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**Research Note**

# Immunodetection of overwintering *Oidium* mycelium in bud scales of *Vitis vinifera*

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Summary: Antigens were isolated from the walls of *Oidium* conidia by a simple extraction method. Polyclonal antiserum was produced against these antigens and an immunofluorescent assay was developed to detect overwintering mycelium of *Oidium* in dormant buds. The results show that a specific detection of *Oidium* mycelium is possible without visualizing other fungal mycelia in dormant buds of grapevines.

Zusammenfassung: Durch einfache Extraktion wurden Antigene aus Konidienwänden von Oidium isoliert. Gegen diese Antigene wurden polyklonale Antikörper hergestellt und ein Immunfluoreszenztest entwickelt, mit dem es möglich ist, Mycelien von Oidium in ruhenden Rebknospen spezifisch und mit hoher Empfindlichkeit nachzuweisen. Andere in Knospen vorkommende Pilzmycelien werden mit dieser Methode nicht dargestellt.

Key words: Vitis, Oidium, Uncinula necator, immunofluorescence.

**Introduction:** Powdery mildew of grapevines, *Oidium tuckeri* (T.: *Uncinula necator*) has become a great problem in German viticulture in recent years. The perennation of the fungus is achieved by ascospores in cleistothecia (WELTZIEN and WELTZIEN 1962) as well as by mycelia found under the bud scales (VAN DER SPUY and MATHEE 1977). Early infections in spring arise from the perennating mycelia in buds while ascospores are of minor importance for the epidemiology of *Oidium* (SALL and WRYSINSKI, 1982). Perennating bud infections are commonly detected by heavily infected shoots found in spring, referred as flag shoots ("Zeigertriebe"). The presence of overwintering mycelium in bud scales has been demonstrated by staining fungal structures with non-specific dyes. When septate mycelia were present, they were usually regarded as *Oidium* but this is an unspecific morphological characteristic true for all higher fungi. We report here an immunological specific detection of *Oidium* mycelium in bud scales of grapevine.This method may serve as an early prognosis of the epidemiology of *Oidium*.

**Materials and methods:** Fungal cultures: *Oidium* infected grapevines were grown in the laboratory with a 14 h light period at 26 °C. Conidia were harvested from heavily infected leaves by sucking them with gentle vacuum into a small membrane filter device (Schleicher & Schüll 009 Microfilter) and stored dry in microtubes at -20 °C. *Botrytis cinerea*, *Alternaria* sp. and *Penicillium* sp. were isolated from grape berries or grapevine tissues and cultivated on potato-dextrose agar (MERCK).

Plant material: Sprouts with dormant buds from several grapevine cultivars grown in the field were collected in late winter and stored at -20 °C. The plants showed strong infection with *Oidium* during summer.

Antigen preparation: Protein from the conidial walls of *Oidium* was extracted by homogenizing 50 mg conidia in 20 ml extraction buffer (15 mM ammonium-bicarbonate, 0.01 % SDS, 0.02 % Triton X-100, pH 8.4) with gentle vortexing. The suspension was sonicated for 3 min, carefully shaken for 30 min at room temperature and centrifuged two times at 8000 *g* for 10 min. The pellets were discarded and fungal protein was isolated from the supernatant by evaporating the fluid in a SpeedVac concentrator (SAVANT). The dried protein pellet was resuspended in 1 ml water and centrifuged in an ultrafiltration filter (ROTH) with a molecular weight cut-off of 10 kDa to remove detergents and residual salts. The remaining protein was resuspended in 1 ml of 1xPBS, 0.01 % N-octylglucoside, 0.05 % sodium azide and taken for immunisation. The yield was up to 1 mg protein per 50 mg of dry conidia depending on the purity of the collected conidia. The protein preparations showed distinct bands and no degradations after electrophoretic analysis on SDS-PAGE.

Antiserum production: A rabbit was immunized (LOEWE BIOCHEMICA) three times with several intracutaneous and subcutaneous injections, each consisting of 1 mg of immunogen with adjuvant, one week and 4 weeks after initial immunization. Blood samples were taken 2, 3 and 6 weeks after the first immunization.

