Comparison of bioassays to biotype grape phylloxera

(Daktulosphaira vitifoliae Fitch) on Vitis spp.

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Summary

Grape phylloxera biotypes exist throughout viticultural regions causing substantial economic losses. In the past different biotyping assays were employed to determine host adaption and potential harm of phylloxera strains or field populations. Standardised and efficient laboratory assays are required to define biotypes according to their aggressivity as well as to make accurate pest management and quarantine decisions. We aim to provide information on the consistency of the three most commonly used assays to accurately identify grape phylloxera biotype. Two phylloxera biotypes (A, C) were tested on two host plants (rootstock ‘Teleki 5C’ V. berlandieri x V. riparia and V. vinifera ‘Riesling’) using three assays: Simple isolation chamber, excised root bioassay and aseptic dual culture bioassay. Insect number, life table and plant-based response parameters (root galling) were compared. The simple isolation chamber and aseptic dual culture bioassay produced consistent results, whereas the excised root bioassay did not. We demonstrated that biotype results depend on whether the technique used is tuberosity- or nodosity-based. Pest management decision based on a single assay may inaccurately assess the phylloxera aggressivity potential. Thus, we recommend using two assay types which allows comparison of both root gall types.

Key words: biotype; Daktulosphaira vitifoliae; grape phylloxera; host-parasite interaction; Vitis.

Introduction

Grape phylloxera Daktulosphaira vitifoliae Fitch biotypes exist throughout viticultural regions causing substantial economic losses in viticultural regions (Corrie et al. 2003, De Benedictis and Granett 1993, Granett et al. 1991, Kocsis et al. 1999). Efficient, standardised and consistent biotyping assays are required to provide precise information on the aggressivity of phylloxera biotypes as an essential factor in phylloxera risk management (Baker et al. 2014).

The term biotype has been used for phylloxera strains varying in their performance and aggressiveness towards host plants and is measured by both host response and phylloxera performance (Forneck et al. 2016b, Granett et al. 1985). An aggressive monophagous phylloxera strain is defined by its host suitability (or host preference) among the Vitis spp. and its insect intrinsic performance defined by (Singer 1986) (Corrie et al. 1997, Forneck et al. 2016b, King and Rilling 1991, Powell et al. 2013). Numerous studies have biotyped phylloxera with regard to their adaption to various Vitis spp. hosts (e.g. Song and Granett 1990, De Benedictis and Granett 1993, Hawthorne and Via 1994, Korosi et al. 2005 and 2010, Herbert et al. 2010) and their performance as assessed by insect survival, development, fecundity and growth as well as host response parameters e.g. gall production on roots (Powell and Korosi 2013) and leaves (Williams and Shambaugh 1988, Forneck et al. 2016b). Due to a lack of consistent screening methods as well as standardized measurements the nomenclature describing biotypes is not standardized and comparable between publications (Korosi et al. 2010a). A recent approach postulated a new classification of phylloxera biotypes (Forneck et al. 2016b) describing seven phylloxera biotype groups (A-G) according to phylloxera performance on Vitis roots and/or leaves and host-plant root response.

Phylloxera biotyping experiments are conducted using a number of techniques (reviewed in Powell et al. 2013) including potted plant assays (Kocsis et al. 2002, Korpás et al. 2006, Pavlousek 2012), simple isolation chambers (Forneck et al. 2001c), aseptic tissue culture bioassays (Askani 1991, Forneck et al. 1996, 2001b and c, Grzegorczyk and Walker 1998) and excised root bioassays (Granett et al. 1983, De Benedictis and Granett 1993, Kocsis et al. 1999 and 2002, Omer et al. 1999, Makee et al. 2004). Sealed potted plant (Korosi et al. 2005) and simple isolation chamber bioassays (Forneck et al. 2001c) monitor galls on both non-lignified root tips (nodosity) and mature, lignified roots (tuberosity) on single or multiple host plants and provided controlled conditions avoiding cross contamination of biotypes. Moreover simple isolation chambers allow real-time observation of the phylloxera-root interaction throughout the bioassay period. Aseptic dual culture bioassays allow the co-cultivation and screening of phylloxera induced nodosities all year round whilst providing insect and host plant optimised as well as sterile growth conditions. Excised root bioassays employ detached lignified root pieces under insect optimised and controlled (non-field) conditions allowing monitoring of life table parameters and assessment of predominant tuberosity formation. Insect performance is studied by demographic life table parameters (e.g. insect reproduction, population growth and life table parameters).
Host plant and insect: Grape phylloxera eggs were collected from two single founder lineages of biotype A (Moselle valley, Germany) adapted to roots of V. vinifera ‘Riesling’ and biotype C (Burgenland, Austria) adapted to roots of rootstocks (V. berlandieri x V. riparia). Both lineages were genotyped by (FORNECK et al. 2016a) and maintained in isolated laboratory conditions on excised roots of Teleki 5C. All bioassays were set up with V. vinifera ‘Riesling’ clone Gm 239 and the hybrid rootstock Teleki 5C’ clone Gm 6–52 (V. berlandieri x V. riparia): Teleki 5C’ was selected because of its role as susceptible standard rootstock cultivar in phylloxera research and particularly among biotyping experiments.

