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Short communication

Vitis coignetiae (Pulliat) shows partial resistance against leaf-feeding phylloxera and may serve to preserve abandoned vineyard habitats

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Preserving wine growing areas on steep slopes is declared a common denominator among the general public and a landscaping concept is needed. The ornamental vine Vitis coignetiae *is suggested to vegetate abandoned vineyards. The propagation potential of phylloxera on* V. coignetiae Pulliat *was assessed in an in vitro study comparing it with the rootstock Teleki 5C (*V. berlandieri x V. riparia*) and* V. vinifera *cv. 'Cabernet Sauvignon'. The results show that* V. coignetiae *has similar host capacities as* V. vinifera *for leaf-feeding phylloxera and thus may be considered a substitute pertaining to grape phylloxera resistance. The potential management of abandoned steep slopes with* V. coignetiae *is discussed and further arguments referring to ecological and landscaping effects are given to serve as decision-making support.*
Keywords: *Vitis vinifera*, rootstock, phylloxera resistance, landscaping, vegetation management, abandoned vineyards

Vitis coignetiae *(*Pulliat*) zeigt partielle Resistenz gegen die blattsaugende Reblaus und kann dazu dienen, aufgelassene Weinberghabitate zu erhalten. *Die Erhaltung von Weinbergen auf steilen Hängen ist ein anerkanntes Ziel in der Öffentlichkeit, und ein landschaftsplanerisches Konzept wird benötigt. Die Zierrebe* Vitis coignetiae *soll unbewirtschaftete Weinberge bewachsen. Das Vermehrungspotenzial der Reblaus auf V. coignetiae (*Pulliat*) wurde in einer in vitro-Studie untersucht und mit dem auf der Unterlage Teleki 5C (*V. berlandieri x V. riparia*) und der V. vinifera -Sorte 'Cabernet Sauvignon' verglichen. Die Ergebnisse zeigen, dass V. coignetieae vergleichbare Wirtspflanzeneigenschaften für blattsaugende Rebläuse hat wie* V. vinifera*. Die Möglichkeit der Bepflanzung aufgegebener steiler Hänge mit V. coignetiae wird diskutiert, und weitere Argumente hinsichtlich Ökologie und Landschaftsplanung werden als Entscheidungshilfen angeführt.*
Keywords: *Vitis vinifera*, Unterlage, Reblausresistenz, Landschaftsplanung, Vegetationsmanagement, unbewirtschaftete Weinberge

Vitis coignetiae *(*Pulliat) ***présente une résistance partielle contre le phylloxéra gallicole et peut servir à conserver des habitats viticoles abandonnés.*** *La conservation de vignobles sur des coteaux escarpés est un objectif public reconnu, et un concept d'aménagement du paysage est nécessaire. La variété d'ornement* Vitis coignetiae *est conçue pour pousser sur des vignobles non exploités. Le potentiel de propagation du phylloxéra sur* V. coignetiae *(*Pulliat*) a été étudié dans le cadre d'une étude* in vitro *et comparé à celui sur le porte-greffes Teleki 5C (*V. berlandieri x V. riparia*) et sur le cépage* V. vinifera *'Cabernet Sauvignon'. Les résultats montrent que* V. coignetieae *présente des caractéristiques de plante hôte pour phylloxéra gallicole comparables à celles de* V. vinifera*. La question de la possibilité de planter des coteaux escarpés abandonnés de V. coignetiae est étudiée, et d'autres arguments relatifs à l'écologie et à l'aménagement du paysage sont présentés en tant qu'aides à la décision.*
Mots clés : *vitis vinifera*, porte-greffes, résistance contre le phylloxéra, aménagement du paysage, exploitation herbagère, vignobles non exploités

Vineyards as characteristic and unique elements of cultural landscapes enrich the human environment by their aesthetic and ecological meaning. Due to increasing productivity costs viticulture on steep slopes is under threat and initiatives exist to develop directives for the preservation of the cultural landscapes worldwide. Very often such abandoned sites are covered with proliferated rootstocks showing leaf galls caused by grape phylloxera (*Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae)] which is still among the most important grapevine pests worldwide (Kocsis et al., 2010). Reports indicate phylloxerated rootstocks in and around vineyards in Austria (Koennecke et al., 2011), Germany (Kopf, 2000; Mueller, 2010), Southern France, Italy (Vorwerk et al., 2006; Forneck et al., 2010) and Hungary (Kocsis et al., 1999 and 2010). These may provide a threat for neighbouring economic vineyards if the population size vastly increases and new biotypes might arise.

