected in crude tuber, stem and culture extracts. Inhibitory substances in crude potato extracts affect LAMP reaction kinetics: for quantification we adopt an analysis method utilizing most probable number statistics. We demonstrate this approach to quantifying *Pectobacterium* directly from potato extracts using an ABI 7900HT Fast Real-Time PCR system to increase sample throughput using closed-tube testing. In its simplest form, LAMP quantification and detection can be performed by end-point analyses of reactions using standard agarose gel electrophoresis and a controlled temperature water bath. This model of pathogen detection and quantification could be used to detect a wide range of other plant pathogens directly from crude sample extracts.

IDENTIFICATION OF NOVEL FUNGAL PATHOGEN-ASSOCIATED MOLECULAR PATTERNS IN *ARABIDOPSIS*.

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Plants are able to sense conserved microbial structures of invading micro-organisms, the so-called pathogen-associated molecular patterns (PAMPs). The detection of these molecules by pattern recognition receptors (PRRs) triggers a powerful immune response in the plant cell characterised by the activation of defence-related genes, the production of antimicrobial molecules and cell wall reinforcement. PAMP detection can also induce systemic acquired resistance and prepare tissues for forthcoming pathogen attack. A series of PAMPs and PRRs have been discovered in recent years, but the list of ligands and receptors is believed to be longer. Broader knowledge about pathogen-sensing will generate interesting prospects in agriculture, as microbial diseases of crops, namely those caused by fungi, are detrimental to food production. We therefore aimed at identifying novel PAMPs from major phytopathogenic fungi. Using classical biochemical purification methods, we fractionated mycelial extracts and culture filtrates and screened them for eliciting activity in the model plant *Arabidopsis*. We initiated the purification to homogeneity of PAMPs contained in the most active fractions, aiming next at their structural and functional characterisation and at searching for their receptors in *Arabidopsis*. Furthermore, we will test (with the help of cooperation partners) whether these PAMPs are recognised and induce immune defences in a series of widely grown crops.

PROTEOMICS APPROACHES IN FUSARIUM SPECIES.

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Infection with *Fusarium* species can have an important impact on agricultural production either due to direct productivity losses (symptomatic infections) or due to secondary damage caused by value-loss of plant products contaminated with *Fusarium* toxins. The availability of the genome sequences of three *Fusarium* species (*F. graminearum*, *F. oxysporum* and *F. verticillioides*) and the increased reliability of techniques for the qualitative and quantitative profiling of proteins in fungal samples is resulting in a number of exciting studies based on proteomic analysis in *Fusarium* species. Here, we describe the work carried out in the comparative proteome analysis of toxigenic *Fusarium* species. A protocol for protein extraction to be used for 2D-DiGE analyses was developed. It is suggested that the analysis of differentially expressed proteins in different growing conditions can help in deciphering the evolutionary differences of chemotypes (functional classification of isolates depending on the type of toxin produced) within the species *F. graminearum*. Moreover, we implemented the use of intact cell MALDI-TOF analysis, a quick method frequently used for classifying organisms, to differentiate the main toxigenic *Fusarium* species found in wheat. The exploitation of this high-throughput method can lead to the establishment of an alternative classification method (to be integrated with DNA information) for discriminating *Fusarium* species and chemotypes.

GLOMUS INTRARADICES, A BIOCONTROL AGENT AGAINST THE MAIZE PEST *DIABROTICA VIRGIFERA VIRGIFERA* LECONTE?

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is an invasive maize pest that has spread over a large area in Europe within the last 17 years. The major damage due to WCR is caused by larval feeding on maize roots, resulting in stalk goose necking of the plants, paving the way to mycotoxin-producing fungi infections, with consequential danger for human health. Because of the ongoing spread of WCR in Europe, interest has arisen to investigate its biology and ecology, which is a key requirement for a successful pest management. The rhizosphere and endosphere are dynamic environments where fungi, bacteria, viruses, nematodes and herbivore insects interact with each other.

In the endosphere, the arbuscular mycorrhizal fungi (AMF) may play an important role in host plant disease resistance. The objective of the present study was to test if inoculation of maize plants with the arbuscular mycorrhizal fungus, *Glomus intraradices,* alters the interaction among plants, indigenous microbial communities in the rhizo- and endosphere of maize, and WCR root feeding and development. The experiment was carried out in a greenhouse, in pots containing Chernozem soil. The maize cultivar used was the commercial line KWS13. Four different treatments were established: (i) control maize plants grown for eight weeks; (ii) maize plants grown in soil inoculated with *G. intraradices* before sowing; (iii) maize plants subjected, after the 4th week of growing, to WCR larval feeding; (iv) maize plants grown in soil inoculated with *G. intraradices* before sowing and subjected after the 4th week of growing, to WCR larval feeding. Eight weeks after sowing, the plants were harvested in order to (i) evaluate the larval feeding effect on the dry biomass of the roots; (ii) investigate the AMF communities in the maize roots by PCR-RFLP; (iii) analyse the fungal and bacterial composition in the rhizosphere and in the endosphere of maize by DGGE (denaturing gradient gel electrophoresis). In parallel, the larvae, which had fed for four weeks on the roots were extracted from soil of treatments III and IV, to evaluate the larval stage and the developmental level of the 3rd instar larvae. Plant root biomass measurements showed that *G. intraradices* did not affect significantly larval feeding of the roots. Analysis of the total number of larvae and of the composition of larval instars revealed that *G. intraradices* did not influence the total number of WCR larvae but reduced significantly their development for the weight of the 3rd larval stage was significantly lower in *Glomus*-treated plants in comparison with the controls (P=0.02). RFLP analysis of AMF populations showed that the soil inoculation with *G. intraradices* reduced the AMF evenness in maize roots almost exclusively to the RFLP type 11 (*G. intraradices*). ITS- and 16S-DGGE analysis showed a specific shift in both bacterial and fungal composition in the roots of plant colonized with *G. intraradices*. The additional dominant bands in the ITS-DGGE profile were identified by sequencing as *G. intraradices.* In conclusion, this work showed that *G. intraradices* significantly reduced larval development. Furthermore, the shift of bacterial communities in the endosphere of *G. intraradices*-inoculated plants, suggests that other microorganisms may be involved in decreasing the developmental level of larvae. However, the colonization of maize plants with *G. intraradices* should be further studied as an option to control WCR larvae in the framework of integrated pest management strategies.

HEALTH PROTECTION OF STRAWBERRY IN MONTENEGRO. N. Latinovic[1], J. Latinovic[1], S. Hrncic[1] and D. Sukovic[2]. *[1]University of Montenegro, Biotechnical Faculty, Mihaila Lalica 1, 20 000 Podgorica, Montenegro. [2]PI Centre for Ecotoxicological Research of Montenegro, 20 000 Podgorica, Montenegro. E-mail: nlatin@ac.me*

During the last three years, strawberry became an important crop in Montenegrin agriculture with more than 1,500,000 plants planted. During 2010, four strawberry fields were monitored, taking into account problems caused by harmful organisms that jeopardize crop health. The most important diseases were strawberry leaf spot (*Mycosphaerella fragariae* (Tul.) Lindau) and grey mold (*Botrytis cinerea* Pers.), while the presence of aphids was detected in only one location. Weeds did not cause significant problems because of plastic mulching along the rows, which made mechanical weeding necessary only between rows. Fungicides for strawberry leaf spot control were azoxystrobin (Quadris), difenilconazole (Score 250) and copper-based products whereas for grey mould they were fenhexamid (Teldor 500), vinclozolin (Ronilan) and cyprodinyl+fludioxonil (Switch). Strawberry fruits were analised for the presence of pesticide residues by GC-MS and/or LC-MS/MS following acetonitrole extraction/partitioning and clean-up by dispersive SPE-Quechers-method, DIN EN 15662:2009-02. Results showed that there were no detectable pesticide residues or they were below the maximum residue level (EU-MRLS; Regulation No. 369/2005), indicating that strawberries grown in the surveyed localites of Montenegro were safe for human consumption with respect to fungicides.

SENSITIVITY OF SIMULATED SURFACE WETNESS DURATION TO METEOROLOGICAL VARIATIONS IN THREE DIFFERENT REGIONS OF THE GRAND-DUCHY OF LUXEMBOURG. A. Mahtour[1], M. El Jarroudi[1], L. Hoffmann[2] and B. Tychon[1]. *[1]University of Liège, 185 Avenue de Longwy, 6700 Arlon, Belgique. [2]Centre de Recherche Public-Gabriel Lippmann, Département Environnement et Agro-biotechnologies, 41 Rue du Brill, 4422 Belvaux, Luxembourg. E-mail: meljarroudi @ulg.ac.be*

Leaf surface wetness duration (SWD) is an important factor influencing the occurrence of winter wheat diseases. Thus, SWD is extremely important for the management of crop protection activities. In order to understand the SWD variability and its influence on winter wheat diseases, the objective of this study was to (i) determine the sensitivity of our model on varying input plant parameters and (ii) evaluate the influence of simulated SWD on meteorological variations in three different climatic regions (Everlange, Obercorn, Schimpach) of the Grand-Duchy of Luxembourg. Hourly weather data for the three sites were obtained from the ASTA (Administration des Services Techniques de l'Agriculture) automatic weather station network. The simulation of surface wetness duration was made with the Surface Wetness Energy Balance (SWEB) simulation model. The agrometeorological inputs of the model are air temperature (°C), relative humidity (%), precipitation (mm), wind speed (m/s) and radiation above the canopy (W/m^2); the output is represented by the fraction (%) of the leaf surface covered by water (the percentage of wet leaf surface, *W*). The *W* parameter is a continuous variable and, was used for comparing estimates of the wet area of a canopy at any given time. The estimated number of wet hours per day (N_n), changes in net radiation, wind speed, temperature, rainfall rate and relative humidity were also taken into account. Each of these measured variables was increased and decreased by 10% (where RH was truncated at 100% if necessary). The model was rerun and the difference in wet hours predicted by the default model (the model without any change in any of the variables), N_{ndef}, and the adjusted model (the model after one variable or parameter had been changed), N_{nnew}, was calculated. The absolute values of these differences were averaged over monthly periods, and referred to as the sensitivity S_+ and S_- for positive and negative changes, respectively, to the variables:

$$S_+(ou\ S_-) = \sum \left| N_{ndef} - N_{nnew} \right|$$

Since S_+ and S_- were very similar, only mean values are given here. In this work, an agrometeorological model known as the Surface Wetness Energy Balance (SWEB) was applied for the simulation of SWD. The model was previously applied in another study for winter wheat cultivars and was adapted for use with agrometeorological data available from standard meteorological monitoring stations. Based on weather data and simulated SWD data, sensitivity analyses were performed to compare the effects of relative humidity, air temperature, wind speed and net radia-

tion on wetness duration over one growing season (March-July) at three test sites. The results indicated that the sensitivities were very similar at three sites and there was no spatial trend (i.e. difference between locations) in the sensitivities. However, the model is most sensitive to relative humidity and differences between 0.5 and 25 h (per month) SWD were found when increasing/decreasing relative humidity by 10%. The model was least sensitive to changes in air temperature, showing differences of only 0.5–2 h (per month) in SWD. Intermediate sensitivity is found for rainfall, net radiation and wind speed. Among the input plant parameter values, SWD was most sensitive to the maximum fraction of canopy allowed as wet surface area, leaf area index, maximum water storage per unit area and least sensitive to crop height. The sensitivity to parameter values was less important compared to the sensitivity to the meteorological variable relative humidity.

POLYMORPHISMS EXISTS IN THE LETTUCE POPULATION OF *SCLEROTINIA SCLEROTIORUM* IN GREENHOUSES. F. Mert-Turk. *Çanakkale Onsekiz Mart University, Agricultural Faculty, Plant Protection Department, Çanakkale 17100, Turkey. E-mail: figen.turk@hotmail.com*

Sclerotinia sclerotiorum is one of the most destructive pathogens of vegetable crops. In Çanakkale (northwest Turkey), lettuce is cultivated in glasshouses between November and the end of March. Afterwards, lettuce is replaced with cucumber which are both important *S. sclerotiorum* hosts so that infected plants can be found throughout the year. We previously showed that there is a great genetic variation within a population of this fungus from rapeseed. Here, we tested if polymorphism exists in the isolates collected from glasshouses. Eighteen isolates were collected at least 10 km apart from each other and anlalysed with 8 microsatellite primer sets. One of them ($(TACA)_{10}$) did not show polymorphism. We found 2 alleles at $(GT)_8$, 3 alleles at $(GA)_9$, $(TATG)_9$, and $(GT)_{10}$ loci. Five loci were observed at $(CATA)_{25}$ and $(TTA)_9$ alleles. The most obvious polymorphism existed at $(AGAT)_{14}(AAGC)_4$ loci revealing 6 alleles. A high level of polymorphism was found in lettuce populations originating from different locations but also within populations from the same glasshouse.

FUSARIUM HEAD BLIGHT AFFECTS THE YIELD BUT NOT THE TOTAL PROTEIN CONTENT OF WINTER WHEAT. F. Mert-Turk[1], F. Kahriman[1], R. Gencer[1] and C.O. Egesel[2]. *[1]Çanakkale Onsekiz Mart University, Agricultural Faculty, Plant Protection Department, Çanakkale 17100, Turkey. [2]Çanakkale Onsekiz Mart University, Agricultural Faculty, Crop Science Department, Çanakkale 17100, Turkey. E-mail: figen.turk @hotmail.com*

Fusarium culmorum is one of the pathogens causing Fusarium head blight (FHB) in winter wheat. The aim of this study was to elucidate the effects of head scab on yield and total carbohydrate and protein content. Four different wheat varieties (Sagittario, Tosunbey, Golia and Yunak) and a DON chemotype of the fungus were employed in this study. Heads were syringe-inoculated with conidial suspensions of the fungus during anthesis. Measuring the diseased spikelets relative to the whole head assessed disease severity. Infection rates differed greatly among the varieties 14 days after inoculation; infected area of the head was as low as 14.3% of the whole head in the cv. Golia, whereas the most susceptible was cv.Yunak where infection progressed up to 60.8% of the whole head. Approximately 47 and 53% of the heads were infested in cvs Saggittario and Tosunbey, respectively. The thousand-kernel weight was reduced significantly in inoculated plots of cvs Sagittario, Tosunbey and Yunak (61.6%, 72.0% and 38.7%, respectively). However, it was similar for inoculated and control plants of cv. Galio. Although there was a significant effect of variety and treatment interactions (P=0.02), protein content of the kernels did not differ among the inoculated and control kernels of any variety tested. The carbohydrate content was significantly reduced by infection in the kernels of cvs Tosunbey and Sagittario (P=0.04).