Simple isolation chamber: Rooted single node cuttings were transplanted in simple isolation chambers (FORNECK et al. 2001c) containing a perlite-peat moss substrate (1:5). Climate chamber growth conditions were set to 25 ± 4 °C, 16 h light. Four months old plants were inoculated with 30 eggs by placing the eggs on a moist filter paper in an open 2 mL reaction tube near the root system. Treatments consisted of 5 to 6 simple isolation chambers for each host-biotype combination. Host plant response was determined by quantifying nodosities.

Aseptic dual culture bioassay: Plants were propagated in vitro from axillary buds, transplanted and cultivated in a ½ Murashige & Skoog agar medium containing sucrose (10 g·L⁻¹), indole acetic acid (1 mg·L⁻¹) and indole-3-butrylic acid (0.5 mg·L⁻¹) adjusted to pH 5.75 (FORNECK et al. 1996). Growth conditions were set to 25 ± 4 °C and 16 h light in an incubator. After 61 d 30–35 eggs were inoculated per in vitro plant. In total 13 plants per treatment were inoculated in separate Petri dishes. Insect number according to the insect developmental life stage (described in GRIESSER et al. 2015). Host plant response (root galling) was determined.

Excised root bioassay: In autumn, field collected root pieces of 15 cm length and 3-4 mm diameter were surface sterilized by soaking them in a fungicide solution (Ridomil™) and a UV treatment (20 min each side) under a sterile bench according to (KOROSI et al. 2010b). Petri dishes were maintained at 25 ± 4 °C and 16 h light in an incubator. Ten phylloxera eggs per root piece (2 root pieces per dish) were inoculated. 21 root pieces were inoculated per host-phylloxera biotype combination. Insect life table parameters: reproduction, development, survivorship, fecundity and egg production were calculated according to KOCSIS et al. (1999) and GRANET et al. (2005) by quantifying living phylloxera individuals according to their developmental life stage (GRIESSER et al. 2015).

Data collection and analysis: Phylloxera life table parameters were recorded for the aseptic dual culture and the excised root bioassay 30 dai (days after inoculation) by counting the phylloxera life stages L1-L5 under a binocular. Based on the phylloxera life stage data population increase, survivorship, development, fecundity and egg production were calculated according to standard equations (KOCSIS et al. 1999, GRANET et al. 2005). Nodosities were counted according to their sizes: N1 < 0.3 cm; N2 0.3-0.6 cm; N3 > 0.6 cm and N4 = several closely aggregated nodosities, FORNECK et al. (2001a) showed that coefficients of living grape phylloxera individuals and nodosity numbers were correlated. For the aseptic dual culture bioassay nodosities were counted 30 dai, whereas for the simple isolation chamber nodosities at 55 dai were correlated to the root dry weight (72 h at 70 °C). Data analysis was executed with SPSS Statistics 21 (IBM) software. Mann-Whitney U tests were performed with p < 0.05.

Results

Based on the biotype classification (FORNECK et al. 2016b) phylloxera biotype A performs better on V. vinifera roots producing both nodosities and tuberosities compared to roots of rootstocks (hybrids of American Vitis ssp.). Biotype C performs better on rootstocks (hybrids of American Vitis ssp.) producing nodosities and pseudotuberosities (POWELL and KOROSI 2013) than on roots of V. vinifera. In terms of experimental time scale, the simple isolation chamber required 55 d, the aseptic dual culture and the excised root bioassays one month for the screening phase post inoculation. Based on our experimental data and experience a minimum of 10 aseptic dual culture plates and 20 excised roots per treatment was sufficient to collect reliable data. Previous experiments employing the simple isolation chamber demonstrated that 5-6 isolation containers are sufficient to biotype grape phylloxera strains correctly.