Grafting susceptible European vines (*Vitis vinifera ssp. sativa)* onto resistant rootstocks was the successful strategy to renew European viticulture and has been working well for over 100 years, however, in the last decades more aggressive biotypes were observed (e.g. Forneck et al., 2001) and so far, no suitable chemical or biological control methods exist. Consequently, spread of leaf galling phylloxera must be prevented by banishing these habitats of susceptible leaf forming rootstocks (*e.g. hybrids of V. berlandieri, V. rupestris and V. riparia*) in scarps and slopes of viticultural areas.

V. coignetiae, also known as "Crimson Glory Vine", "Scharlachrote Rebe" or "Yama Budoe" is a vigorous large deciduous tendril climber with slightly lobed, broad ovate leaves. In Europe, *V. coignetiae* is used as an ornamental plant and legally not considered a wine producing cultivar. The plant has some meaning in the grape production in Japan (Morinaga, 2001) as its juice is valuable due to the polyphenols and anthocyanins in the fruit (Takayama et al., 2009). Further, a high fungi and drought tolerance was reported (Okamoto et al., 2004) and *V. coignetiae* has been used for hybridization with *V. vinifera* to increase its resistance to *Erisiphe necator* and grey mould in Japan (Yamakawa et al., 1991).

Little information exists on the plant resistance against leaf gall phylloxera of *V. coignetiae* (Wapshere et al., 1987) apart from personal observations. The aims of this work were (1) to screen grape phylloxera performance on leaves of a *V. coignetiae* accession and (2) to provide a critical consideration regarding its use in extensive landscaping of abandoned vineyards.

Material and Methods

Insect material

A single founder lineage of phylloxera (*Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae)) was obtained from a leaf galling rootstock in Großhöflein (Austria) and has been maintained on caged potted plants under greenhouse conditions since 2007. Plants were watered and fertilized (5 ml/3 l; FERTY Spezial Mega (PLANTAN, Regenstauf, Germany); NPK + Mg + micronutrients (18 + 12 +18 + 12)) depending on the weather conditions. For the *in vitro* bioassay phylloxera eggs were collected from leaf galls of the rootstock Teleki 5C (*V. berlandieri x V. riparia*).

In vitro bioassay

As *V. coignetiae* is extremely hard to root from dormant or green cuttings, the installation of a greenhouse bioassay eligible to screen for phylloxera resistance (e.g. Forneck et al., 2001; Pavlousek 2012)

was technically not possible. Plantlets of *Vitis coignetiae* Pulliat, *V. vinifera* L. cv. 'Cabernet Sauvignon' and the rootstock Teleki 5C (V. berlandieri Planch. x *V. riparia* Michx.) were vegetatively micropropagated in magenta culture vessels as described in Forneck et al. (1996) using a modified MS medium (2.4 g/l MS salts, 15 g/l sucrose, 10 g/l plant agar, pH-value 5.75) and kept in a growth chamber at 24 ± 3 °C and a 16 h photoperiod (19.4 W/m²). To promote rooting the media for *V. coignetiae* and *V. vinifera* cv. 'Cabernet Sauvignon' included the hormones indole-3-acetic acid and indole-3-butyric acid (1 mg/l each). Phylloxera eggs were surface sterilized using 0.5 % peracetic acid as described in Kolberg et al. (2011) increasing the centrifugation step from 30 sec to 60 sec between the washing steps to allow eggs to sediment further. Four to five weeks old plantlets of each variety (N = 10-11 per variety) were inoculated in a randomized order in two independent runs. After six weeks all boxes were opened and inspected for the total number of leaf galls, total number of eggs and number of sucking phylloxera (separated in adults and nymphs).