AN RNA INTERFERING CONSTRUCT TARGETED AT THE TRICHOTHECENE BIOSYNTHESIS GENE *TRI6* AFFECTS MYCOTOXIN PRODUCTION IN THE PLANT PATHOGENIC FUNGUS *FUSARIUM CULMORUM*. M. Orrù[1], B. Scherm[1], V. Balmas[1], F. Spanu[1], E. Azara[2], G. Delogu[2], T.M. Hammond[3], N.P. Keller[4] and Q. Migheli[1]. *[1]Dipartimento di Protezione delle Piante-Unità di Ricerca Istituto Nazionale Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. [2]CNR, Istituto di Chimica Biomolecolare, Sezione di Sassari, Traversa La Crucca 3, Località Baldinca, Li Punti, 07040 Sassari, Italy. [3]Division of Biological Sciences, University of Missouri, Columbia, USA. [4]Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI, USA. E-mail: qmigheli@uniss.it*

Fusarium culmorum is a major fungal pathogen of wheat, causing two forms of disease, namely crown and foot rot (CFR) and Fusarium head blight (FHB). Significant yield losses are reported and the grain often becomes contaminated with mycotoxins. Among the most bioactive compounds are trichothecenes; type B trichothecenes, including deoxynivalenol (DON), also known as vomitoxin, with its derivatives monoacetyldeoxynivalenols (3-AcDON, 15-AcDON), nivalenol (NIV) and its acetylated form 4-acetylnivalenol or fusarenone X. In this study, we have tested the efficacy of an RNA silencing construct targeted at the regulatory gene *TRI6* to suppress mycotoxin production in the plant pathogenic fungus *F. culmorum*. A series of *TRI6* IRT transformants were obtained from two highly virulent strains of *F. culmorum*, namely MCf 21 wild type and its nitrate reductase deficient mutant MCf 21 nit1. We have focused our HPLC-MS analysis on DON and its acetylated derivatives 3-AcDON and 15-AcDON. An Agilent Technologies 1100 series LC/MSD equipped with a diode-array detector and an autosampler (G1313A) was used for LC separation. Chromatographic separation was achieved using a Luna C18 column fitted with a 3 µm C18 security guard cartridge. Type B-trichothecenes were extracted using a liquid/liquid extraction step. In a preliminary experiment, the average (mean of six replicates ± SD) production of type B trichothecenes (ng ml^{-1} culture filtrate) by this strain was 212.6±74.2 DON, 523.0±98.9 3-AcDON, and 16.3±3.9 15-AcDON after 14 d of growth in Vogel's medium at 25°C. In MCf 21 wild type-derived transformants # 252 and 253, the production of type B trichothecenes was reduced by 95 and 100%, respectively. In transformants # 266 and 282 type B trichothecene production was reduced to 3.1 and 5.4% with respect to the recipient strain. Surprisingly, transformants # 255, 256, 257, and 260 produced 1,370-2,770% more trichothecenes than the MCf 21 wild type strain, corresponding to average concentrations ranging from 12,008±2,637 to 24,341±5,400 ng ml^{-1} of culture filtrate. Among TRI6 IRT transformants deriving from the nitrate reductase mutant MCf 21 nit1, only # 5 did not differ from the recipient strain for the production of type B trichothecenes, while in transformant 112 the trichothecene production was reduced by approximately 90%. In all the other TRI6 IRT transformants (i.e., # 14,

106, 111, 114, and 127), the production of type B trichothecenes was completely suppressed.

PUCCINIA STRIIFORMIS ANALYSIS BY SEQUENCE RELATED AMPLIFIED POLYMORPHISM. **M. Pasquali[3], H. Komjati[2], D. Lee[1] and R. Bayles[1]**. *[1]National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 0LE, UK. [2]Szent Istva´n University, Plant Protection Institute, Gödöllö, Hungary. [3]Centre de Recherche Public- Gabriel Lippmann, Département Environnement et Agro-biotechnologies, 4422 Belvaux, Luxembourg. E-mail: pasquali@lippmann.lu*

Yellow rust, caused by the fungal pathogen *Puccinia striiformis*, is a major disease of wheat in temperate-cool climates. The pathogen exists as a large number of 'physiologic races' possessing virulence for different resistance genes in host cultivars. The traditional method used for testing rust populations using a set of differential cultivars is time-consuming. As such, any molecular tool that offers a quick method for primary screening yellow rust populations would be welcomed. Ideal markers used for pathogen identification would be based on the genes responsible for virulence. Here we present the use of a promising molecular approach for identifying and developing race-specific markers. The technique, SRAP, is based on the use of degenerate random primers. It has been shown that the primers used, preferentially target the exon–intron junction of genes in plants. To identify markers that can be useful to differentiate races of the pathogen, a subset of twelve single pustule isolates was first tested for pathogenicity and secondly analysed for their molecular profile obtained using the SRAP technique with 9 primer combinations. It was possible to generate a 30% higher level of polymorphism compared to AFLP and it was possible to identify bands specific for single isolates that differ for the avirulence genes. These results suggest that SRAP may be suitable for the identification of pathogencity-related markers in pathogens such as yellow rust and represents the first application of the technique to the order Uredinales.

COMPARISON OF _FUSARIUM_ GENETIC CHEMOTYPING METHODS. **M. Pasquali, M. Beyer, T. Bohn and L. Hoffmann.** *Centre de Recherche Public-Gabriel Lippmann, Département Environnement et Agro-biotechnologies, 41, rue du Brill, 4422 Belvaux, Luxembourg. E-mail: pasquali@lippmann.lu*

Fusarium chemotyping is an essential tool for characterizing *Fusarium* populations causing Head blight on wheat and other cereals. The chemotype determination of *F. graminearum* and *F. culmorum* was shown to improve the precision of prediction of *Fusarium* toxin contamination in the field. Moreover, it is useful for defining the population structure of the pathogens within a field. Chemotyping methods for trichothecene type B discrimination in *Fusarium* have been developed for *F. graminearum* and *F. culmorum.* All methods are based on differences in the *tri* cluster that encodes the genes necessary to synthesize toxin variants (nivalenol, 3 acetylated deoxynivalenol and 15 acetylated deoxynivalenol). Three methods were compared, using DNA obtained from previously isolated *F. graminearum* strains as developed by Ward *et al*. (2002), Quarta *et al*. (2006) (derived from Jennings *et al*., 2004), and Li *et al*. (2008). The methods, all based on polymorphisms of the *tri* cluster between the three known chemotypes are targeting *tri 3*, *tri 7*, *tri 12* and *tri 13*. In order to verify specificity of the chemotyping tests and consistency of the markers, a set of 110 isolates from the CRP-GL collection belonging to *F. graminearum*, *F. culmorum* as well as control strains belonging to *F. poae* and *F. avenaceum* were analysed using the three above cited methods. PCR programs were modified to increase specificity of Phusion Taq, raising temperature of the annealing and denaturing steps. The three methods were not always consistent in chemotype attribution. In particular, differences were observed at the level of amplification specificity. When primers were used to amplify *F. poae* and *F. avenaceum* DNAs aspecific products were seldom amplified by Ward *et al*. (2002) and Quarta *et al*. (2006) methods. Wang's method showed a lack of specificity being unable to distinguish correctly all the 3ADON and 15ADON isolates. This suggests that *tri13* intron size cannot be used to distinguish acetylated chemotypes. This work highlights the fact that genetic chemotype determination requires a continuous monitoring of markers and that targeting different regions of the cluster may be the most reliable strategy for correct chemotype attribution. Moreover, none of the tested markers can be used on direct plant extracts that are often contaminated by multiple *Fusarium* species, giving raise to aspecific cross reaction products.

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PATHOGENICITY AND MYCOTOXIN PROFILE OF _FUSARIUM TEMPERATUM_, AN EMERGENT PATHOGEN OF MAIZE IN EUROPE[#]. **J. Scauflaire[1], M. Gourgue[1], A. Callebaut[2], L. Pussemier[2] and F. Munaut[3].** *[1]Université Catholique de Louvain, Earth and Life Institute, Applied Microbiology, Laboratory of Mycology, Croix du Sud 3/6, 1348 Louvain-la-Neuve, Belgium. [2]Centre d'Etude et de Recherches Vétérinaires et Agrochimiques, Leuvensesteenweg 17, 3080 Tervuren, Belgium. [3]Université Catholique de Louvain, Earth and Life Institute, Applied Microbiology, Mycothèque de l'Université Catholique de Louvain (BCCMTM/MUCL), Croix du Sud 3/6, 1348 Louvain-la-Neuve, Belgium. E-mail: Francoise.Munaut@uclouvain.be*

In a recent study, a population of *Fusarium* strains isolated from maize, closely related to *F. subglutinans,* was described as a new species, *Fusarium temperatum* J. Scauflaire et F. Munaut. In several temperate regions of Europe, the *F. temperatum:F. subglutinans* ratio is very high in the fields, suggesting that *F. temperatum* competes with its sister species *F. subglutinans*. This raised the question of the contribution of this novel species to the final ear rot or stalk rot symptoms observed on maize plants at harvest, as well as to the potential mycotoxin contamination. The pathogenicity of *F. temperatum* to maize plant has been tested in greenhouses and preliminary results confirm its ability of to cause stalk rot and seedling malformation with a virulence similar to that of *F. subglutinans*. Currently, studies are in progress to elucidate the mycotoxigenic potential of *F. temperatum*.

[#] *Patholux Poster Award 2010*

EXTRACELLULAR DEGRADATION OF AFLATOXIN BY CERTAIN FUNGI PREVIOUSLY IDENTIFIED AS AFLATOXIN B1 BIODESTRUCTORS. **N. Zhemchuzhina[1], L. Shcherbakova[1], O. Mikityuk[1], T. Nazarova[1], B. Campbell[2].** *[1]Russian Research Institute of Phytopathology, RAAS, B. Vyazemy, Moscow 143050, Russia. [2]USDA, ARS, WRRC, Plant Mycotoxin Research, Albany, CA, USA. E-mail: zemch@mail.ru*

Several species of the genus *Aspergillus* are common opportunistic fungal pathogens of plants. They often infect many economically important crops in the field, and can also develop on agricultural feedstock and food products during post-harvest. These fungi also produce mycotoxins, most notably aflatoxins, which possess hepatotoxic, carcinogenic, teratogenic and mutagenic properties. *Aspergillus flavus* is one of the main aflatoxin producers. Pre- and post-harvest infection by *A. flavus* can result in contamination of food products with aflatoxin B1 (AFB1). Like other aflatoxins, there is currently no feasible method to decontaminate AFB1. Thus, AFB1 contamination is a serious food safety issue, in addition to being an economic problem, on a global scale. The only current remedy is to physically remove contaminated products from the processing stream. However, this is expensive and can result in an extensive loss of product. Chemical methods for detoxification, such as ammoniation under high pressure and temperature, have obvious drawbacks. Therefore, development of an environmentally appropriate and safe method for decontamination of agricultural material contaminated with aflatoxin would be a major advancement for food safety and quality. One promising approach for decontamination is enzymatic catabolism of aflatoxin by microbial "biodestructors" which occupy ecological niches conjointly with toxigenic *A. flavus* strains. We previously showed that 28 out of 41 fungal micromycetes screened that co-colonize natural substrates with aflatoxigenic *A. flavus*, were able to degrade AFB1. The most active biodestructors catabolized 80-98% of AFB1 added to media. Our current study shows that some of these biodestructors produce aflatoxin-degrading enzymes extracellularly. Strains of *Phoma glomerata, Ph. exigua*, *Ph. chryzanthemicola*, *Trichoderma sp.*, *T. viride*, *Cladosporium sp.*, *Chaetomium sp.*, *Ulocladium sp.*, *Colletotrichum atramentarium*, *C. coccoides*, *Ophiobolus sp.*, *Gliocladium roseum* and *Verticillium tenerum* were grown on liquid Czapek with casein hydrolyzate at 28°C, at 200 rpm for 7 days. Mycelia were removed from cultural liquid by filtration through filter paper. This filtrate was sterilized by further filtration through a sterile 0.22 μm Millipore membrane. The resulting filtrate of culture liquid (FCL) was used for further studies. AFB1 between 5 to 10 m/kg, dissolved in 95% ethanol, was added in 1 ml samples to FCLs. These samples were incubated at 28°C for 3 days under aseptic conditions. Amounts of AFB1 remaining in the FCLs were quantified by HPLC and compared to that of controls (water and aflatoxin). Results indicated the *Phoma* fungi were the most promising sources of AFB1-degrading enzymes. FCLs of *Ph. chryzanthemicola*, *Ph. glomerata* and *Ph. exigua* removed about 32, 66 and 99% of the added toxin, respectively. FCLs of some other biodestructors, such as *Gliocladium roseum*, *Colletotrichum atramentarium* and *Ulocladium* sp., also contained extracellular catabolizing activity against AFB1. AFB1 content in the FCLs of these fungi showed a decline of 43.5 or 62% in AFB1, as compared to controls. Interestingly, although the *Cladosporium* sp. and *Chaetomium* sp. showed impressive toxin degrading ability under culture, their respective FCLs were inactive.

IMPACT OF PLANT PATHOGENS ON FOOD QUALITY OF AGRICULTURAL CROPS AND WINE

DAY 2

EFFICACY OF SPRAYS APPLIED AGAINST POWDERY MILDEW *(ERYSIPHE NECATOR)* DURING A CRITICAL PERIOD FOR INFECTIONS OF CLUSTERS OF GRAPEVINES *(VITIS VINIFERA)*

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SUMMARY

Erisyphe necator is one of the most destructive pathogens of grapevines and is normally controlled by seven and more fungicide treatments until veraison of grapes. Two fungicide regimes comprising either 3 or 7 sprays per season were compared in a German vineyard between 1999 and 2010. The trials always included: (i) 3 fungicide sprays, one before flowering, the second during blossoming and the third at berries diameter 2 mm [so called "Open Window Period" (OWP) sprays]; (ii) 7 sprays which included OWP sprays of (i) plus one spray before and three afterwards. The trials were carried out with a single line tunnel-sprayer in 4 replicates, each comprising 12 vines of highly susceptible varieties. Experimental rows were separated by untreated rows on both sides. Although in most years, natural initial inoculum was present, in 2008-2010, the initial inoculum was supplemented with artificial infections on the separating untreated spacer lines. Even under extreme disease pressure, 3 sprays in OWP had nearly the same and always more than 90% of the preventive effect of seven sprays.

Key words: Erysiphe necator, fungicide treatments, grapevine, control.

INTRODUCTION

Powdery mildew, caused by *Erysiphe necator*, is one of the most widespread and destructive diseases of grapes. Most cultivars of the European wine grape (*Vitis vinifera*) are highly susceptible to it (Pearson and Gadoury, 1992). Due to a lack of commercial cultivars with adequate resistance, powdery mildew control strongly relies on 7 or more fungicide applications.

Investigations by Stark-Urnau and Kast (1999) and Gadoury *et al.* (2003) indicated that developing grapevines bunches are highly susceptible to infections only between one week before anthesis and the point of time when berries reach a diameter of 2 mm. An intelligent use of fungicides to manage grapevine powdery mildew should use knowledge of the dynamics of fruit susceptibility to infection. To determine the impact of fungicidal protection during this critical period, fungicide treatments were applied in repeated field trials that included a comparison of two spray regimes differing in the number and timing of applications.

MATERIALS AND METHODS

Starting in 1999, experiments were conducted at Weinsberg (Germany) in vineyards of the highly susceptible cvs Silvaner, Trollinger (syn. Vernatsch) and Cabernet Dorsa (Table 1).

The vines were planted at 1.2 m distance on the row and 2.0 m between rows and were trained in a half bow trellis system with a stem height of 0.9 m. All trials were carried out with four replications in a randomised block design, with plots of 12 vines in one row separated by untreated rows on both sides of the experimental rows.

Fungicides were applied with an experimental tunnel-sprayer that allowed a precise treatment of single rows. In most years, natural initial inoculum was present, because untreated rows of the previous year were used for the experiments. In 2008-2010, however, the initial inoculum was supplemented by the addition of powdery mildew on infected vines grown in a glasshouse in 12 cm diameter pots. These vines carried about 10 completely infected leaves. At the stage of three unfolded leaves (BBCH13), two infected pots were fixed in each plot on a trellis of the experimental plots.

The trials included two spray regimes and an untreated control. As to regime 1, one spray was applied about one week before flowering (BBCH55), another during anthesis (BBCH64-66) and a third at berry size = 2 mm diameter (BBCH73). In 2010, the additional regime 1a was tested, which included 5 sprays of wettable sulfur during the BBCH55-65 period. Regime 2 consisted of 7 (1a = 9) sprays including the 3 sprays of regime 1 plus one earlier spray and three later sprays using different products (preferably triazols) and other powdery

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Table 1. Fungicides used in the trials and their active ingredients.

