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Development of a botanical plant protection product from *Larix* by-products to protect grapevine from *Plasmopara viticola*

Extracts from European Larch (*Larix decidua*) were shown to be efficient to control grapevine downy mildew (*Plasmopara viticola*) under controlled and field conditions. Larixyl acetate and larixol were identified as the active compounds.

Introduction

- There is an urgent need to replace copper fungicides with more environmentally friendly alternatives, e.g. plant extracts.
- Bark containing many secondary metabolites is a by-product of the forestry industry and is highly suitable as a raw material to produce botanical plant protection products (high availability, low costs, renewable resource, good degradability expected).
- Evaluating bark extracts from several forestry species for fungicidal activity (EU-ForestSpeCs), we identified extracts from *Larix decidua* (Fig. 1) containing the active compounds larixol and larixyl acetate (Fig. 2) as promising candidates[1].
- The aim of the follow-up project EU-ProLarix was to reduce the gap between an innovation and the registration of a marketable product.
- Here we present data on efficacy of larch extracts against *Plasmopara viticola* under *in vitro*, controlled and field conditions.

Material and Methods

- *Larix decidua* extracts were gained from larch turpentine (removal of diterpene acids by precipitation with 2-Amino-2-methyl-1-propanol) or by extracting bark.
- Larixol and larixyl acetate were isolated from extracts.
- Extracts were formulated as emulsifiable concentrates.
- *In vitro* fungicidal activity against zoospores of *Plasmopara viticola* was tested in serial dilutions in 96 well plates.
- Controlled conditions bioassays: grapevine seedlings grown in the greenhouse were treated with serially diluted products before inoculation (disease assessment 6 d post inoculation).
- Field experiments were performed in 3 experimental vineyards in CH-Frick, I-San Michele all'Adige and GR-Veria in completely randomized block designs (n=4) at concentrations of 1-5 g extract L^{-1} spray broth. In GR (2015) and I (2015), one or four treatments were made with a copper fungicide to reduce overall disease pressure.

Results

- Activity of larch extracts and active compounds against *P. viticola in vitro* (MIC_{100} 6-23 µg mL^{-1}) and in *planta* under controlled conditions (EC_{50} 0.21-0.36 mg mL^{-1}) (Tab. 1) was promising.
- In vineyards, two different formulations of a turpentine extract (LAR-016, LAR-042) reached efficacies of 49-68 % in a stand-alone strategy and 76 %-82 % in a low-copper strategy (Tab. 2). Formulations of two different bark extracts reached efficacies of 53 % (extract 2014: LAR-024) or 95 % (extract 2017: RS-84), respectively.

Fig. 1: European larch (*Larix decidua*); photo: Barbara Thürig, FiBL

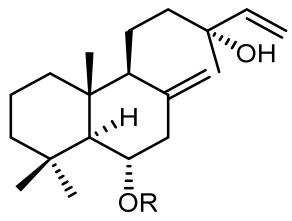

Fig. 2: Structure of the active compounds larixol (R=OH) and larixyl acetate (R=Ac)

Tab. 1: Concentrations needed (i) to completely inhibit zoospore germination and activity (MIC_{100}) *in vitro*, and (ii) to reduce area with downy mildew symptoms on grapevine leaves under controlled conditions by 50 % (EC_{50}).

	in vitro		Grapevine-*P. viticola* controlled conditions	
Product	MIC_{100} (µg*mL^{-1})	N §	EC_{50} (µg*mL^{-1})	N §
LAR-016*	23† (14;38)‡	3	210† (200;220)‡	3
Larixyl acetate	6 (4;9)	8	250 (120;470)	4
Larixol	14 (9;22)	7	360 (160;780)	2

† Mean; ‡ Upper and lower limits of the 95 % confidence interval; § Number of independent experiments; * formulated extract based on larch turpentine

Tab. 2: Efficacy of different, formulated larch extracts based on turpentine or bark, and a copper control against grapevine downy mildew in experimental vineyards in three experimental vineyards (CH, I, GR) by the end of the season (August).

Formulation	Extract based on	2015			2017	2018
		CH	I	GR	CH	CH
LAR-016	Resin	58 %	76 %	82 %		
LAR-042	Resin	68 %				
LAR-024	Bark					
LAR RS-84	Bark				95 %	78 %
Cu^{2+}		89 %	86 %	90 %	98 %	87 %

Conclusions / Outlook

- Formulated larch extracts showed good efficacy against grapevine downy mildew under field conditions.
- Larch extracts represent valid candidates for copper reduction in organic vineyards.
- Their development into a sustainable plant protection product might be feasible and is ongoing.

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