Data analysis

The cumulative number of leaf galls was analyzed using a repeated Poisson regression model while the total number of leaf galls, the total number of eggs in leaf galls as well as the total number of nymphs and adults in leaf galls were analyzed using a simple Poisson regression model. In all analyses possible overdispersion was accounted for and robust variance estimation was used. The autoregressive covariance structure was used in the repeated model. Level of significance was set to 5 % adjusted for multiplicity by the Bonferroni-Holm procedure. Due to technical reasons leaf galls were not assessed after three weeks in the first run. Furthermore some *V. vinifera* cv. 'Cabernet Sauvignon' plants had to be opened after five weeks already. Those were considered as missing data in the statistical analysis. Posthoc analyses for cumulative number of leaf galls were performed to assess onset of differences between Teleki 5C and *V. coignetiae* or *V. vinifera*, respectively. This was done by stepdown testing at 2.5 % significance level restricting the data to 5, 4, 3 and 2 weeks. Due to these missing values a missing data analysis was performed using multiple imputations based on 30 sets. SAS version 9.3 was used for all analyses.

Results and Discussion

Phylloxera performance of *V. coignetiae* leaves

In vitro bioassay was performed to screen for phylloxera resistance as done before (e.g. Grzegorczyk et al., 1998; Forneck et al., 2001; Kellow et al., 2004; Makee et al., 2004). *In vitro* bioassays tend to enhance susceptibility on leaves and roots due to the particular microenvironment existing in the boxes (Forneck et al., 1996; Grzegorczyk et al., 1998) and according to our knowledge it has never been reported that a decrease of susceptibility was measured *in vitro* compared to greenhouse or field conditions. An accession of *V. coignetiae* has been used originating from a discontinued collection of the DLR Rheinpfalz (Germany) that was conserved at a nursery. The additional test plants were chosen for comparison reasons: Teleki 5C as susceptible for leaf galling; *Vitis vinifera* cv. 'Cabernet Sauvignon' as partially resistant against leaf galling.

A significant difference for the cumulative number of leave galls was observed among treatments between Teleki 5C and *V. coignetiae* (adjusted p-value < 0.0001) as well as between Teleki 5C and *V. vinifera* (adjusted p-value = 0.0007). No significant difference was observed between *V. coignetiae* and *V. vinifera* (adjusted p-value = 1). Assessing the time point when the number of leaf galls started to differentiate among treatments, a significant difference between Teleki 5C and *V. coignetiae* was observed after 2 weeks and between Teleki 5C and *V. vinifera* after 3 weeks (Fig. 1).

Between Teleki 5C and *V. coignetiae* a significant difference was observed for the total number of eggs (adjusted p-value < 0.0001), but not for any other of the assessed parameters (all adjusted p-values ≥ 0.0842). Comparing Teleki 5C and *V. vinifera* all parameters differed significantly from each other (total number of eggs: adjusted p-value = 0.0008; total number of adults: P < 0.0001; total number of nymphs: P = 0.0015). No difference was observed for any of the parameters between *V. coignetiae* and *V. vinifera* (all adjusted p-values = 1) (Fig. 2 and 3). The performed missing data analysis did not give any major differences to the complete case analyses (data not shown). Furthermore the number of egg producing adult phylloxera differed per leaf gall among the varieties. Whereas sometimes up to three adults were sitting in one leaf gall of the variety Teleki 5C only one per leaf gall

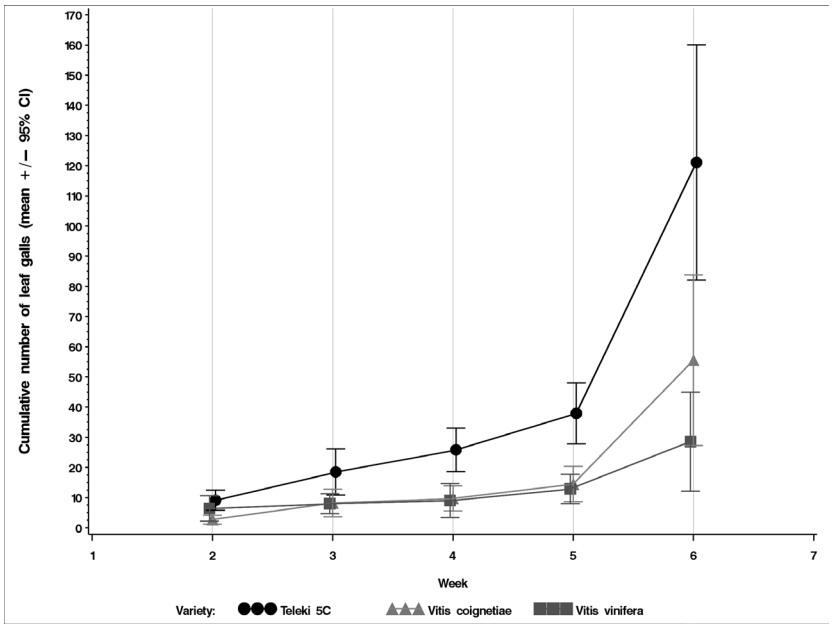

Fig. 1: Cumulative mean number of leaf galls (95 % confidence intervals) (fresh and dead ones) over a period of six weeks