Trade name	Ingredient	Group
Cabrio Top	(Metiram) + Pyraclostrobin	Strobilurines
Collis	Boscalid + Kresoxim-methyl	Carboxianilides + Strobilurines
Discus	Kresoxim-methyl	Strobilurines
Flint	Trifloxistrobin	Strobilurines
Kumulus	Sulfur	-
Systane 20 EW	Myclobutanil	Azoles
Talendo	Proquinazid	Quinoazolines
Topas	Penconazol	Azoles
Vento	Quinoxifen + Myclobutanil	Chinolines + Azoles
Vento Power	Quinoxifen + Fenarimol	Chinolines + Azoles
Vivando	Metrafenone	Benzophenones

mildew fungicides (Table 1 and Table 2).

All fungicides (Table 1) were applied at the dose recommended in Germany for the relevant phenological stage. Powdery mildew disease severity was scored on 100 bunches per plot at veraison in 7 classes (0, >0-5, >5-10, >10-25, >25-50, >50-75, >75%).

Table 2. Vine variety and fungicides used in the different years. Treatments 2, 3, 4 are regime 1. Regime 2 includes all treatments (1 to 7).

Year	Treatment No.	1	2	3	4	5	6	7
	Vine cultivar	BBCH 19	BBCH 55	BBCH 65	BBCH 73	BBCH 75	BBCH 77	BBCH 79
1999	Silvaner	Discus	Discus	Discus	Discus	Discus	Discus	Discus
2000	Silvaner	Vento	Vento	Vento	Vento	Vento	Vento	Vento
2001	Silvaner	Flint	Flint	Flint	Flint	Flint	Flint	Flint
2002	Silvaner	Flint	Flint	Flint	Flint	Flint	Flint	Flint
2003	Trollinger	Topas	Flint	Vento	Flint	Topas	Topas	Topas
2004	Trollinger	Kumulus	Flint	Discus	Vento	Topas	Topas	Topas
2005	Trollinger	Kumulus	Topas	Vento	Collis	Discus	Topas	Systhane 20 EW
2006	Cabernet Dorsa	Kumulus	Flint	Flint	Flint	Vento	Systhane 20 EW	Topas
2007	Cabernet Dorsa	Kumulus	Talendo	Cabrio Top	Talendo	Vivando	Systhane 20 EW	Systhane 20 EW
2008	Cabernet Dorsa	Kumulus	Talendo	Cabrio Top	Talendo	Vento Power	Systhane 20 EW	Systhane 20 EW
2009	Cabernet Dorsa	Kumulus	Vivando	Cabrio Top	Vivando	Vento Power	Systhane 20 EW	Systhane 20 EW
2010	Cabernet Dorsa	Kumulus	Vivando	Cabrio Top	Vivando	Vento Power	Systhane 20 EW	Systhane 20 EW
2010a	Cabernet Dorsa	Kumulus	Kumulus (2x)	Kumulus (2x)	Kumulus	Kumulus	Kumulus	Kumulus

RESULTS

Powdery mildew severity in untreated plots ranged from 1% in 2005 to 65% in 2009. No infection was found in 2003 and 2006. Spray regime 1 (3 sprays during the period of extreme susceptibility) had almost the same effect as that of spray regime 2 (7 sprays in all 10 evaluable experiments) (Table 3). The differences in powdery mildew reduction (RW%) were low: 1.3% on the average over the 10 experimental years.

DISCUSSION

Our findings do not support the widespread use of a developmental threshold at the beginning of sugar accumulation of grapevine berries (Flaherty *et al.*, 1982; Pearson and Goheen, 1988; Travis *et al.*, 1994). Thus, the results of more than 10 years indirectly support the previous findings of Stark-Urnau and Kast (1999) and of Gadoury *et al.* (2003) about ontogenetic resistance development of berries a few days after fruit set of grapes.

Untreated vines at both sides of a vineyard as in plots of these trials represent a worst-case scenario. Using the most efficient fungicides during the period of high susceptibility (BBCH55-BBCH73), it was possible to produce healthy grapes even under such an extreme disease pressure. Three fungicide applications using the best fungicides provided nearly the same preventive effect as 7 sprays (Table 3). If less effective fungicides like wettable sulphur are used during this period, vine-growers need to implement a very tight spray schedule as shown in 2010a with Kumulus WP. Spraying twice a week during this period is a problem, because vine-growers are very busy in with a lot of other vine treatment work.

Powdery mildew symptoms were believed by German vine-growers to be the result of infections of the last 10 days as it is true for the downy mildew, whose infections are clearly visible at the end of the incubation period. However, before powdery mildew symptoms become visible with the naked eye, several fungal propagation cycles must occur. Due to this delay, the precise timing when an infection occurred is hard to determine by the growers. Our data suggest, that sprays during the sensitive period of plant development from

Table 3. Effect of different spray regimes (complete season vs. 3 sprays in the period of highest susceptibility).

Year	Untreated	Regime 1 (3 sprays)		Regime 2 (7 sprays)	
	Severity (%)	Severity (%)	RW (%)	Severity (%)	RW (%)
1999	42	9	78.6	4	90.5
2000	20	2	90.0	1	95.0
2001	18	0	100.0	0	100.0
2002	13	7[2]	46.1	7[2]	46.2
2003	0	*	*[3]	*	*[3]
2004	9	0	100.0	1	88.9
2005	1	0	100.0	0	100.0
2006	0	*	*[3]	*	*[3]
2007	22	1	95.5	0	98.6
2008	68	2	97.1	1	98.4
2009	65	2	96.9	1	98.5
2010	23	0	98.7	0	100.0
2010a		*	*	0	100.0[3]
Mean over 10 years[3]			90.3		91.6

* Not evaluated; RW%= reduction in relation to control

[2] Gap caused by technical problems (no sprays during BBCH 55-69)

[3] Without 2003, 2006 and 2010a

Fig. 1. Sporulation of powdery mildew (*Erisyphe necator*) on the rachis of a grapevine bunch.

BBCH55 to BBCH73 are sufficient to control the disease on grape clusters and that further sprays provide hardly any benefit. For a better understanding of this problem we propose to introduce the name of "Open-Window-Period" (OWP) for the period starting one week before flowering and ending a few days after berries reach 2 mm in diameter (period from BBCH55 to BBCH73). A vine-grower should keep this window covered by his spray program. During OWP, growers need an absolutely effective and sure powdery mildew spray program. Therefore, we recommend using the best fungicides during this period. Other fungicides may only be used for further sprays to reduce the production of cleistothecia on the leaves in order to reduce the overwintering potential.

Since an accident with the tunnel sprayer caused technical issues, a gap in the spray schedule of 18 days beginning 10 days before flowering (BBCH55) occurred in 2002. The next spray in all treatments was at the end of anthesis (BBCH69). The effect of three sprays (RW%) was only 40% in spite of a relatively low disease pressure in this year. Yet, 7 sprays had the same low effect. These technical problems in 2002 showed clearly, that errors in this period are extremely risky in that infections could not be cleared by further sprays.

During OWP, growers need to work very accurately against powdery mildew closing the open window, especially if weather conditions are suitable fungal attacks. The biggest problems may be caused by infections at the beginning of this period. If the rachis is colonized by the fungus, sporulation will occur when young berries are extremely susceptible (Fig. 1).

During the OWP, fungicides against powdery mildew should be used at short spray intervals, especially if the weather conditions are suitable for the fungus. All sprays at other times had a very low relevance for the final disease levels. These notions were integrated into the expert system OiDiag 2.2 as an ontogenetic part index (Kast and Bleyer, 2010). The strong decrease of index values after fruit set demonstrates the decreasing susceptibility of grape clusters.

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EFFECTS OF PROHEXADIONE-CALCIUM ON GRAPE CLUSTER STRUCTURE AND SUSCEPTIBILITY TO BUNCH ROT *(BOTRYTIS CINEREA)* IN cv. GRÜNER VELTLINER

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SUMMARY

In Austria plant growth regulators have long been used on grapevines for induction of loose clusters and avoidance of *Botrytis cinerea* attacks. The purpose of this work was to examine the effect of a plant growth regulator containing prohexadione-calcium as active ingredient based on various parameters. The experiment was conducted on cv. Grüner Veltliner. Results were analysed on the basis of the following parameters, cluster weight and lenght, berry and seed weight, number of berries and seeds, evaluation of infestation severity by *B. cinerea*, and FTIR analysis. In addition, the bending index of the berries was recorded. which describes the suppleness of the clusters. The four main test variants were: (i) control, (ii) prohexadione-calcium alone, (iii) prohexadione-calcium with a botryticide and (iv) botryticide alone. Regarding the effect of prohexadione-calcium, results showed that there was a significant difference in cluster compactness. The increased number of small clusters additionally resulted in a significant increase in suppleness and reduced compactness. In 2009, *B. cinerea* infestation in the control was less than in the other treatments; in 2010, no significant difference was found. In 2010, the application of prohexadione-calcium led to increased must densities, compared with the control plots.

Key words: bunch rot, plant growth regulator, grape cluster structure, chemical control, grey moud

INTRODUCTION

Botrytis cinerea, the causal agent of grey mould or botrytis bunch rot of grapes is responsible for significant economic damage worldwide and is present in vineyards as part of the environmental microflora. Infection of grape clusters often occurs at blooming, followed by a period of latency, during which the pathogen is present (quiescent or latent) inside the berry without causing apparent disease symptoms until grape berries begin to ripen (McClellan *et al.*, 1973; Nair *et al.*, 1995; Pezet and Pont, 1986). The berries can be damaged by cracked cuticles as a result of pressure inside the berry and physical damage as induced by insects, hail or wind which, under wet conditions, lead to disease expression (bunch rot) and crop loss. Flower infection is believed to be an important stage in the epidemiology of *B. cinerea* in grape (Nair *et al.*, 1995), but little is known about the relationship between early infection and latency and its importance for disease control.

B. cinerea is commonly associated with humid conditions and temperatures of 10-25°C, the presence of water on the surface of grape berries being ideal for germination and mycelial growth (Ribéreau-Gayon *et al.*, 2006).

In the past few years in Austria, problems have appeared with *B. cinerea* damage depending on the climate and the canopy structure. Even vineyards with a very good integrated pest management programme had problems with *B. cinerea* before harvest. The timing of the latest possible botryticide application as well as possible residues in the wine were matter of debate. In Austria, two botryticide sprays with active ingredients of different classes are allowed, registered compounds being. boscalid, cyprodinil/fludioxonil, fenhexamid, mepanipyrim and pyrimethanil. The plant growth regulator Regalis (active ingredient prohexadione-calcium; i.e. 3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate) is registered to reduce cluster compactness of cvs Rheinriesling, Sauvignon blanc and St. Laurent. The active ingredient is absorbed and translocated in the plant, inhibiting gibberellin biosynthesis. Prohexadione-calcium is known to interfere with the 3-β-hydroxylation of GA_{20} to GA_1. The net effect is a reduction of immobile, biologically active GA_1 and an increase in the level of mobile, but inactive GA_{20} (Evans *et al.*, 1999; Graebe, 1987). High doses of prohexadione-calcium and other acylcyclohexanediones inhibit the formation of anthocyanins in flowers and other parts of higher plants. Representative targets for these compounds include 2-oxoglutarate-dependent dioxygenases and, in particular, of flavanone 3-hydroxylase (FHT), involved

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in the biosynthesis of anthocyanidins and other flavonoids (Rademacher *et al.*, 1992). Prohexadione is structurally similar to 2-oxoglutaric acid, enabling the compound to inhibit 2-oxoglutarate-dependent dioxygenases, which are involved in the formation of growth active gibberellins and in flavonoid metabolism (Rademacher, 2000). Our objectives were to quantify the response of grapes to prohexadione-calcium and to determine whether reductions in berry size or crop yield could be related to increased fruit and wine quality.

MATERIALS AND METHODS

Experimental design. The experiments were conducted in the years 2009 and 2010 on field grown grapevines (*Vitis vinifera* cv. Grüner Veltliner on Kober 5BB rootstock). The experimental vineyard was located at the Federal Research Station Agneshof, Klosterneuburg, (Austria). The vineyard was planted with a vine-by-row spacing of 1.20 × 3 m in south orientation, with a permanent green ground cover. The vines were trained to espalier with two vertically positioned canes. Soil and canopy management and grape protection strategy for the year 2009 and 2010 [six applications of: sulphur 0.3%, sulphur 0.3% and dithianon 0.05%, azoxystrobin/folpet (combined product) 0.2%, proquinazid 0.025% and cymoxanil/dithianon (combined product) 0.1%, metalaxyl-m/folpet (combined product) 0.2% and metrafenone 0.02%, cymoxanil/dithianon (combined product) 0.1% and meptyldinocap 0.06%] were carried out as in commercial vineyard. In the evenings of the 9 June 2009 and 22 June 2010, respectively, when sixty percent of the flowerhoods were fallen (BBCH 66) (Lancashire *et al.*, 1991), prohexadione-calcium (1,8 kg/ha; 400 l water) with citric acid (0,1%) was applied with a motorized backpack sprayer (STIHL SR 420). Two applications of botryticides (mepanipyrim 0.12%) were done at BBCH 73 – BBCH 75 (Table 1). All experimental treatments were replicated in four randomized plots consisting of 10 vines.

Weather data. Data were recorded on an ADCON Weather Station located on the trial site.

Grape bunch length. Length was measured of two bunches of the third shoot, counted from the basis of the cordon. If the third shoot was weak, then bunches of the next shoot were considered.

Table 1. Nature of treatments and dates of application.

Number	Treatment	Application date
1	Control	
2	Prohexadione-calcium	9 and 22 June 2010
3	Prohexadione-calcium and Mepanipyrim	9 and 22 June 2010 14 and 19 July 2010
4	Mepanipyrim	14 and 19 July 2010

Bending index. The bending index was developed to assess the compactness of grape clusters. The index was determined using the following scale: 1= firm, 2= flexible, 3= bending up to a maximum of 45°, 4= bending up to a maximum of 90°, 5= bending above 90°. One hundred grapes clusters were analyzed in each treatment for grape bunch length and bending index.

Weight parameter. In 2009 and 2010 the weight of the crop was measured with a laboratory scale on twelve vines per treatment and replicates.

Berry diameter. The diameter of all berries from three clusters per treatment was recorded. To this aim, berries were cut with a scalpel through the middle and measured with a ruler.

***B. cinerea* disease serverity.** The severity of *B. cinerea* attacks was assessed according to the EPPO Standards [PP 1/17 (2)] on 100 bunches on 15 September 2009 and 13 September 2010.

Fourier transform infrared spectroscopy. Bunch ripeness was determined on 100 berries per treatment with Fourier transform infrared spectroscopy (FTIR), which is used for routine quantification of wine and grape parameters (for example sugar concentration). A Foss instrument (WineScan FT 120, Type 77110 with sampling 5027) was used to analyse the must samples (Griffiths and de Hasseth, 2007).

Data analysis. SPSS Statistics 17.0 was used for data analysis. Significance of main effects was determined by analysis of variance (ANOVA) and Tukey.

RESULTS

There was no significant difference between the treatments on cluster length (Fig. 1). Likewise, cluster weight did not differ significantly in the variants (ANOVA, significance = 0.350) (Fig. 2).

When looking at the frequency with which a certain berry diameter was observed, a significantly higher number of berries with a diameter of 3 mm was found in the variant prohexadione-calcium. Similarly, a higher number of berries with a diameter of 8-10 mm was found in this treatment as compared with the control (Fig. 3).