Simple isolation chamber provided correct biotype information for both strains tested regarding host and non-host species (Figure, a). However the monitoring and screening phase required more time. It allowed regular visual monitoring throughout the trial. The information on different nodosity stages would allow further interpretation on the structure of the population screened, since correlation
Comparison of bioassays to biotype grape phylloxera

A comparison of bioassays to biotype grape phylloxera of gall and gall stages has been established (FornEck et al. 2001a). However, for biotype determination this is not of primary relevance. Aseptic dual culture bioassay provided correct biotype information for both strains. However the susceptibility of both hosts is increased, resulting in lower significant differences in number of insects compared to the simple isolation chambers (Figure, b and c). Excised root bioassay provided incorrect biotype information for strain C. (Figure, d and Tab. 1). In contrast to the other two bioassays biotype C performed in the excised root bio assay superior on 'Riesling' than on its native rootstock host. Biotype A was biotyped correctly (Figure, d and Tab. 1). The most widely used phylloxera screening technique is the excised root bioassay, which promotes the host susceptibility of V. vinifera 'Riesling'. This bioassay, when insect screening was done after one insect generation, lacked nodosity formation due to the employment of merely lignified and detached roots. Therefore it may underestimate rootstock susceptibility in terms of nodosity formation. As a consequence the excised root bioassay alone was critical for resistance screening of host plants.

Biotype consistencies: Despite the fact that the bioassays were based on different evaluation parameters, biotype A was tested in accordance to its native host preference for V. vinifera 'Riesling' among all three assays (Tab. 2). For Biotype C, adapted to rootstocks (V. berlandieri x V. riparia), the excised root assay provided inconsistent data, classifying the strain as a more aggressive biotype E (performing superior on roots of both V. vinifera and rootstocks). Tab. 2 described and summarized the biotyping results obtained by the three bioassays and added information about infestation intensities. Conclusively the choice of the bioassay had a significant impact on the biotyping result of the tested phylloxera strain.

Discussion

In the aseptic dual culture bioassay gall formation was present on the non-host 'Teleki 5C', however significantly less than on the native host (Figure, b). Plants are comparably younger and grow in aseptic media under grape phylloxera optimized conditions. Physical barriers against pathogens and parasites such as peridermal layers (Du et al. 2011), might be less developed allowing the insect to form a compatible host-parasite interaction even on non-host roots. However our experiments verified the suitability
of the aseptic dual culture bioassay to biotype phylloxera strains, if host performance (root galling) was applied as a quantitative parameter (Figure b and c).

There are constrains in literature about the validity and the efficient number of biological replicates required for experiments with potted plants (Passioura 2006). Unpublished pretrials employing the same grape phylloxera strains and hosts in the simple isolation chamber bioassays demonstrated that 5-6 isolation containers were sufficient to biotype grape phylloxera strains correctly. Although phylloxera growth conditions in the simple isolation chambers were optimized prior to this experiment, the simple isolation chamber bioassay required more time post inoculation to set up an evaluable phylloxera population, possibly due to secondary difficulties for the insect influencing host-parasite interaction e.g. soil matrix and temporal availability of susceptible root tips (Forneck et al. 2001c).

The absent nodosity formation was likely the major reason for the lack of comparability among the three bioassays tested. Another possible explanation was the limited host response on excised roots compared to assays employing the complete plant system. Granett et al. (2001) reported that detached roots host significantly higher phylloxera populations due to vine related mortality factors leading to an overestimation of phylloxera virulence and an underestimation of rootstock tolerance. The excised root assay was invented to test for tuberosity formation, which is essential to differentiate among biotypes that are more aggressive on tuberosities (Forneck et al. 2016b, Granett et al. 1987). Our results showed that the excised root bio assay, which excluded complete plant response, produced distinct biotyping results. Furthermore published parameters such as nodosity formation or life table parameters to evaluate host performance of phylloxera biotypes varied among the applied bioassays in their intensity. However, when biotyping the same phylloxera strain the simple isolation chamber and the aseptic dual culture produced consistent and reliable results.

**Conclusion**

In agreement with (Powell and Korosi 2013) we conclude that for biotyping unknown phylloxera strains adequately a combination of bioassays covering nodosity...
and tuberosity formation is suggested. However depending on the purpose of the research it might be advisable to either in- or exclude bioassays based on the capacity of the biotype to induce tuberosities or pseudotuberosities on mature roots.

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