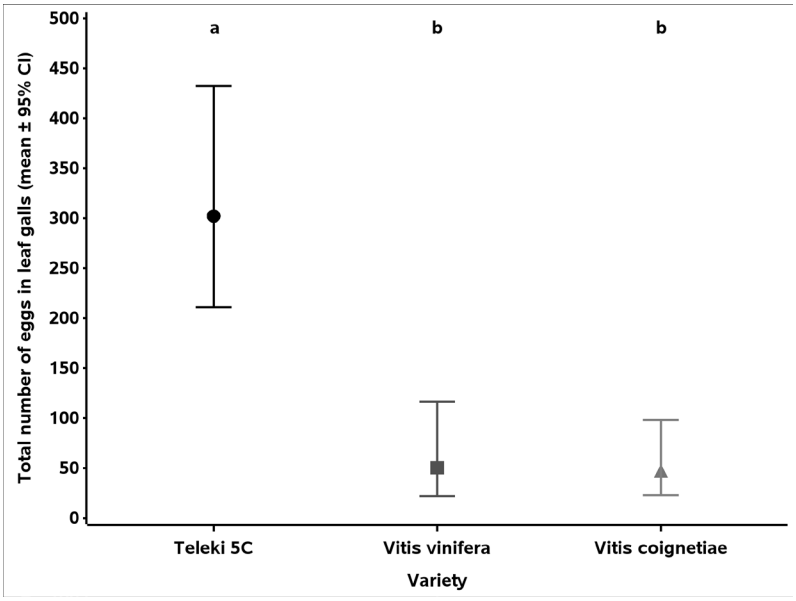

Fig. 2: Estimated means and 95 % confidence intervals of total number of eggs in leaf galls from Poisson regression; different letters indicate statistical significance at adjusted 5 % level.

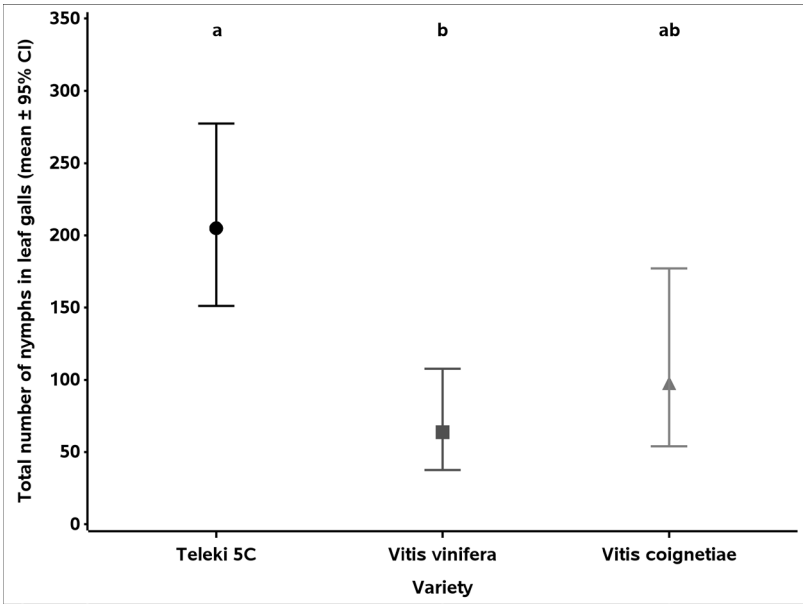

Fig. 3: Estimated means and 95 % confidence intervals of total number of nymphs in leaf galls from Poisson regression; different letters indicate statistical significance at adjusted 5 % level.

could be found for *V. coignetiae* and *V. vinifera*. After four weeks the first plantlets of *V. vinifera* started to change their leaf color from green to reddish. Fertile phylloxera were observed for all varieties on nodosities after six weeks (Teleki 5C: 10 / 11 plantlets; *V. coignetiae*: 6 / 11 plantlets; *V. vinifera*: 8 / 10 plantlets). Based on these findings it can be concluded that under the in vitro bioassay regime *V. coignetiae* and *V. vinifera* have partial resistance to leaf-galling phylloxera and Teleki 5C is susceptible. Nevertheless more studies need to be conducted to test different phylloxera lineages and biotypes as it is well known that they differ in their fitness (e.g. Forneck et al., 2001; Griesser and Forneck, 2009; Herbert et al., 2010).