As for the density index, in the year 2009, the treatment with prohexadione-calcium + botryticide differed significantly α=0,000 (α>0.05) from the control and the variant prohexadione-calcium and botryticide, which was the treatment with the loosest grapes. In 2010,

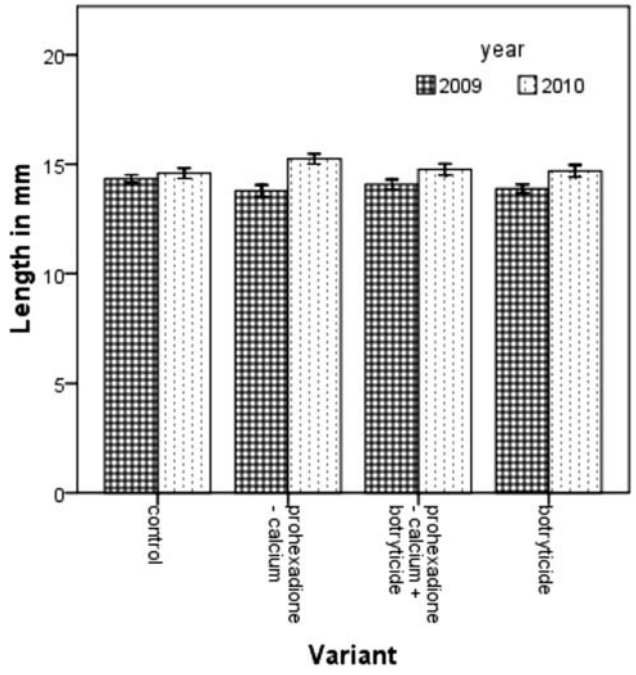

Fig. 1. Cluster length measured before harvest. Values are mean ± standard error (n=100).

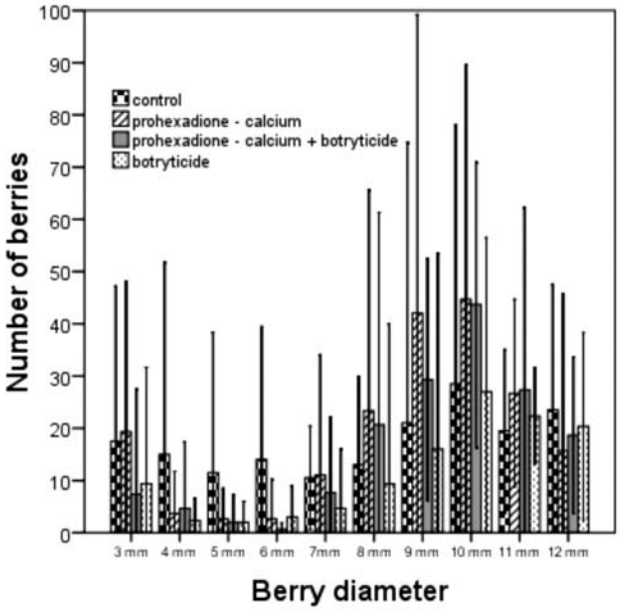

Fig. 3. Frequency histogram of the observation of different berry diameters (n= 3 grapes) in the treatments control, prohexadione - calcium, prohexadione - calcium + botryticide, botryticide.

there was no significant difference at all between treatments (Fig. 4).

In 2009, *B. cinerea* infestation in the control was less than in the other treatments [α=0.029 (α>0.05)], whereas in 2010 no significant difference was found, α=0.110 (α>0.05) (Fig. 5).

As to sugar concentration, in 2009 the variant prohexadione-calcium had one degree less (17 KMW) than the other variants (18 KMW). In 2010, the control had a sugar concentration of 13.5 "Klosterneuburger Mostwaage" (KMW). The highest sugar concentration was observed in the treatment prohexadione-calcium with 14.3 KMW as compared with the botryticide treatment (11.9 KMW) (Fig. 6).

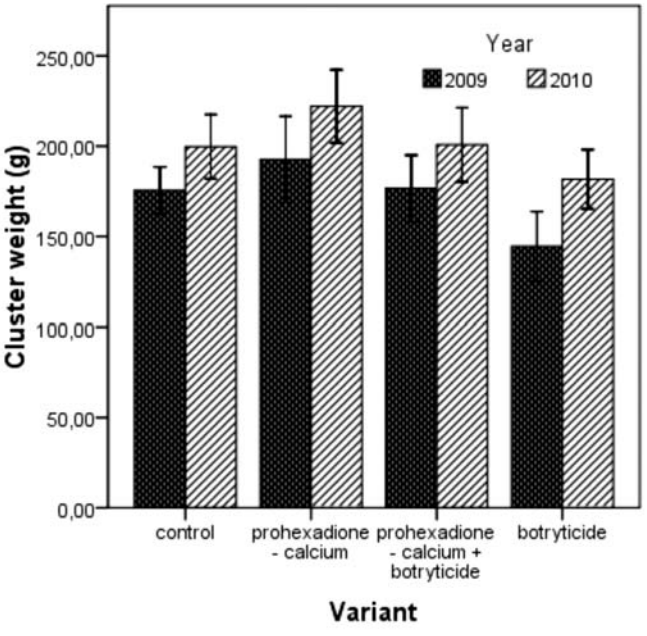

Fig. 2. Cluster weight measured at harvest. Values are mean ± standard error (n=12).

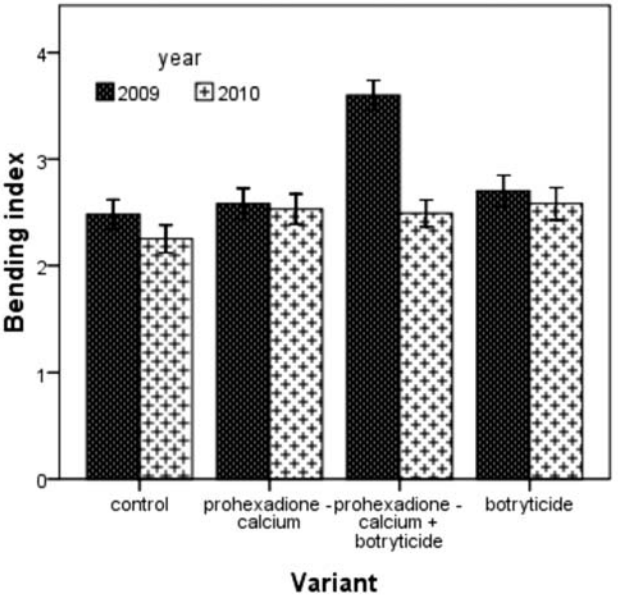

Fig. 4. Bending index used to evaluate the compactness of the clusters. Higher numbers indicate a less compact structure (bars=standard errors; n=100).

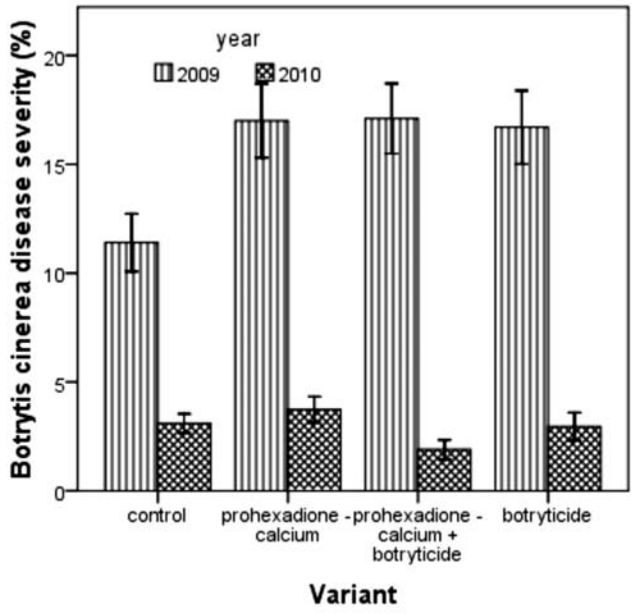

Fig. 5. *B. cinerea* disease severity in the four different variants (bars=standard errors; n=100).

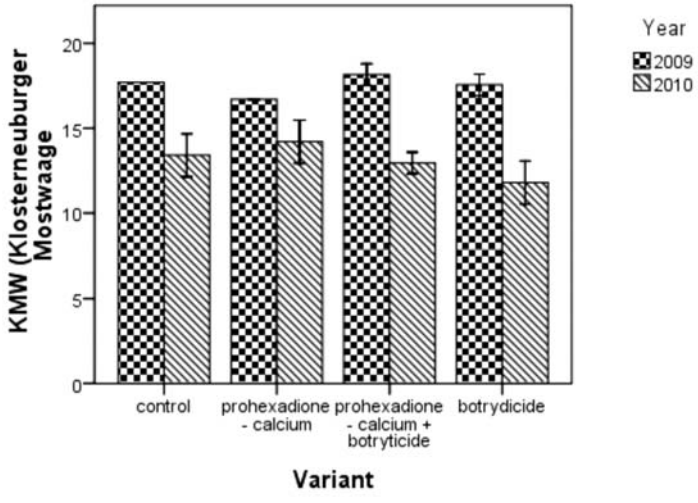

Fig. 6. Sugar concentration measured in KMW (Klosterneuburger Mostwaage). Values are mean ± standard error (n=100).

DISCUSSION

The field experiment showed that disease pressure strongly influences results. The health of the grapes is a primary goal of quality-oriented viticulture. *B. cinerea* is difficult to control because it has a variety of modes of attack, diverse hosts as inoculum sources, and it can survive as mycelium or conidia. For these reasons, the use of any single control measure is unlikely to succeed (Williamson *et al.*, 2007). *B. cinerea* infection is dependent on some kind of damage to the berry skin to provide a point of entry, for example parasite attack, hail damage and strong water absorption which can lead to rapid bursting of the berries or dislodged berries that are imprisoned inside the grape cluster. The thick cuticle of the grape berry usually provides a mechanical barrier against *B. cinerea* infection (Ribéreau-Gayon *et al.*, 2006). Another reason for *B. cinerea* infection can be grape compactness, which leads to burst berries. This depends on the variety, on whether there is a high number of remaining berries after blooming, but also on the size of individual berries (Kast, 2007).

In 2008, prohexadione-calcium yielded less compact grapes, hence preventing *B. cinerea*.attacks. Thus, it improved quality and quantity of the yield without risk for consumers or the environment. As mentioned, prohexadione-calcium is a gibberellin biosynthesis inhibitor whose mode of action differs from that of other gibberellin biosynthesis inhibitors currently used in agriculture. Many of these growth regulators, including the quaternary ammonium compounds, substituted pyrimidines, norbornenodiazetine derivates, and triazole derivates (Graebe, 1987) function by interrupting the synthesis of gibberellin early in the biosynthetic pathway, specially at the synthesis of ent-kaurene. The use of another plant growth regulator (gibberellic acid) leads to the extension of the stems and blossom drop, which can lead to yield reduction and should be avoided. Longitudinal growth can be increased, although not without limits, since a genetically caused border exists (Spies and Hill, 2008). In this experiment, no changes could be observed in length growth following the use of prohexadione-calcium. A correlation between berry weight and number of berries was found although the results showed that the treatment prohexadione-calcium resulted in many small berries with a diameter of 3 mm, which generally reduces compactness. It was confirmed that the use of prohexadione-calcium leads tendentially to more berries with a diameter around 8-10 mm as previously observed by Haas *et al.* (2009). However, in the present study, no significant difference was found in the induction of loose grape clusters and the subsequent infestation of *B. cinerea*. Prohexadione-calcium had minimal effects on pH or titratable acidity and on sugar content of musts in 2009, in agreement with Lo Giudice *et al.* (2004) who reported that an immediate postbloom application had no impact on soluble solids (Brix). In 2010, control bunches had a sugar concentration of 13.5 "Klosterneuburger Mostwaage" (KMW) whereas a higher sugar content was observed in the treatment prohexadione-calcium with 14.3 KMW.

In conclusion, the data presented here show that the result of the application of a plant growth regulator depends on different environmental parameters, among which the exact timing of application during flowering, the rainfall and the pathogen's infestation pressure.

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THE BIOGENIC AMINE HISTAMINE: PHYSIOLOGICAL EFFECT AND CONCENTRATIONS IN WINE

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SUMMARY

Wine samples were collected from German wineries during a two-year study (2007/2008) and their histamine concentration was determined. The data from 2007 showed that between two wineries either no histamine or a low concentration of it was found when with starter cultures (*Oenococcus oeni*) were used or maximum values of 1,01 up to 7,30 mg/l were detected when spontaneous MLF (malolactic fermentation) occurred. In the 2008 survey maximum histamine concentrations ranged between 1,85 and 2,24 mg/l when the musts were inoculated with starter cultures and 2,77 to 3,37 mg/l in the case of spontaneous MLF. The two-year study showed that inoculation with starter cultures reduced concentrations of histamine in wine but if no spontaneous MLF-bacteria were present, there was no histamine in the wines.

Key words: amines, histamines, health, wine quality.

INTRODUCTION

The term biogenic amines covers a group of 20-30 compounds of biological origin that are formed in human, animal and plant tissues and may occur in food containing protein. Some of these amines play a role in biological functions, are important precursors of vitamins or other substances, and may trigger physiological responses. In food, they originate from microbial breakdown of amino acids. High contents of biogenic amines can therefore be found especially in spoiled food, in some cases also in fermented food such as cheese, cold meats, wine or sauerkraut. Biogenic amines ingested with food are normally harmless, since they are broken down by enzymes in the small intestine mucosa (Gappmaier, 2000). Negative effects are only expected in special situations, e.g. in cases of very high intake of histamine and tyramine through food, individual hypersensitivity, or in conjunction with medicines (Askar and Treptow, 1986). Biogenic amines are physiologically active substances that perform important functions, although in higher concentrations they may trigger adverse health effects or even toxic effects. From a nutrition-toxicological perspective the main issues are histamine intoxications and risk of high blood pressure caused by tyramine. The best-known representative of biogenic amines is histamine (Eder, 2003). It has numerous functions in the human organism. Particularly noteworthy is its role in defensive reactions. Histamine is released whenever a cell in the body is damaged. This results in increased permeability of the blood vessels, enabling immune defence cells (e.g. white blood cells) to enter damaged tissue from the blood supply to counteract a possible infection. In addition to these desired effects, the immunological response to alien proteins (allergens) also results in a release of histamine, which, in the case of allergies, causes undesired symptoms such as itching, swelling or a contraction of the respiratory tract (asthma). A similar effect is caused by the venoms of bees, wasp and hornets, which also contain histamine, in addition to other substances (Eder, 2002). Although various barriers limit the intake of orally supplied biogenic amines, food with a high content of such amines can lead to health problems. In particular, this may be the case if the body's regulatory systems are impaired by medication or a chronic intestinal disease. Symptoms can usually be explained by the effects of histamine and tyramine. Little is known about adverse health effects of other biogenic amines (Beutling, 1996). Histamine can also be classified as a poison, since in high concentrations it can trigger a state of shock and therefore have a life-threatening effect. The tolerance limit is around 10 mg, although this may fluctuate widely. In terms of health disorders a distinction is made between histamine intolerance and acute histamine intoxication. Histamine intolerance occurs when histamine as a tissue hormone is produced naturally in the body and as a messenger substance causes allergic reactions. In approxymately 1% of the human population (mainly women) the consumption of even small quantities of food containing histamine can lead to pseudo-allergic responses due to intolerance. These health problems are

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due to increased histamine levels in the plasma or tissue (Biegantski *et al.*, 1983). The symptoms may vary between different individuals and can include abdominal cramps, diarrhoea, flatulence, feverishness, reddening of the skin, skin rashes, itching, runny nose, headache, exhaustion, fatigue, asthma, mental aberration or even aggressive behaviour. The symptoms usually start around 45 min after intake and may require hours to subside. The normal histamine level in the blood is 1 ng/ml. Higher levels indicate increased histamine intake or reduced histamine breakdown (Gappmaier, 2000). Histamine intoxication can be caused by intake of large histamine quantities (100-1000 mg) and can lead to acute symptoms of poisoning within 30-60 min, such as nausea, vomiting, diarrhoea, migraine, asthma, low blood pressure, giddiness, dizziness and circulatory collapse. In persons with histamine intolerance the reactions can be particularly severe, while good histamine tolerance results in weaker reactions (Taylor *et al.*, 1984; Hui *et al.*, 1985). Children are more vulnerable than adults, since their enzyme system is not yet fully developed. Histamine intoxications are most frequently caused by fish and seafood, but can also by triggered by cheese, poultry, sauerkraut, cold meats, beer or wine (Beutling, 1996).

