Studies of *Verticillium* Wilt in Hops

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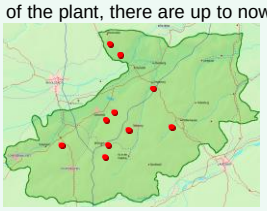
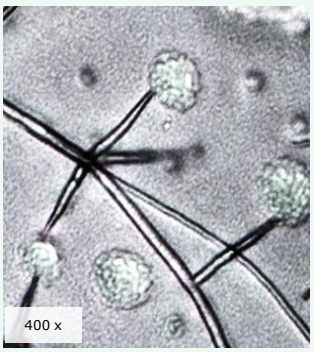
Introduction

Wilt symptoms on hops

In Germany hop growing is an export oriented agricultural business producing 30 % of the world hop crop including aroma and high alpha cultivars. Since 2005 an outbreak of more severe hop wilt has resulted in massive yield losses in some regions of the Hallertau. For the first time, all varieties, even the tolerant ones have been affected. The occurrence of lethal *Verticillium* strains in England (Keyworth 1942) and Slovenia (Radišek et al. 2003) showed how dangerous this soil-borne fungus is. Once the *Verticillium* fungus has infected the root system of the plant, there are up to now no effective chemical treatments for this disease. Two types of *Verticillium albo-atrum* - mild and lethal - have been described (OEPP/EPPO Bulletin, 2007). A risk management tool combining several strategies should be established. First a rapid molecular system to detect *Verticillium* in bine and soil without cultivating the fungus should be developed. Several organisms should be tested as possible biological control agents (BCA) (Berg et al. 2009) to protect the hop plants. Furthermore, agronomical approaches e.g. reduced fertilization, testing biofumigation will be performed.

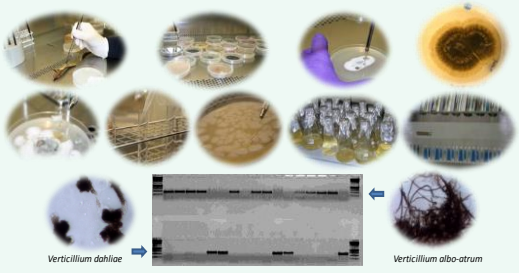


Regions in Hallertau with severe wilt

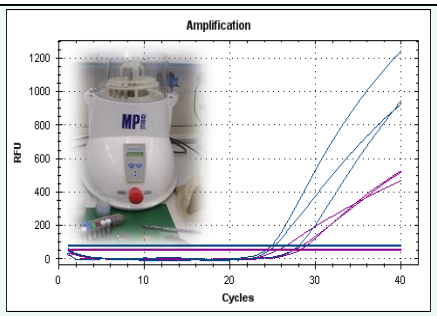


Verticillium albo-atrum isolated from hop

Material and Methods



Classical, but time-consuming method to determinate the *Verticillium* species. The DNA from 150 single-spore isolates was used for a further genetic differentiation by AFLPs applying various enzyme systems: *EcoR*I/*Mse*I; *EcoR*I/*Msp*I; *Pst*I/*Msp*I.



Rapid in planta *Verticillium* detection by multiplex qPCR after homogenizing the hop bine and isolating fungi DNA by commercial extraction kits
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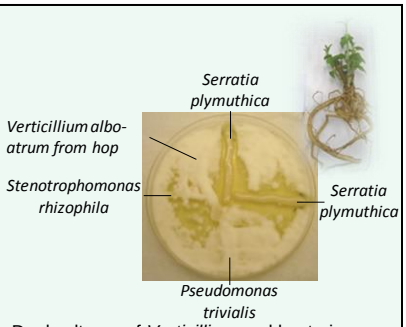
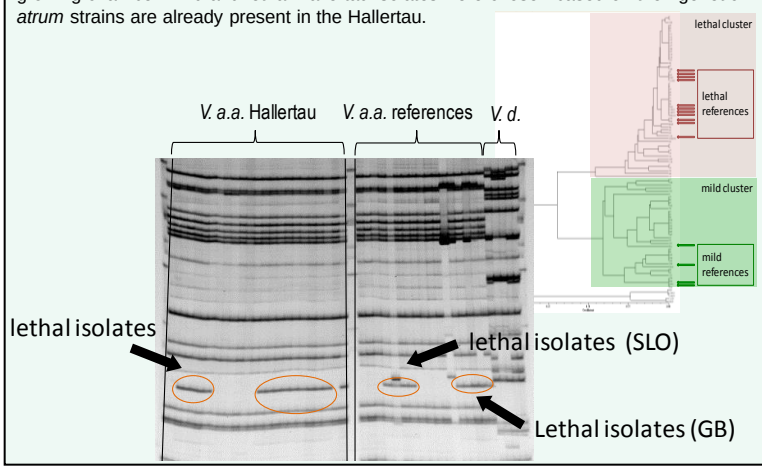