We want to point out that even if the reproduction potential was significantly diminished on *V. coignetiae* compared to Teleki 5C plant leaves, fertile eggs were detected in leaf galls on 82 % of the plantlets. Neither plantlets from Teleki 5C nor from *V. coignetiae* seemed to be considerably affected by a phylloxera infestation during the bioassay, whereas the performance of phylloxera on *V. vinifera* cv. 'Cabernet Sauvignon' was restricted in all of the assessed parameters.

Interpretation of our findings needs to be carefully done. Field resistance of *V. coignetiae* against leaf-feeding phylloxera has been proposed (Schrank, pers. comm.) and is supported by our results, since the reproduction potential is comparable with the one of the *V. vinifera* cultivar tested. However if population densities get extremely high phylloxera leaf galling cannot be excluded in *V. coignetiae* nor in *V. vinifera* at this point. Being able to proliferate on *V. coignetiae* to some minor extent might bear the risk that the insect adapts and potentially enters commercial vineyards. Studies to understand the resistance of further accessions of *V. coignetiae* against phylloxera should continue involving more accessions of this species.

Considering the suitability of *V. coignetiae* in abandoned vineyards and effects on landscaping

The primary objective of replanting is to establish a site providing visual impression of a vineyard site and preserving characteristic ecological niches and microclimate with no threats to neighbouring commercial vineyards through migrating pests occurring in abandoned vineyards. Such vineyard-like habitats planted with *V. coignetiae* may house diseases (viruses, phytoplasms) and serve as inoculum for neighbouring vineyards through vectors without being symptomatic. In the case of flavorescence dorée some authors

recommend clearing of abandoned vineyards and abandoned rootstocks since they can be sources of infected vectors (Pavan, 2012). Up to date few scientific information on the resistance of *V. coignetiae* against powdery mildew (*Erysiphe necator syn. Uncinula necator*) or against downy mildew (*Plasmopara viticola*) exist. Foliar resistance against *P. viticola* of *V. coignetiae* was rated very high in similar ranges as *V. amurensis* and *V. cinerea* (Cadle-Davidson, 2008). According to a similar study *V. coignetiae* is susceptible against *U. necator*, also variation exists among the accessions and the authors indicate knowledge gaps in the host-pathogen interaction due to environment and potential strains of U. necator (Cadle-Davison et al. 2011).

Vineyards as characteristic and identical elements of cultural landscapes enrich the human environment by their aesthetic and ecological meaning. Behaviour patterns of existing *Vitis* species in Europe may assess the prospects in invasive risks. *V. berlandieri*, *V. rupestris* and *V. riparia* are established neobiota in Southern Europe. In Austria *V. cinerea*, *V. rupestris* and *V. riparia* as well as *V. vinifera ssp. vinifera* occasionally spread on their own, the populations however are not stable; *V. riparia locally* is established in Austrian river wetlands (Fischer et al., 2008). During the last decade increase of evergreen and thermophilic species has become evident in Middle Europe. Therefore using ornamental plants of temperate zones as *V. coignetiae* must be handled carefully.

Conclusion

According to our results *V. coignetiae* shows partial resistance against phylloxera on leaves and thus may be an potential substitute for replanting abandoned vineyards. Further studies on the resistance to other pathogens and vectors need to be done to complement this finding. The ecological interactions and effects to the landscape should be screened in long-lasting experiments prior to generally advise such concepts. At this point replanting abandoned vineyards with *V. coignetiae* seems to be a feasible alternative for the wine growing areas of Germany (Valley of Moselle, Middle Rhine, Ahr) and Austria (Wachau, Kamptal, Steiermark) to preserve cultural landscapes for ecological and economic (tourism) reasons, although the economic and ecological use needs further confirmation.

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