There is currently no limit value for histamine in wine. Various European studies (Straub *et al.*, 1993; Masqué *et al.*, 2007; Herbert *et al.*, 2005) and research projects are currently examining a wide range of aspects, which are discussed in the international technical literature. These projects investigate the formation and occurrence, as well as the avoidance of biogenic amines in wine. Researchers study the viticultural factors (nitrogen fertilization, grape variety, water availability, grape health) and the oenological conditions (spontaneous fermentation, pure yeast fermentation, microbiological contamination of the must, spontaneous MLF, MLF starter cultures, wine aging). In terms of histamine formation and occurrence the focus is on microbiological conversion during malolactic fermentation. As early as 1985, Davis *et al.* (1985) showed that there are significant differences with regard to the formation of biogenic amines within the family Lactobacteriaceae, within the genera that are relevant for wine production, i.e. *Lactobacillus*, *Pediococcus* and *Oenococcus oeni*. The highest amine levels, particularly histamine, were observed with representatives of the genera *Pediococcus* and *Lactobacillus*. In addition to genetic constitution, the occurrence of amino acids, i.e. the precursors of the amines, is a further significant factor. Which species will develop from which genus during wine production depends mainly on the pH value of the wine. Bacteria from the genera *Pediococcus* and *Lactobacillus* (spontaneous MLF) usually multiply at pH values of 3.4 or higher and therefore represent a particular risk in wine production.

The aim of this work was to determine the concentration of histamine in German wines in the autumn 2007 and 2008 either inoculated with MLF starter cultures or undergoing spontaneous MLF.

MATERIALS AND METHODS

Determination of biogenic amines was according to Smit and Ansorge (2005). HPLC with fluorescence detection was done with HP Agilent 1100 Series. The wine samples (15 µl) were injected under a pressure of 220-240 bar and 35°C column temperature on a PerfectSil Target column (250 × 3 mm). Eluent A was 100% acetontrile and eluent B was diluted (1:3) in tris buffer at pH 8.5. The detection was at 254 nm and the emission at 51 nm with a fluorescence detector. HPLC validations of histamine concentration were done at 0.5%, 1%, 2.5%, 5%, 7.5% and 10% standards (dilution with 0.2 N perchloric acid). The wine sample preparation was done in three steps: an extraction step with 10 mM diamino heptane and 0.2 N perchloric acid in relation 1/3 mixed during 60 min, followed by incubation at 4°C for 20 min. The second step was a derivatisation: 300 µl of extract from the extraction step were added to 200 ml Na_2CO_3 and 400 µl dansyl chloride and mixed at 60°C for 1 h in a thermocycler. The cleaning step was a solid phase extraction with the J.T. Baker SPE-System. Washing in the Baker SPE-System was done twice with 3 ml methanol and twice with 3 ml bidistilled water; elution was finally done with 2 ml methanol.

In autumn 2007, 42 wine samples were taken after MLF in the winery GI and 42 samples in GII. The 42 wine samples of each winery consisted of 21 white and 21 red wine samples. Samples (100 ml) were collected at 2/3 height of the tank. Controlled MLF in white wines was done with the commercial product Viniflora CH35 LS and in red wines with Viniflora Oenos LS.

In autumn 2008, 42 samples, 21 of white and 21 of red wine .were colleted in each winery (GI, GII, GIII, GIV, GV, GVI) after MLF, in the same quantity and with the same modality as above. Controlled MLF was also as specified above.

RESULTS

In winery I (GI) 1 million litres of wine were treated with direct inoculation of starter cultures after alcoholic fermentation. MLF was spontaneous in the whole wine batch. In winery II (GII) 1.4 million litres were treated with direct inoculation of starter cultures and in 4.2 million litres MLF was spontaneous. In wines inoculated with starter cultures histamine were either not detected or occurred only in low concentrations. The highest

Table 1. Histamine concentrations at two large wineries (GI & GII) in autumn 2007 with sampling after MLF. Maximum, minimum and mean values are shown. "Controlled" indicates the samples that were treated with direct inoculation of starter culture. "Spontaneous" means that the MLF occurred without addition of starter cultures.

Wineries	G I (controlled)	G I (spontaneous)	G II (controlled)	G II (spontaneous)
Histamine max. value (mg/l)	1.11	7.30	0.00	1.01
Histamine min. value (mg/l)	0.00	0.89	0.00	0.07
Histamine mean value (mg/l)	0.63	2.75	0.00	0.49

concentration (maximum value) in GI was 1.11 mg/l, whereas in GII no histamine was found in any of the analysed wine samples (Table 1). In GI the mean histamine value in spontaneously fermented samples was 2.75 mg/l, but in samples with controlled malolactic fermentation the histamine level was only 0.63 mg/l. The same was observed in GII since samples with spontaneous MLF had a mean histamine level of 0.49 mg/l, whereas no histamine was found in any of samples with controlled MLF. It is interesting to note that in spontaneous samples from GI the maximum histamine level was 7.30 mg/l, while the maximum value for GII was 1.01 mg/l.

Table 2 shows the histamine concentrations in autumn 2008 detected in six large wineries with a total volume of 5.5 million litres. On the average, the wines with starter cultures showed somewhat lower mean and maximum values (Table 2). This is illustrated by the mean histamine values of 1.12 mg/, 1.17 mg/l and 1.41 mg/l for GI, GIII and GIV respectively. Slightly higher histamine concentrations were found in the wines where spontaneous MLF had occurred. The values were 1.38 mg/l, 1.58 mg/l and 1.60 mg/l from GII, GV and GVI, respectively.

DISCUSSION

In both studies (autumn 2007 and 2008) the addition of direct inoculation with starter cultures reduced histamine development. On the other hand, an addition of starter cultures is no guarantee for histamine-free wines. One of the main causes for histamine formation is likely the natural wine flora, which is present in any winery. Only pasteurization or flash pasteurization of the grape mash can reduce the natural flora sufficiently so as to eliminate histamine in wines with direct inoculation of starter cultures. In any case, it should be noted that in smaller and medium-sized wineries fluctuations in histamine concentrations were significantly larger.

To conclude, in the absence of MLF, histamine-free wine can be produced through application of direct inoculation of starter cultures. Our studies have shown that the use of direct inoculation of starter cultures results in lower histamine concentrations than in wines with spontaneous MLF. These wines exhibit larger variations (minimum/maximum values).in histamine content.

Table 2. Histamine concentrations at six large wineries (GI-GVI) in autumn 2008 with sampling after MLF. Maximum, minimum and mean values are shown. "Controlled" indicates samples that were treated with a direct inoculation of starter culture. "Spontaneous" means that the MLF occurred without addition of starter cultures.

Wineries	G I (controlled)	G II (spontaneous)	G III (controlled)	G IV (controlled)	G V (spontaneous)	G VI (spontaneous)
Histamine max. value (mg/l)	1.85	2.95	2.24	1.87	3.37	2.77
Histamine min. value (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00
Histamine mean value (mg/l)	1.12	1.38	1.17	1.41	1.56	1.60

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GRAPEVINE-*PLASMOPARA VITICOLA* INTERACTION. **H.H. Kassemeyer**. *Staatliches Weinbauinstitut, Merzhauser Strasse 119, 79100 Freiburg im Breisgau, Germany. E-mail: hannsheinz.kassemeyer@wbi.bwl.de*

The grapevine pathogen *Plasmopara viticola* belongs to the oomycetes, which differ from the true fungi. *P. viticola* originates from the south-east of the USA, where it occurs on wild grapevine species such as *Vitis aestivalis* and *V. riparia.* In 1878, symptoms of downy mildew caused by *P. viticola* were detected for the first time in Europe in the south-west of France. The following disastrous pandemic of downy mildew in Europe revealed the high susceptibility of the European *Vitis vinifera* cultivars. In the opposite, the wild American species express a more or less effective resistance. Recent findings on the interaction pathogens/host plants reveal new insights in defence response of the plants and disclose new aspects about susceptibility and resistance. The aim of this work was to characterize both resistance and susceptibility to *P. viticola* of grapevine genotypes. We are studying the infection process and the host plant response at the molecular, biochemical and structural level. For this purpose, we are looking for genes in the pathogen and the host plant involved in the particular interaction. In this context we have cloned and sequenced putative defence genes such as PR proteins and have followed the course of colonization of host tissue with microscope observations. To analyse the kinetics of defence response and the transcriptional activity of PR proteins after a challenge infection by *P. viticola,* a quantitative PCR (qPCR) by means of real time PCR was developed. The quantification of the PR protein activation revealed differences in the time course of the transcription of a β-1,3 glucanase, belonging to the PR2 family (*V*PR2). Transcription of *V*PR2 was activated within the first 12 h post inoculation (hpi) in the resistant genotypes resulting in a continued approximately 20 to 30 fold induction. In susceptible genotypes only a slight increase of the *V*PR2 transcript occurred not until 48 hpi. By means of epifluorescence microscopy (EFM) and low temperature scanning electron microscopy (LTSEM) the specific infection stages were analyzed. In the susceptible genotype, *P. viticola* established itself within 12 hpi in host tissues and formed a substomatal vesicle with primary hyphae. At that time, the primary hyphae had already grown in the intercellular spaces of the host plant mesophyll. After 24 hpi, primary hyphae quickly colonized the intercellular spaces and branched. At 48 hpi, the branched hyphae eventually formed a mycelium. In the resistant genotype, however, the pathogen ceased its further development after 24 hpi and no mycelium was formed. The comparison of gene induction with the course of infection and colonization of the host tissue by *P. viticola* showed a distinct pathogen development in resistant and susceptible genotypes. In the former, *P. viticola* was able to form penetration structures and a few haustoria, but mycelial growth and development of haustoria was not as abundant as in the susceptible genotypes. Molecular and microscopic studies indicated that in resistant genotypes the defense response was activated within a short time after the first contact between host and pathogen. We assume that the first contact between the penetration peg and the guard cells during the penetration process plays a crucial role in resistance induction. The inhibition of further pathogen development after the formation of the first haustoria in the resistant genotypes supports this assumption.

MOLECULAR AND CYTOLOGICAL RESPONSES OF GRAPEVINE AGAINST DOWNY MILDEW CAUSED BY *PLASMOPARA VITICOLA*. **M. Selim[1,2], G. Langen[2], B. Berkelmann-Löhnertz[3], K.H. Kogel[2] and D. Evers[1].** *[1]Centre de Recherche Public-Gabriel Lippmann, Department Environment and Agro-Biotechnologies, 41 Rue du Brill, 4422 Belvaux, Luxembourg. [2]Research Centre for BioSystems, Land Use and Nutrition, Justus Liebig University Giessen, 26 Heinrich-Buff-Ring, 35392 Giessen, Germany. [3]Geisenheim Research Center, Institute of Biology, Department of Phytomedicine, 1 von-Lade-Strasse, 65366 Geisenheim, Germany. E-mail: selim@lippmann.lu; evers@lippmann.lu*

Downy mildew, caused by *Plasmopara viticola,* is one of the most destructive grapevine diseases in Europe and in the eastern half of the United States. In 1996, almost 10% of the sales value of the global fungicide market was for grapevine's downy mildew. Therefore, induced resistance to *P. viticola* is an appealing method for controlling the disease in a more sustainable way. This work aims at inducing the plant's defence mechanisms against *P. viticola* by some (biogenic and abiogenic) elicitors, through a protective method, where the elicitor is applied before the infection, as well as through a curative method, where the elicitor is applied after the infection. Disease severity was recorded to assess the elicitor's efficacy. The level of induced resistance was estimated through assessment of callose deposition as well as through a study of the expression of some defence-related genes. Disease severity analysis showed that the protective treatment was more efficient than the curative treatment. Callose deposition was more abundant in the protective than in the curative treatment. At the molecular level, the gene expression of some defence related genes (*chitinase1b, lipoxygenase, stilbene synthase*) was analysed using real-time RT-PCR. Results showed differential expression of these genes between the different treatments. For example, in the curative treatment, non infected plants (only elicited) showed a higher expression of the above-mentioned genes in comparison with the infected plants.

NEW STRATEGIES FOR MILDEW RESISTANCE BREEDING IN GRAPEVINE. **R. Eibach and R. Töpfer.** *Federal Research Centre for Cultivated Plants, Julius Kuehn-Institute, Institute for Grapevine Breeding, Geilweilerhof, 76833 Siebeldingen, Germany. E-mail: rudolf.eibach@jki.bund.de*

Since the introduction of phylloxera and mildew diseases from North America to Europe during the second part of the 19th century, grape breeders around the world have been engaged in the introduction of resistance traits existing in wild American and Asian *Vitis* species into the gene pool of European quality vines. These activities have led to remarkable success for cultivars with high wine quality and a high degree of resistance against downy and powdery mildews were obtained, thus allowing a considerable reduction of plant protection. Recent research on the genetics and inheritance of resistance traits have led to the identification of different loci for both downy and powdery mildew. These loci can be tagged by molecular markers so that the use of marker-assisted selection (MAS) allows the identification in a crossed population of genotypes that have inherited resistance-related loci from their parents. When crossing parents with different resistance loci, MAS allows seedling selection within the offspring that carries resistance loci from both female and male parent. It is expected that this kind of pyramiding resistance loci in new cultivars increases resistance level with respect to resistance sustainability. Currently, the breeding programme is directed to pyramiding six resistance-related loci, three of them related to downy mildew and the other three related to powdery mildew. The first breeding lines established with the combination of four loci (two each for downy and powdery mildew) exhibit a very high degree of resistance to both mildews. One of these lines was used for a further cross for adding an additional powdery

mildew-related locus. The use of MAS disclosed the existence of seedlings with pyramided five resistance loci within this population.

MATRIX METALLOPROTEINASES ARE INVOLVED IN PLANT RESISTANCE TO *BOTRYTIS CINEREA*. P. Zhao[1,2], D. Liu[2], G. Langen[2] and K.H. Kogel[2]. *[1]College of Horticulture, Northwest A&F University, Taicheng Road 3, 712100 Yangling, China. [2]Institute of Phytopathology and Applied Zoology, Heinrich-Buff-Ring 26-32, University of Giessen, 35392 Giessen, Germany. E-mail: karl-heinz.kogel@agrar.uni-giessen.de*

Botrytis cinerea causes massive losses to grapevine, strawberry and tomato production. A thorough understanding of the plant defences against *Botrytis* may provide new approaches for disease prevention, thereby securing food quality under changing global conditions. The genome of *Arabidopsis thaliana* contains five matrix metalloproteinases (*At1-MMP* to *At5-MMP*) and their gene expression profiles after *B. cinerea* inoculation were investigated. Using RT-PCR analysis, we found expression of both *At2-MMP* and *At3-MMP* upregulated in *Arabidopsis* leaves after *B. cinerea* infection. Because *At2-MMP* showed the strongest pathogen-responsiveness, we tested the *Arabidopsis at2-mmp* mutant for disease resistance. We found that *at2-mmp* was more susceptible to *B. cinerea* than wild-type plants. Consistently, ectopic over-expression of *At2-MMP* in *Arabidopsis* increased resistance to *B. cinerea* compared to control transformants. We addressed the question whether resistance to *B. cinerea* depended on the salicylate (SA) or jasmonates (JA)/ethylene (ET) defence pathway. *B. cinerea* induced an expression of *At2-MMP* in the signaling-defective mutants including *NahG, jar1.1, ein2-1, npr1-1* comparable to wild-type plants. These data suggest that *B. cinerea*-induced expression of *At2-MMP* might be independent of SA, JA and ET signaling. *At2-MMP* has typical characteristics of other plant and animal MMPs, i.e. the recombinant protein exhibited myelin basic protein proteolytic activity, and was inhibited by the zinc-chelator EDTA in a dose-dependent manner. Considering the conservation and wide distribution of matrix metalloproteinases in the plant kindom, we speculate a general role of MMPs as modulators of plant defences.