Dual cultures of *Verticillium* and bacteria were performed as first step to test microorganisms as possible bio-antagonists against hop wilt. Colonization of bacteria on hop roots.

Results

Cultivation of 150 single-spore isolates was successful. *Verticillium albo-atrum* infection was confirmed microscopically and molecularly. Molecular analysis using AFLP-markers showed differences between lethal and mild *Verticillium* types, collected in different European regions. A 134bp AFLP-fragment can be associated with the lethal *Verticillium* type. Parallel to the molecular approaches artificial infection tests with selected *Verticillium* isolates and hop varieties were performed in a growing chamber. Mild and lethal Hallertau isolates were chosen based on their genetic fingerprint. With both methods it could be confirmed that lethal *V. albo-atrum* strains are already present in the Hallertau.



Isolate	Days after infection	Cultivar						Mean
		Celeja	Perle	H. Tradition	N. Brewer	H. Magnum	Wye Target	
P55 (Hallertau mild)	38	1	0	0	0	1	0	0,4
	52	2	0	1	0	1	0	0,8
	66	**3**	**1**	**2**	**1**	**1**	**0**	**1,6**
P83 (Hallertau mild)	38	2	1	1	0	0	0	0,8
	52	3	1	2	0	0	0	1,2
	66	**3**	**1**	**3**	**1**	**1**	**0**	**1,8**
Zup (SLO mild)	38	0	0	1	0	0	0	0,2
	52	2	0	1	0	0	0	0,6
	66	**2**	**1**	**2**	**1**	**0**	**0**	**1,2**
P10 (Hallertau lethal)	38	3	2	2	1	0	0	1,6
	52	4	3	2	1	1	0	2,2
	66	**5**	**3**	**4**	**3**	**1**	**1**	**3,4**
P15 (Hallertau lethal)	38	4	1	1	1	1	0	1,6
	52	5	2	3	1	1	0	2,4
	66	**5**	**3**	**5**	**3**	**2**	**1**	**3,8**
T6 (SLO lethal)	38	1	2	3	0	1	0	1,4
	52	3	3	4	1	2	1	2,8
	66	**5**	**4**	**5**	**2**	**2**	**1**	**3,8**
11055 (GB lethal)	38	3	1	1	1	0	0	1,2
	52	4	3	3	1	1	1	2,6
	66	**5**	**3**	**4**	**3**	**1**	**1**	**3,4**

Conclusion and outlook

It can be concluded that inside the Hallertau new lethal strains have developed. Furthermore a monitoring of hop wilt infections of the Hallertau hop growing region has to be continued with an improved rapid molecular test directly from the bines. Several microorganisms will be tested as possible bio-antagonists against the *Verticillium* fungus. Currently, breeding lines and wild hops are being tested for wilt-resistance in highly infected hop fields. In addition, tests with agronomical measurements and biofumigation are being performed.

References

Berg G. 2009: Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol* 2009 , **84:**11-18; **European and Mediterranean Plant Protection Organisation (OEPP/EPPO) Bulletin**, 2007. *Verticillium albo-atrum* and *V. dahliae* on hop, 528-535. – ; **Keyworth**, **W.G.** 1942: *Verticillium* wilt of the hop (*Humulus lupulus*) Ann. Appl. Biol. 29: 346-357. – ; **Radišek, S., Jakše, J., Simoncic, A., Javornik, B.** 2003. Characterization of *Verticillium. albo-atrum* field isolates using pathogenicity data and AFLP analysis. Plant Disease 87(6); 633-638.

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