Immunodetection protocol: Bud scales were carefully removed from the buds, fixed in 80 % acetone for 15 min and washed for 15 min with PBS. The bud scales were incubated in PBS + 5 % BSA for 3 h and washed three times with PBS. Antiserum against *Oidium* was diluted 1:50 in PBS + 1 % BSA, added to the scales, incubated for 1 h and washed away three times with PBST. The bud scales were incubated 1 h with anti-rabbit IgG FITC conjugate (SIGMA) diluted 1:100 in PBST, washed five times with PBST and transferred to a microslide covered with a mounting medium containing equal parts of PBS and glycerol with 5 % n-propylgallate. For the visual analysis of the specimen a ZEISS microscope with epifluorescence was used. All steps were carried out at room temperature.

Nonspecific staining of fungal structures: The fluorescent dye Fungiqual A was provided by CIBA CORNING DIAGNOSTICS. Bud scales were stained for 5 min with the dye, washed two times with PBS and analysed with epifluorescence microscopy.

**Results and discussion:** Investigation of grapevine bud scales using indirect immunofluorescent technique revealed hyphae with a strong fluorescence on the surface (Figure, a). Analysis of mycelia and conidia from different moulds common in the vineyard, e.g. *Penicillium*, *Botrytis* and *Alternaria*, by indirect immunofluorescent microscopy showed only very slight signals. This obser-

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vation was confirmed by protein analysis using western blot technique (data not shown). These data suggest that overwintering *Oidium* mycelium can be easily demonstrated in bud scales. Other fungal mycelia and spores found in dormant buds, e.g. *Botrytis*, are not recognized by this method. Generally, fungal mycelia are visualized with non specific dyes like lactophenol anilineblue or Fungiqual A . The latter is a specific dye for cellulose and chitin which is also appropriate for staining fungal mycelia in buds (Figure, b). However, a definite diagnosis of *Oidium* mycelium in dormant buds is only achieved by immunofluorescence technique.

The investigation of grapevine buds by the method described above enables to forecast infections much more earlier than it is possible by the observation of flag shoots. Thus, a more precise method is available to decide the right moment of disease control.

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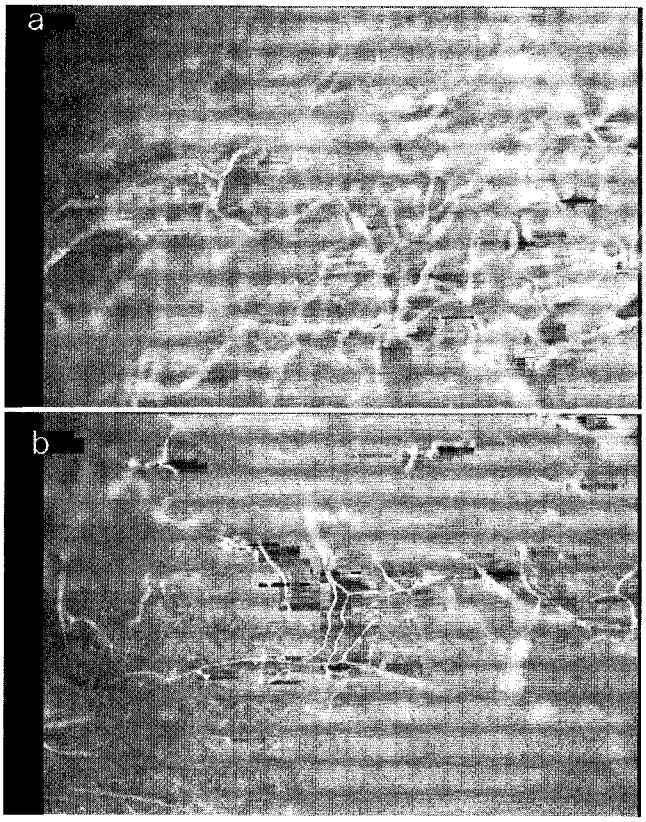


Figure: *Oidium* mycelium on grapevine bud scale tissue visualized specifically by indirect immunofluorescence. 500 x magnification (**a**). Fungal mycelium on grapevine bud scale tissue stained non specifically with Fungiqual A. 125 x magnification (**b**).