RAIN-INDUCED GRAPE BERRY SPLITTING: A PROBLEM OF SURFACE WATER TRANSPORT? T. Becker[1], A. Kortekamp[1], F. Louis[1] and M. Knoche[2]. *[1]Kompetenzzentrum Weinforschung am Dienstleistungszentrum Ländlicher Raum Rheinpfalz, Breitenweg 71, 67435 Neustadt, Germany. [2]Leibniz University Hannover, Institute for Biological Production Systems, Herrenhäuser Strasse 2, 30419 Hannover, Germany. E-mail: moritz.knoche@obst.uni-hannover.de*

Cracking of grape berries is thought to result from increased volume and turgor that strains the berry skin beyond its limit of extensibility. Water transport may occur through the vascular system of the vine or through the surface of the berry. We focused on transport through the surface of detached cvs Chardonnay, Müller-Thurgau, and Riesling berries. Sealing the receptacle including the pedicel/fruit juncture with silicon rubber decreased water uptake by about 80%, but transpiration by only 20%. Thus, water uptake in the receptacle region was rapid and must have occurred by viscous flow, but transpiration through the surface was slow and occurred by diffusion. The water potential of cv. Riesling berries decreased throughout development from -0.52 (±0.18) MPa at 20 DAFB to -1.56 (±0.04) MPa at maturity. In the same time period, the permeability of the skin decreased, averaging 4.1 (± 1.2) and 1.6 (±0.0) nm/s in osmotic water uptake and transpiration, respectively. Comparison of our data with those on transport through the vascular system of table grape cv Italia [Lang and Thorpe (1989), downscaled by a factor of 4 because of difference in mass] revealed that on a rainy day, the increase in mass caused by water uptake through the surface may account for up to 38.5% of the increase in berry volume, but transpiration may reduce the mass gain by up to 61.7%.

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SECONDARY METABOLITE PRODUCTION IN ESCA-ASSOCIATED FUNGI AND IMPACT OF FUNGICIDES ON THE BIOSYNTHESIS RATE. J. Fischer[1], E. Birner[2], M. Merz[2], J. Rether[2], L. Antelo[1], A.J. Foster[1], T. Opatz [3] and E. Thines[1]. *[1]Institute of Biotechnology and Drug Research, Erwin-Schroedinger-Strasse 56, 67663 Kaiserslautern, Germany. [2]BASF SE, 7114 Limburgerhof, Germany. [3]Johannes Gutenberg University Mainz, Department of Organic Chemistry, Duesbergweg 10-14, 55128 Mainz, Germany. E-mail: fischer@ibwf.de*

Esca is a destructive disease of grapevine caused by several endophytic fungi, mainly *Phaeoacremonium chlamydospora*, *Phaeomoniella aleophilum* and *Fomitipora mediterranea*. It has been suggested that phytotoxins are secreted by the fungi inducing disease development in the leaves and bunches. Several toxins produced by Esca-associated fungi have been reported. To characterize phytotoxic metabolites produced by the different pathogenic fungi, they were grown individually and in co-culture in submerged cultures. Several metabolites were identified by bioactivity-guided isolation and HPLC-MS as well as by NMR-analysis. The isolation and identification of the compounds were based on phytotoxic, cytotoxic and antimicrobial activities. Several fungal secondary metabolites were identified which have not been reported for Esca-associated fungi. Amongst the bioactive metabolites identified there were siderophores, e.g. triacetylfusigen as well as linoleic acid, methylemodin, phaeofuran and methoxycoumarin. The production rates of the bioactive secondary metabolites were analysed under stress conditions, such as heat stress, salt stress or stress induced by sublethal concentrations of F500, a fungicide of the strobilurin class. It was found that the application of the fungicide at sublethal concentrations under salt stress conditions resulted in a significantly lower production rate of phytotoxic compounds.

MODELLING THE EFFECT OF PLANT GROWTH AND SUSCEPTIBILITY ON THE DEVELOPMENT OF A PLANT DISEASE EPIDEMIC: POWDERY MILDEW OF GRAPEVINE. A. Calonnec. *INRA-Bordeaux, UMR INRA-ENITA 1065 Santé Végétale, BP 81, 33883 Villenave d'Ornon, France. E-mail: calonnec@bordeaux.inra.fr*

Vitis vinifera is highly susceptible to many pathogens. According to a survey by the European Commission, in 2007, growers in Europe used 70,000 tons of fungicides for grape protection. In term of investment, downy mildew (33%) and powdery mildew (22%) are the main concern, preceding grey mould (9%) and insects (16%), and are responsible for 533M€ expenditure in Europe (data from Bayer CropScience 2005-2008). It is therefore ur-

gent for the grapevine pathosystem to move towards an integrated production of grapes giving priority to production systems that are economically viable with respect to the environment. Understanding the factors that trigger the development of an epidemic is essential if we are to create and implement effective strategies for disease management. Modelling is a key approach allowing to handle various scenarios for pathogen, host, and/or crop management. We have to differentiate empirical models from mechanistic ones. Empirical models tend to summarize the general relationships among the host, the pathogen and the environment and can be used to infer the underlying biology of a system without directly identifying causality (De Wolf and Isard, 2007). For example, they look for the relationships between climatic variables and the appearance of disease symptoms. These kinds of models usually trigger one stage of the disease cycle (e.g.. primary infection, dormancy, etc.). Most of the time, for the grapevine pathosystem, these models are weather-driven with no or little input variables linked to the disease (e.g. source of primary inoculums). They can be developed to predict the risk of disease appearance for one region but need to be calibrated to be useful for another region. They often lack in predicting a level of risk. Mechanistic models provide a convenient means to combine a number of sub-models representing unique parts of the disease cycle to discover causal relationships between the components of the system. These models are used to explore the relationship in the pathosystem for a wide range of scenarios to find the most favourable or unfavourable conditions for disease development or to identify part of the disease cycle that needs further experiments. These types of models are however usually not appropriate for disease risk prediction. The grape-powdery mildew pathosystem is characterised by a polycyclic pathogen capable of explosive multiplication, a host population with a high degree of spatial structure at the field level and with a complex architecture at the individual plant level, exhibiting rapid changes over time. Different kinds of models have been developed, either empirical models, to predict the primary inoculum risk based on more or less complex rules and data (Kast, 1997; Gubler *et al.*, 1999; Gadoury *et al.*, 1990) or mechanistic models to describe the secondary infections or the epidemics' development (Chellemi and Marois, 1992; Sall, 1980). A brief review of these models can be found in Legler *et al.* (2010). However, none of these models are yet able to predict the time and amount of primary inoculum and the protection against the disease is very often systematic. Because of the tight relationship between powdery mildew and its host (Doster and Schnathorst, 1985; Gadoury *et al.*, 2003) and of the spatial localization of primary inoculum on the vine stock, we hypothesized that the dynamic changes in crop structure and susceptibility should be considered as key factors for explaining variability in the severity of epidemic behaviour. Interactions between diseases and vine growth were investigated in several studies (Evans *et al.*, 2006; Gadoury *et al.*, 2001; Zahavi *et al.*, 2001) and we could show that the high heterogeneity in disease progression makes the spatial disease prediction very difficult (Calonnec *et al.*, 2009). Then, we devised a simulation model to better understand the vine/powdery mildew interactions and to explore how the host development and management can modify disease spread. The model is an epidemiological simulation coupling vine growth with the dispersal and disease dynamics of *Erysiphe necator* at the vine stock scale (Calonnec *et al.*, 2008). It is mechanistic, with sub-models either coming from the literature or from empirical data. The model allowed simulating the spatio-temporal dynamics of host growth and epidemic development beginning from a range of climatic conditions, production systems and initial conditions for the density and location of the pathogen. Particularly, the model takes into account shoot topping, which has for effect, to enhance the development of secondary shoots and the emergence of new susceptible leaves during the epidemic process. Input variables are environmental (temperature, wind speed and direction) or related to the pathogen (location and onset of primary infection). Input parameters characterise the crop system (number of buds, distance between buds, shoot topping, vigour), and conditions of growth for the vine and the pathogen. Output describes, at each time step, number, age and pattern of the healthy and infected organs, infected and infectious leaf area and aerial density of spores released. A focus will be done on the bases of the model and the sensitivity of the epidemic to variation of parameters of pathogen, plant growth or crop management as well as the relationship between host and disease variables at key periods in the epidemic process for different conditions of vine vigour.

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ELABORATION AND VALIDATION OF A DOWNY MILDEW FORECAST MODEL REGARDING SOIL-BORNE INFECTIONS. **B. Berkelmann-Loehnertz[1], O. Baus[1], H. Hassemer-Schwarz[2] and C. Fruehauf[3].** *[1]Geisenheim Research Center, Section of Phytomedicine, Von-Lade-Strasse 1, 65366 Geisenheim, Germany. [2]Deutscher Wetterdienst (German Meteorological Service), Kreuzweg 25, 65366 Geisenheim, Germany. [3]Deutscher Wetterdienst (German Meteorological Service), Centre*

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Downy mildew, caused by *Plasmopara viticola*, is one the most important grape diseases. A dense spraying schedule is required to prevent severe crop losses. Since many years, consumers and environmentalists demand low input strategies. An essential tool for fungicide reduction programs is the use of forecast models supporting local extension work. Until the end of the 80s and the beginning of the 90s of the last century, downy mildew forecast was based on the secondary or leaf borne life cycle of *P. viticola*. The start of the epidemic (primary infection) was estimated by simple, mostly temperature based rules. In the following years a fundamental gap between forecast results and disease development on untreated monitoring sites was apparent. In order to elucidate this essential hindrance, genetic analyses of soil surface adjacent and more distant oil spots and detailed epidemiological studies were conducted. Based on this, the significance of soil-borne infections later in the growing period could be identified for the first time. Up to now, the assumption was that soil-borne infections seem to be triggered by strong rainfall and splash events. The next step was to elaborate an additional disease model that integrates soil-borne infections. Therefore, different sub-models were created: (i) dynamics of oospore ripening and germination, (ii) splash algorithm, and (iii) primary infection index. To realise this, an existing micro climate model used for arable crops was adapted to the specific conditions in vineyard rows. Together with the items of the secondary disease model, these new components are managed by AMBER (Agrarmeteorologische Beratungsverfahren), the agrometeorological advisory services of the German Weather Service. Necessary input parameters are the recorded data of the local weather stations, mounted in the vineyards, and the meteorological data of the numeric weather forecast. In order to optimise spraying intervals the simulation of leaf appearance and leaf area development of primary shoots was integrated as well (Schultz, 1992). Based on the model output, days with soil-borne and/or leaf-borne infections can be compiled. Individual lengths of incubation periods can be outlined. With the evaluation of the requested data all steps required for model editing could be finalised. The last step is the validation of the new model. In the Rheingau region, automatic weather stations are mounted in numerous vineyards and three monitoring sites are available for the collection of epidemiological data in untreated plots. These historical data were used for validation purposes. Of special interest is the key-date scheduling of the primary infection. Data processing clearly indicates, that a high degree of congruence was obvious for severe downy mildew years as well as for growing periods with low disease pressure. For the years 2009 and 2010 forecast results reflected extremely precise downy mildew epidemics in different areas of the Rheingau region. In summary, the new Geisenheim downy mildew model enables to predict the date of the primary infection and further soil-borne infections. Together with the infestation data of the secondary disease cycle the complete epidemiology within a growing season can be displayed for the growers. The negative impact of false negative and/or false positive forecast results is out of focus now. Twice a week, growers are provided with the disease model output, weather forecast data, management advices, specific information on pests and diseases as well as on leaf area development via internet.

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IMPACT OF SPRAY TIMING AND FUNGICIDE RESISTANCE ON FUNGICIDE EFFECTIVENESS AGAINST GREY MOULD IN THE VINEYARDS. A.N. Petit[1], P. Vatsa[1], A.S. Walker[2], P. Leroux[2], M.L. Panon[3], C. Clément[1], F. Fontaine[1] and N. Vaillant-Gaveau[1]. *[1]Université de Reims Champagne-Ardennes, Unité de Recherche Vignes et Vins de Champagne EA 2069, Laboratoire de Stress, Défenses et Reproduction des Plantes, UFR Sciences Exactes et Naturelles, Moulin de la Housse, B.P. 1039, 51687 Reims Cedex 2, France. [2]INRA, UMR 1290 BIOGER CPP, Route de St Cyr, 78000 Versailles, France. [3]Comité Interprofessionnel du Vin de Champagne, 5 Rue Henri Martin, BP 135, 51204 Épernay Cedex, France. E-mail: parul.vatsa@univ-reims.fr*

Fungicides are used to control *Botrytis cinerea,* the agent of grey mould of grapevines. Three preventive applications of fungicides are recommended to reduce the risk of the disease: at the end of flowering (BBCH 68), bunch closure (BBCH 77) and beginning of veraison (BBCH 81). The hydroxyanilide derivatives are among the most effective fungicides, registered to control *B. cinerea*. In our study, their effectiveness was examined in relation to spray timing during the course of five years (2002-2007) at different crop stages and fungicide resistance in grapevine. Interestingly, it was observed that the earlier the application of these fungicides, the better was the protective effect against *B. cinerea*. The efficiency of hydroxyanilide derivatives was followed according to the application stage: BBCH 68, 77 and 81. When the presence of *B. cinerea* fungicide resistant strains was evaluated at harvest, it was found that they were similar among treatments and years, except for a class of multidrug resistant strains (MDR 2) whose frequency increased after fungicide applications. Although current spray programmes including fenhexamid (a hydroxyanilide derivative) appear to control bunch rot at the current MDR frequency, a propagation of MDR 2 strains might lead to a decline in disease control. Our results have shown that a standard programme of three fungicide (Fenhexamide: Teldor; Fludioxonil: Geoxe-Syngenta; Pyrimethanil: Scala-BASF) applications provided the best control of *B. cinerea* in the Champagne region, in comparison with a single treatment of Teldor at any of the crop stages. More precisely, the application of Teldor at flowering (BBCH 68) seems to be the most effective against bunch rot disease.

PRODUCTION OF BIOGENIC AMINES BY GRAPE- AND MUST-ASSOCIATED MICROORGANISMS. H. König. *Johannes Gutenberg-University, Institute of Microbiology and Wine Research, 55099 Mainz, Germany. E-mail: hkoenig@uni-mainz.de*

Due to health concerns regarding biogenic amines we have investigated German red and white wines from different grape varieties by high-performance-liquid chromatography using Strata-X-C and Bond ElutSCX cation exchange cartridges before derivatization of the amines with ortho-phthaldialdehyde. Twelve biogenic amines were detected using heptylamine as internal standard. The detection limit ranged from 0.5 mg/l for histamine to 0.1 mg/l for the other amines under investigation. Up to eight biogenic amines were found in German wines. The mean values of histamine were lower than those of most other biogenic amines like tyramine, phenylethylamine, ethanolamine and isopentylamine. In addition, we present a general overview about the diversity of biogenic amine-forming lactic acid bacteria (LAB) as a function of different winemaking techniques in recent young wines from Palatinate. We have investigated the potential of bacterial isolates from young wines produced by different technologies of the vintage 2008. According to our results, more than half

of the bacterial isolates from 53 red and white wine samples were able to produce biogenic amines. Tyramine, histamine and ethylamine were the major biogenic amines detected. Bacterial strains were identified by specifically amplified polymorphic DNA–polymerase chain reaction (SAPD-PCR), a molecular fingerprinting method based on the amplification of species-specific gene sequences.

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ESTIMATION AND QUANTIFICATION OF COPPER AND TEMPERATURE EFFECTS ON GROWTH OF VINE SPOILAGE FUNGI AND GEOSMIN PRODUCTION BY *PENICILLIUM EXPANSUM*.

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Copper is an active component of many fungicides used in agriculture and especially in vineyard treatments. The effect of copper (II) ions on the growth of two grape rot fungi, *Botrytis cinerea* and *Penicillium expansum* was evaluated. Both fungi were grown on Czapek agar medium, at 15, 20 and 25°C, with different concentrations of copper added, and the radial growth rate was determined. The combined effects of copper, temperature and carbon dioxide, on geosmin production by *P. expansum* were also studied. An experimental device hermetically closed was developed for easy monitoring of fungal growth. Experiments were carried out according to a Doehlert matrix. Depending on the temperature, these fungi grew up to 10 mM copper. *B. cinerea*, a fast grower, had higher growth rates than *P. expansum*, i.e.: 1.50 mm×day^{-1} and 0.20 mm×day^{-1} respectively, in the presence of copper 4 mM. At the Geosmin production by *P. expansum* depended on copper level. In particular, there was an increased production up to a rate between 1 and 1.2 mM (63.75 and 76.50 mg l^{-1}), a decrease at higher concentrations and total inhibition of production beyond 5.2 mM (331.50 mg l^{-1}). Under experimental conditions of Doelhert matrix, effects were established of interactions between factors. Contrary to copper, temperature and CO_2 had negative effects on geosmin production. Measurement of individual and interaction effects of these factors on *B. cinerea* and *P. expansum* development will contribute to a more appropriate use of pesticides to prevent organoleptic defects of biological origin. Depending on the temperature, the Bordeaux mixture (0.16 to 0.32 mM copper) used in vineyards may have a positive effect on the growth of these fungi and even a positive effect on geosmin production by *P. expansum*.

IMPACT OF GRAPE VARIETY AND WINE TYPE ON THE PERCEPTION THRESHOLDS OF ROT-RELATED MUSTY AND MOULDY OFF-FLAVOUR COMPOUNDS IN WINE.

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In the last years, an increasing amount of wines suffering from musty and mouldy off-flavours caused by rot on grapes was reported by different authors (Darriet *et al.*, 2000, La Guerche *et al.*, 2006; Eder *et al.*, 2008). Among others, the compounds geosmin, 2-methylisoborneol (MIB) and 1-octen-3-ol were identified as the cause of an earthy, mouldy or mushroom like off-flavour in wine. Geosmin (trans-1,10,dimethyl-trans-9-decalol), is a metabolite of various actinomycetes and fungi. In wine, its source can be linked to the effect of rot on grapes, especially secondary rot inducers like *Penicillium* spp. can produce high geosmin amounts in infected grapes. Geosmin is stable during alcoholic fermentation and is transferred into wine (La Guerche *et al.*, 2006). Along with geosmin, 2-methylisoborneol, and 1-octen-3-ol are known as compounds closely linked with the growth of moulds (Simpson, 2005). 1-octen-3-ol is a typical mould metabolite responsible for a typical fungal and mouldy flavour. MIB, a metabolite of *B. cinerea*, some *Penicillium* spp. and *Streptomyces* spp. has a flavour mainly described as earthy, musty and camphor (Kugler and Rapp, 1997; Hesford and Schneider, 2002; La Guerche *et al.*, 2006). During a research project at the Geisenheim Research Center (Germany), the effect of wine style and grape variety on perception thresholds of geosmin, 2-methylisoborneol, and 1-octen-3-ol was tested in different sensory trials. Three white wines from Germany [cvs Gewürztraminer (sweet), Riesling (dry) from Rheingau, and cv. Riesling (dry) from Mosel] and two red wines, one from Germany [cv. Pinot noir (dry) from Rheingau] and one from south-east Australia (cv. Merlot, dry) were spiked with different amounts of the above mentioned compounds and evaluated by a panel consisting of 15 experienced tasters. Perception thresholds for geosmin in the tested white wines were between 40 and 45 ng/l and up to 180 ng/l in the red wines. For the 1-octen-3-ol the perception thresholds for the white and red wines were between 6 and 12.5 µg/l. As to 2-methylisoborneol, the panellists found perception thresholds between 12.5 and 35 ng/l.

Darriet P., Pons M., Lamy S., Dubourdieu D., 2000. Identification and Quantification of Geosmin, an Earthy Odorant Contaminating Wines. *Journal of Agriculture and Food Chemistry* **48**: 4835-4838.

Eder R., Weingart G., Brandes W., 2008. Occurrence of geosmin in Austrian wines, reduction by means of special filter layers and estimation of sensory threshold values. *Book of Abstracts Grapelux, Remich 2008*: 14.

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La Guerche S., Douphin B., Pons M., Blancard D., Darriet P., 2006. Characterization of some mushroom and earthy off-odours microbially induced by the development of rot on grapes. *Journal of Agricultural and Food Chemistry* **54**: 9193-9200.

CHARACTERIZATION AND REMOVAL OF OFF-FLAVOURS IN WINES.

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Geosmin is an unacceptable molecule because of the earthy-mouldy taste it gives to wines. Geosmin is produced by some *Penicillium* spp. in general and by *Penicillium expansum* in particular. Its biosynthesis needs a contamination by *Botrytis cinerea* (La Guerche *et al.*, 2005). The production of geosmin can occur with a very small percentage of *Botrytis* contaminated berries.

Thus, classical methods used to estimate the *Botrytis* disease severity can be inadequate to detect a potential geosmin production risk. Moreover, the detection threshold of geosmin in wine is around 60 ng/l (Darriet *et al.*, 2000). As long as contamination from the vineyard cannot be avoided, must or wine treatment will be an important issue. A large diversity of oenological devices is proposed to the winemakers, including activated carbons, filtration, chitosan and some old well-known practices (oil, cream, etc.). In the present work, some of these products were tested for their potential to remove geosmin on geosmin-spiked white wine. After Headspace Solid Phase MicroExtraction, post-treatment geosmin contents were analysed by Gas Chromatography coupled to Mass Spectrometry (HS-SPME GC-MS). The first trial was done on a micro-scale filtration unit. It included several paper filters and the Filtrox TX-R. In the second trial, chitosan (50 and 100 g/hl), deodorant and bleaching activated carbons were tested at doses of 20 and 40 g/hl. The active carbon GOTA gave the best results. At 20 g/hl, the efficiency was 81%, while at 40 g/hl it was 97%. Filtrox TX-R eliminated 97% of the initial geosmin quantity. The Siha active carbon Fa used at 40 g/h gave an efficiency of 86%. The efficiency of six other carbons ranked between 0 and 61%, which made them less interesting. The tested chitosan had a quite low efficiency, with a maximum of 22%. To confirm these first results, the same experiments will be repeated on a more sensitive and selective GC-MS/MS system, coupled with tasting by trained people to determine the incidence of the treatment on the sensorial profile of the wine.

La Guerche S., Chamont S., Blancard D., Dubourdieu D., Darriet P., 2005. Origin of (-)-geosmin on grapes: on the complementary action of two fungi, *Botrytis cinerea* and *Penicillium expansum*. *Antonie van Leeuwenhoek* **88**: 131-139.

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OPTIC-MECHANICAL ELIMINATION OF QUALITY-DECREASING PARTICLES OUT OF MACHINE HARVESTED GRAPES AS A QUALITY MANAGEMENT METHOD IN VITICULTURE.

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The consequence of climate change is a creeping alteration in viticulture. Increasing pH values cause microbiological hazards. Primary, secondary and tertiary fungal infections lead to negative sensory changes as well as contamination with mycotoxins and biogenic amines. Limits for these substances are either already defined or under evaluation. Insects like the multicoloured Asian ladybeetle *Harmonia axyridis* also cause off-flavours. Until now, selection of grapes after harvest had no importance in German viticulture. Because of the low concentration of rotten components, the financial efforts were too high. Hand picking allowed selection in the vineyard, but since more and more harvesting machines are being used, even in steep slope viticulture, another solution must be found. Available systems of automatic mechanical sorting only partly fulfill the necessary criteria concerning efficiency, careful handling of the grapes and hygienic conditions. Sorting systems with optical methods, long known in food industry, can comply with the required criteria. These sorters operate with RGB colour camera and/or laser-induced fluorescence systems. Prior to sorting, the machine has to be taught with "good" and "bad" product. A computer compares camera and laser pictures with the given data and sorts the grapes by blowing out bad products. Using the camera system, green and yellow berries can be separated to create different wine types. Even the acceptable grade of rot on a single berry can be defined. The processing in laser engineering is still going on. Chlorophyll detection via laser-induced fluorescence is now already a standard. A new optical laser technology for the detection and rejection of aflatoxin- contaminated products is already in use. In the near future an analysis of each berry via NIR-laser should be possible.

PRODUCTION OF OCHRATOXIN A AND GEOSMIN BY DIFFERENT SPECIES OF *ASPERGILLUS* AND *PENICILLIUM* IN GERMAN GRAPE-GROWING REGIONS.

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Investigations in German grape-growing regions have shown that a lot of potential toxinogenic *Aspergillus* and *Penicillium* species are present in soil, wood and on bunches. Studies from 2004 to 2006 showed that the main species causing green mold on grapes are *Penicillium expansum* (93%), *P. minioluteum* (4%) and *P. crustosum* (2%). Some of the isolates of *P. expansum* and *P. crustosum* can produce geosmin, a volatile secondary metabolite responsible for earthy-musty off-flavour in wine. Two of the investigated isolates of *P. crustosum* produced *in vitro* ochratoxin A (OTA), a known cancerogen and teratogen. In some years (especially warm vegetation periods) even *Aspergillus* can be found on grapes. A screening of *Aspergillus* species found in soil and on wood showed that about 25% of the 300 investigated isolates were Ochratoxin A producers *in vitro*. About 80% of these ochratoxinogenic isolates were identified as *A. niger/awamori*, 14% as *A. tubingensis* 5% as *A. japonicus* and <5% as *A. wentii. A. carbonarius*, that is supposed to be the main source of OTA contamination in wine of southern countries, has not been identified in German vineyards until now. OTA contamination was found in some German wine, but its content was lower than the allowed maximum concentration of 2 µg/l.

MOLECULAR IDENTIFICATION OF *BOTRYTIS CINEREA* AND *PENICILLIUM EXPANSUM* IN LUXEMBOURG.

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Botrytis cinerea, the causal agent of grey mould, causes important economic losses to viticulture. It is often present as latent infections that can develop into damaging symptomatic infections in the plant under appropriate environmental conditions (Suarez *et al.*, 2005). Important *B. cinerea* infections can not only cause yield losses but can also have detrimental effects on the organoleptic properties of the wine. Other fungi, such as *Penicillium* spp., also infect grapes and are a common component of the grape microbiota in many regions. Their presence has been associated with off-flavour development and mycotoxin production in wines. Morphological and physiological characterisation of fungal species for classification is currently completed by molecular identification. Among DNA-based molecular methods, amplification of conserved regions using primers designed on conserved genes such as the rDNA internal transcribed spacer (ITS) or the β–tubulin have proved effective. Several fungal strains of *B. cinerea* and *P. expansum*, isolated from Luxembourgian vineyards along the river Moselle were cultured on malt extract agar (MAE) prior to DNA extraction. PCR amplification was carried out using two sets of primer pairs each for ITS (ITS1 and ITS4,

ITSU5 and ITSR2) and β-tubulin (Bt1a and Bt1b, Bt2a and Bt2b) (Glass and Donaldson, 1995). These primers were able to discriminate genera and species. For grey mould, homology searches of amplicons confirmed the identification of *B. cinerea*; for *Penicillium*, most of the strains were *P. expansum*, whereas some strains were identified as *P. minioluteum* by ITS primer amplification.

Suarez B.M., Walsh K., Boonham N., O'Neill T., Pearson S., Barker I., 2005. Development of real-time PCR (TaqMan) assays for the detection and quantification of *Botrytis cinerea* in planta. *Plant Physiology and Biochemistry* **43**: 890-899.

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FAST SCREENING FOR PRESENCE OF MUDDY/ EARTHY ODORANTS IN WINE AND IN WINE MUST USING A HYPHENATED GAS CHROMATOGRAPHY-DIFFERENTIAL ION MOBILITY SPECTROMETRY (GC/ DMS). M. Camara[1], N. Gharbi[1], E. Cocco[2], C. Guignard[2], M. Behr[2], D. Evers[2] and P. Orlewski[1]. *[1]Centre de Recherche Public-Gabriel Lippmann, Department of Research in Automotive Equipments. [2]Centre de Recherche Public-Gabriel Lippmann, Department Environment and Agro-Biotechnologies, 41 Rue du Brill, 4422 Belvaux, Luxembourg. E-mail: orlewski@lippmann.lu*

Rapid, hyphenated detection techniques involving a gas chromatograph (GC) coupled to a classical time-of-flight ion mobility spectrometer (IMS), or more recently, to a micro-machined, miniature differential ion mobility spectrometer (DMS) offer a very interesting potential for the *in-situ* detection of many kinds of volatile organic compounds (VOCS) (Eicemean, 2002) and notably those appearing in the headspaces of selected foodstuff. Early, time effective and mobile detection of off-smelling earthy and muddy microbial volatile organic compounds like geosmin and methyl-isoborneol (MIB) in grape must and in wine is of interest for winemakers, especially in more eastward and rainy wine regions. Indeed, the moist and rainy weather is among the main factors favouring infection of grapes by simultaneously occurring fungal pathogens such as *Botrytis cinerea* and *Penicillium expansum*, which frequently results in the emergence of off-flavours in the must and wine. In the present work, we report the preliminary result of a study aiming at a fast, quantitative screening for geosmin in the headspace of wine and of grape must. We employed a field-deployable GC/DMS (Sionex, DMx) orthogonal detector, in parallel with a laboratory gold standard solid phase micro-extraction/gas chromatography/mass spectrometry (SPME-GC/MS) analysis has been taken as a reference. Prior to screening of wine headspaces, a series of GC-DMS spectra of geosmin/MeOH solution at known concentrations allowed to set-up optimal experimental conditions for geosmin detection. In spite of H_2O and EtOH saturated headspace in further examined wine samples, both positive and negative geosmin peaks were successfully identified in recorded spectra of two spiked white wines (Riesling and Elbling). We also detected the presence of geosmin in a red wine sample that naturally (not spiked) contained a high amount of it. Concentrations estimated from observed product ion abundance were in line with SPME-GC/MS results yielded with parallel investigations. Preliminary results have shown the presence of geosmin in concentrations down to 5 ng/l in white wines at the Df dispersion field of 23kV/cm, with a flash desorption of the trap column at 250°C and with a steady GC column temperature of 110°C. A very rapid sample setup time and short instrument duty cycle below 5 min, together with its high analytical sensitivity below the human olfactory (geosmin) threshold of 50 ng/l (Boutou and Chatonnet, 2007) are making the GC/DMS detection technique potentially suitable for *in situ* geosmin screening directly during the wine-making process.

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RESISTANT PROFILES OF *PLASMOPARA VITICOLA* POPULATION IN THE LUXEMBOURGIAN GRAPE-GROWING REGION. F. Giraud[1], D. Molitor[2], M. Bleunven[1], L. Hoffmann[2] and D. Evers[2]. *[1]Laboratoire BIORIZON (STAPHYT Groupe), Rue Magendie/Site Bordeaux Montesquieu, 33650 Martillac, France. [2]Centre de Recherche Public-Gabriel Lippmann, Département Environnement et Agro-Biotechnologies (EVA), 41 Rue du Brill, 4422 Belvaux, Luxembourg. E-mail: fgiraud@staphyt.fr*

Downy mildew, caused by *Plasmopara viticola* (Berk. et M.A. Curtis) Berl. et De Toni, is an economically important disease of the grapevine in many parts of the world. It affects both leaves and fruits, and is of particular importance in climates with abundant rainfall, high relative humidity, and long periods of wetness on leaves and fruits. In Luxembourg, downy mildew is one of the major constraints to grape production. Disease management of downy mildew in most geographic areas generally requires the application of fungicides several times during the growing season, starting as soon as five leaves are unfolded (BBCH 15) in the cultivars typically grown in Luxembourg, i.e. Rivaner, Elbling, Riesling, Auxerrois, Pinot gris and Pinot blanc. One problem in the management of epidemics is the appearance of fungicide resistance in pathogen populations. Indeed, the introduction of selectively active site-specific fungicides into agricultural production systems has regularly been followed by the development of resistant fungal strains, decreasing the efficacy of chemical products. The situation becomes worrying in specific locations where more than one half of the natural population is resistant to one or more class of fungicides. Because of the cost of such frequent treatments, the desire to reduce pesticide levels in the environment and to preserve the fungicide utility and prolong their efficacy, considerable efforts have been made to counteract fungicide resistance. An essential part of such management strategies is the monitoring of fungal pathogen populations for their sensitivity to crop-protection compounds. These monitoring efforts should be based upon comparisons with baseline sensitivity data and the techniques must be suitably precise and reliable to detect relevant shifts in sensitivity within populations. In the BIORIZON laboratory the sensitivity of *P. viticola* has been monitored for over a decade, allowing comparison of sensitivities to some of the most commonly used fungicides (CAA, carboxylic acid amides; PA, phenyl-amides; QoI, quinone outside inhibitors). To study the current resistance status in the Luxembourgian grape-growing regions, leaf samples were collected from about 20 vineyards along the river Moselle in August 2010. Samples were analysed using biological tests (excised leaf disc assays and/or microplate assay with conidia growth inhibition). The main objective of this study was to measure the sensitivity of *P. viticola* populations to three classes of fungicide (CAA, PA, QoI) and to detect resistant populations in the Moselle area. To our knowledge, this preliminary study is the first report of the resistance/sensitivity profile of the agent of downy mildew of grapevines in Luxembourg.

GROWTH PROMOTION AND PATHOGEN SUPPRESSION ON POTTED VINES (*VITIS VINIFERA* cv. RIESLING) DUE TO APPLICATION OF *PIRIFORMOSPORA INDICA*. **E. Kecskeméti[1,2], K.-H. Kogel[2], B. Berkelmann-Löhnertz[1].** *[1]Geisenheim Research Center, Department of Phytomedicine, Von-Lade-Strasse 1, 65366 Geisenheim, Germany. [2]Justus-Liebig-University Giessen, Institute of Phytopathology and Applied Zoology, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany. E-mail: Elizabeth.Kecskemeti@fa-gm.de*

Effects of growth promotion and pathogen suppression by treatments with the beneficial fungus *Piriformospora indica* have been shown in several host-pathogen-interactions. The aim of this study was to observe specific colonization patterns of *P. indica* on the roots of potted vines (*Vitis vinifera* cv. Riesling) together with the effects on root development and plant growth. Additionally, pathogen suppressive properties of *P. indica* were studied in the pathosystems *V. vinifera-Plasmopara viticola* (causal agent of downy mildew) and *V. vinifera-Guignardia bidwellii* (causal agent of black rot). Under greenhouse conditions, two different approaches for the application of *P. indica* were compared: (i) treatment of the growing medium with *P. indica* chlamydospores at the beginning of the cultivation process of grape cuttings; (ii) treatment of young grape plants by watering with *P. indica* chlamydospores at weekly intervals. Inoculation suspensions were adjusted at 50 and 5,000 spores/ml, respectively. After inoculation, the presence of *P. indica* on plant roots was checked by molecular techniques. Comparison with a reference strain confirmed complete homology with genes of the 18S ribosomal RNA and with a putative β–tubulin (NCBI server database). Results showed that *P. indica* is able to colonise the roots of potted vines. Additionally, microscopic examination (in combination with different staining techniques) was used to detect *P. indica* and to characterize its colonization behaviour in the young roots. Chlamydospores were formed mostly intracellularly. The hyphae of *P. indica* had a diameter of 0.7 to 3.5 µm. Also the surface of inactive roots were colonized by *P. indica* hyphae. They covered the partly dead rhizoplane in a rhizomorphic pattern. Three weeks after inoculation, vegetative growth parameters were assessed. Vines treated with the the lower chlamydospore suspension produced longer shoots than those inoculated with higher *P. indica* concentrations. These differences, however, were not significant. Root mass (fresh weight; dry matter) of treated vines was significantly higher compared to the control vines. This effect was evident at higher chlamydospore concentration. The impact of the inoculation method was not evident. In the treated plots, the number of leaves and the fresh weight of shoots, were not significantly affected compared to the control plants. Regarding pesticide reduction programs and low input cultivation systems, the pathogen suppressive properties of *P. indica* may be of interest also for viticulture In the pathosystem *V. vinifera-G. bidwellii* these effects were present, but not significantly different compared to the plants of the control plot. Disease severity on the leaves of potted vines was reduced from 60% (control plot) to 40% in vines treated with higher concentrations of *P. indica* chlamydospores. In the case of *P. viticola*, no disease suppressive effect was obvious. Further studies will be conducted in order to improve these effects and to work out an approach for early rootstock treatment and/or field application of *P. indica*.

EFFECTIVENESS OF CYPRODINIL + FLUDIOXONIL MIXTURE AGAINST *BOTRYTIS CINEREA* AND ITS RESIDUES IN GRAPES AND WINE. N.D. Köycü[1], N. Özer[1] and N. Delen[2]. *[1]Namık Kemal University, Department of Plant Protection, Faculty of Agriculture, Tekirdag 59030, Turkey. [2]Ege University, Department of Plant Protection, Faculty of Agriculture, Bornova-Izmir 35100, Turkey. E-mail: dkoycu@hotmail.com*

Grey mould caused by *Botrytis cinerea* is a common disease of grapevines in Turkey. In this study, the effectiveness of cyprodinil+fludioxonil on *B. cinerea* was evaluated in two experimental vineyards in which cvs Emir and Zinfandel were grown. In addition, the residues of this mixture were determined in grapes and wine. Cyprodinil+fludioxonil was applied with two different spraying programs. Program I included spraying at the following stages: post-bloom, berries pea-size, veraison and 21 days before harvest. Program II was conducted at the stages of veraison and 21 days before harvest. In program I, the mixture was effective more than 70% in both cultivars due to decreased flower infections, whereas program II showed very low effect in disease control. The concentration of cyprodinil+fludioxonil on grapes and in wine made with fruits from both spraying programs were much lower than the value of maximum residue limit (MRL) enforced in Turkey and the European Union.

IMPORTANCE OF THE TIME OF TREATMENT ON PHOMOPSIS CANE AND LEAF SPOT CONTROL IN GRAPEVINES. N. Latinovic and J. Latinovic. *University of Montenegro, Biotechnical Faculty, Mihaila Lalica 1, 20 000 Podgorica, Montenegro. E-mail: nlatin@ac.me*

Phomopsis cane and leaf spot (*Phomopsis viticola* Sacc.) is a disease that occurs every year in vineyards of Montenegro, causing damages of various extent. In 2010, weather conditions were favourable for *Phomopsis* development. Average temperatures at the onset of vegetation, when grapevines are most susceptible to infections, were between 11°C and 16.5°C and rainfalls were 4.4 mm/m^2 to 254 mm/m^2, depending on the locality. A survey carried out in some Montengrin vineyards in 2010 revealed a different disease incidence on grapevine shoots. Therefore, the first four internodes of 100 shoots were examined in nine vineyards of five localities. Influence of grapevine development phase, time of treatment and rainfalls on disease severity was also assessed. Products with active ingredients: mancozeb, fosetyl-Al+folpet, propineb and copper were used for disease control. Results showed that disease severity varied from 0% to 27.5%. Considering data on the timing of treatment, is was established that the infection rate was extremely low or nil in all vineyards where the treatment was done at the appropriate time, i.e. at onset of vegetation before the first rain.

CROP CULTURAL AND CHEMICAL PRACTICES TO CONTROL GREY MOULD OF GRAPES. D. Molitor[1], M. Behr[1], S. Fischer[2] and D. Evers[1]. *[1]Centre de Recherche Public-Gabriel Lippmann, Department Environment and Agro-Biotechnologies, 41 Rue du Brill, 4422 Belvaux, Luxembourg. [2]Institut Viti-Vinicole, Section Viticulture, B.P. 50, 5501 Remich, Luxembourg. E-mail: dmolitor@lippmann.lu*

Grey mould or bunch rot on grapes caused by *Botrytis cinerea* is an economically important disease worldwide. Beside direct crop losses, *B. cinerea* attacks may lead to off-flavours, unstable colour, oxidative damages, premature aging and difficulties in clarification of the wine. Moreover, other fungi and bacteria can readily invade grey mould-infected clusters, which further contributes to the occurrence of off-flavours. In the last decades, the frequency of massive grey mould attack has increased. In order to address this problem in the vineyard, different control strategies

were tested in the years 2007 to 2009 in commercial vineyards along the Luxembourgian part of Moselle. The trials were conducted in cvs Pinot blanc and Pinot gris, which are typically grown in Luxembourg and are highly susceptible to grey mould due to their compact cluster structure. The aim of the studies was the development of sustainable management practices for efficient bunch rot control strategies. The investigated management practices consisted of: (i) leaf removal in the cluster zone directly after flowering (BBCH 71) on the north or east sides of the rows; (ii) application of the plant growth regulator Regalis (a.i. prohexadione-Ca) at full blooming (BBCH 65); (iii) application of the botryticide Teldor (fenhexamid) at cluster closure (BBCH 77), singly or as several combinations of them. The impact of these different strategies on cluster morphology, harvest parameters, such as yield, sugar level and total acidity, grape health status and possibility to prolong the ripening period were investigated. The use of Regalis led to a considerably more flexible cluster structure and to a slight decrease of grey mould severity. The reduction of the bunch rot infestation level was similar to that obtained by a single application of the botryticide Teldor at BBCH 77. Leaf removal also reduced cluster density and was more efficient against *B. cinerea* than the chemical treatments (reduction of grey mould disease severity of more than 50% in average). Presumably, the reduction of assimilation leaf area led to a disaggregation of cluster structure. Furthermore, the better sun and wind exposure allowed for a faster drying process. Thus, leaf removal in the cluster zone shortly after bloom can be recommended as a standard measure in integrated as well as in organic bunch rot protection strategies. The best loosening effect on the cluster structure as well as the best *B. cinerea* reduction efficiency were achieved when leaf removal and a Regalis application were combined. However, this combination reduced the yield, depending on the variety, the year and the location of the vineyard, by about 20% in average. The combination of leaf removal and botryticide application showed comparable efficiencies, though the yield reductions were lower. Simulations of *B. cinerea* epidemic indicated that all treatments tested might enable a longer persistence of the bunches on the vines due to lower infection. Thus, the presented strategies can be recommended to maximize wine quality in two ways, i.e. reduction of fungal contamination and/or improvement of grape maturity. The longest potential harvest delay (around 10 days in average) until reaching an assumed threshold of 5% disease severity was achieved by combining leaf removal and the application of Regalis or a botryticide.

LENGTH OF THE INCUBATION PERIOD OF *GUIGNARDIA BIDWELLII*. **D. Molitor[1,2], C. Fruehauf[3], O. Baus[1] and B. Berkelmann-Loehnertz[1].** *[1]Geisenheim Research Center, Section of Phytomedicine, Von-Lade-Strasse 1, 65366 Geisenheim, Germany. [2]Centre de Recherche Public-Gabriel Lippmann, Department Environment and Agro-Biotechnologies, 41 Rue du Brill, 4422 Belvaux, Luxembourg. [3]Deutscher Wetterdienst (German Meteorological Service), Centre of Agrometeorological Research, Bundesallee 50, 38116 Braunschweig, Germany. E-mail: dmolitor@lippmann.lu*

Black rot, caused by *Guignardia bidwellii*, is a grape disease of North American origin first recorded in Europe at the end of the 19th century. Since 2002, black rot became established in the northern grape-growing areas of Germany and currently represents regionally one of the major fungal grape diseases. The length of the incubation period is of special interest for scheduling curative as well as protective control measures. To determine the impact of temperature conditions and of the developmental stage on the length of *G. bidwellii* incubation period, studies were conducted at the Geisenheim Research Center in 2006 to 2008. The aim was to develop algorithms to determine the length of the incubation period based on cumulative degree-days after infection. Current knowledge tells that the incubation period of *G. bidwellii* on both leaves and clusters of cvs Riesling, Muller-Thurgau is strongly correlated with temperature conditions. For the mathematical description of this correlation, a new temperature-based model to calculate the incubation period length was developed. The following criteria have to be taken into consideration when calculating the temperature sums: (i) mean daily temperatures (t_{av}) below 6°C are included in the average sum calculation with an assigned value of 0°C; (ii) when the mean daily temperature is above 6°C, (t_{av}-6)°C is included in the calculation; (iii) when mean daily temperatures rise above 24°C, 18°C (i.e. 24 minus 6°C) will be considered in the average sum calculation. First disease symptoms appear on the leaves after reaching a threshold temperature sum of 175 cumulative degree-days. The length of the incubation period on clusters is additionally affected by the developmental stage. Until reaching the phenological stage "berries begin to touch" (BBCH 77), the length of the incubation period on cv. Riesling clusters is as long as on leaves. With ongoing development, the length of the incubation period increased continuously. Due to this, after reaching the stage "majority of berries touching" (BBCH 79), a correction factor for cluster phenology has to be taken into account to calculate the temperature sum thresholds for the occurrence of first symptoms. This specific information on the current state of fungal development in the host-plant and the expected date of the occurrence of new symptoms opens up innovative options for a more precise scheduling of fungicide based strategies to control grape black rot.

INNOVATION TO CONTROL DOWNY MILDEW ON GRAPES. **L. Triebus**. *Bayer CropScience, Alfred Nobel Strasse 50, Building 6100, 40789 Monheim, Germany. E-mail: ludger.triebus @bayercropscience.com*

Downy mildew, caused by *Plasmopara viticola*, is one of the most important and damaging diseases on grapes that has considerable negative economic impact worldwide. Bayer has invested significant resources over the last few years to develop a new alternative fungicide that meets all the needs of modern agriculture in providing improved disease control. PROFILER is the new, innovative technology developed by Bayer CropScience, that combines the properties of two active ingredients with different modes of action to give outstanding efficacy against downy mildew. Firstly, fluopicolide a new active ingredient provides both curative and anti-sporulant activity to control the disease at several points in the *P. viticola* life cycle. Fosetyl-Al, the active ingredient in the well established product Aliette, has complete systemic properties moving in both xylem and phloem as well as providing strong indirect stimulation of the plant's natural defense system. Fluopicolide is active at low dose rates against all oomycete-induced diseases and has been shown not to be cross-resistant to any other commercial fungicide used in grapes. From the trial results obtained during five years of intensive testing in vineyards of France, Italy, Germany, Spain and Portugal, PROFILER applied at 2.25 to 3.0 kg/ha, throughout the season or three times around the blooming period, showed its excellent efficacy, superior to commercial standards, to protect leaves and bunches against downy mildew, even when applied at 14 day spray intervals. As part of our anti-resistance management strategy, a maximum of three applications of PROFILER are recommended per crop per season in grapes from blooming up to bunch closure, with a spray interval of 12 to 14 days.

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Index of Authors

Edizioni ETS Pisa, 2011

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Book chapter

Alleweldt G., 1987. The contribution of grapevine breeding to integrated pest control. In: Cavalloro R. (ed.). Integrated Pest Control in Viticulture, pp. 369-377. A.A. Balkema, Rotterdam, The Netherlands.

Thesis or Dissertation

Hammer P., 1992. Mechanisms of resistance to infection by *Botrytis cinerea* in rose flowers. Ph.D. Thesis. Pennsylvania State University, University Park, USA.

Proceedings

Mortensen K., Makowsky R.M.D., 1989. Field efficacy of different concentrations of *Colletotrichum gloeosporioides* f.sp. *malvae* as a herbicide for round-leaved mallow (*Malva pusilla*). In: Delfosse E.S. (ed.). *Proceedings of the 7th International Symposium on Biological Control of Weeds, Rome* 1988: 523-530.

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