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Aseptic dual culture of grape (*Vitis* spp.) and grape phylloxera (*Daktulosphaira vitifoliae* FITCH).

by

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S u m m a r y : An aseptic dual culture of grape phylloxera (*Daktulosphaira vitifoliae* FITCH) and grape vine (*Vitis* spp.) was developed. This method permits continuous observation of phylloxera feeding and the whole plant response on a dynamic basis. The plant/parasite interaction of three testplants (*V. vinifera* L., var. Riesling, SO 4 (*V. berlandieri* PLANCH. x *V. riparia* L.) and *V. riparia*, var. Gloire de Montpellier) are demonstrated by observing post-infectious reactions of the host- and population dynamics of the parasite. Different stages of phylloxera could be observed including nymphs, winged phylloxera (alatae) and sexual male phylloxera. Several potential applications for this aseptic dual culture are demonstrated.

K e y w o r d s : Daktulosphaira vitifoliae, phylloxera, Vitis, aseptic dual culture.

Introduction

Grape phylloxera (*Daktulosphaira vitifoliae* FITCH) has been recognized as the major pest insect problem for grapes since its accidental introduction to Europe in the 1860s. The planting of vines grafted on resistant rootstocks derived from resistant *Vitis* species is the only long-term control of phylloxera. The breeding and use of resistant rootstocks began near the end of the 19th century and the best of those rootstocks remain in use today. The fact that phylloxera continues to impact viticulture in California (WALKER 1992), Germany (RÜHL 1995) and Switzerland (REMUND and BOLLER 1994) and the need for rootstocks with resistance to other soil-borne problems in addition to phylloxera, necessitates further studies of the phylloxera / grape interaction.

Past studies have investigated this interaction in field trials, with potted and containerized greenhouse plants, in excised root studies in laboratory bioassays and aseptic tissue culture of roots, callus or foliage. These methods allow the study of the host and parasite interaction. However, none of these methods allows continuous observation of the phylloxera feeding and whole plant response. This paper presents a method to observe the interaction of plant roots and shoots with phylloxera on a dynamic basis. It is a rapid technique which is seasonably independent, provides controlled conditions, and requires less space as well as lower inoculum volumes than conventional greenhouse methods.

Materials and methods

G r a p e m i c r o p r o p a g a t i o n : Green cuttings of *Vitis vinifera* L., var. Riesling, and the rootstock SO 4 (*V. berlandieri* PLANCH. x *V. riparia* L.) and *V. riparia*, var. Gloire de Montpellier, were cut into one-node-segments of 3-4 cm length and 0.5 cm diameter. After harvesting, these cuttings were immediately surface-sterilized by immersion in a 1.5 % NaOCl-solution containing a drop of liquid detergent. Treated shoot pieces were rinsed in sterile water six times and inserted into culture tubes (25 mm x 100 mm) containing 20 ml of media and closed with a cap (Kap-UTS (K25), Bellco, USA). The medium consisted of 50 % MS (MURASHIGE and SKOOG 1962), mineral salts (Gibco Lab, Grand Island, NY), supplemented with 50 % MS vitamins (Gibco Lab), 10 g sucrose, 1 mg/l indole-3-acetic acid (Sigma) and 7 g/l plant tissue agar (A-1296, Sigma). The pH was adjusted with KOH or HCl to 5.7. Tubes containing plants were kept in a growth chamber at 27 °C with a 16-h light period.

P h y l l o x e r a e g g s o u r c e : Cultured plants were inoculated with phylloxera eggs gathered from a mixture of laboratory based colonies. These phylloxera strains were collected from a variety of rootstocks and locations throughout California and maintained in the laboratory on root pieces of *V. vinifera*, var. Cabernet Sauvignon (DE BENEDICTIS and GRANETT 1992). The eggs ranged from one- to 4-day-old when used for inoculation.

D u a l c u l t u r e : The rooted nodal cultures were placed in magenta culture vessels (Magenta GA-7 Vessel, Sigma) prior to inoculation with phylloxera. The vessels were filled with 25 ml modified MS media supplemented with additional 2 g/l agar (Sigma). As the agar cooled and solidified after autoclaving the vessels were stored at an 45° angle resulting in a media corpus with a sloped surface covering 50 % of the bottom (Fig. 1). A two-node segment of the micropropagated plants was inserted in the media filled vessels and incubated in a growth chamber as described above. After two weeks the containers were tipped by 90° in order to promote root growth towards the media-free side of the vessel. Optimal host plants for inoculation

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had 4-5 roots of 3-4 cm length in the media-free space as well as a root system, functioning as an independent source for nutrition. Prior to inoculation the shoot tip was trimmed above the third apical node to decrease transpiration and to obtain space for further growth. Fifty phylloxera eggs were sterilized as described by GRZEGORCZYK and WALKER (1995) and gently spread over the roots with an autoclaved (50 min, 121 °C) brush.

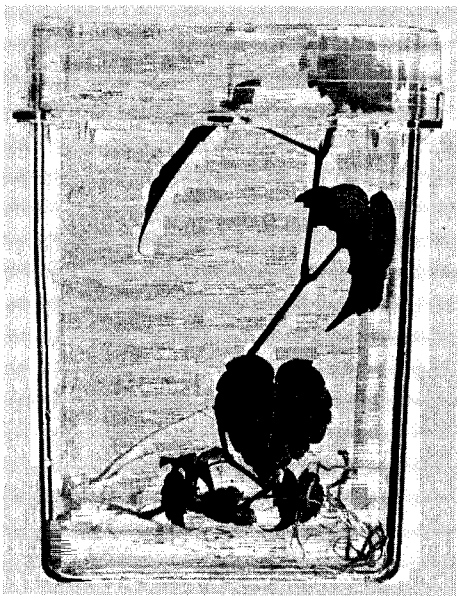

Fig. 1: *Vitis* sp. in culture vessel prior to inoculation with surface sterile phylloxera eggs.

Evaluation: The screening based on the evaluation of three phylloxera-inoculated testplants with five replications each, and was performed on a 2- to 3-day basis noting the progress of the plant/parasite interaction and the post-infectious reactions of the plant as well as the population dynamics of phylloxera. A suitable technique was developed including numeric, categorical and binary data (FORNECK 1995). The size (length, diameter) and the number of the nodosities were assessed directly by measuring, whereas their color and angle of bending were categorized with predefined scales. Fecundity of phylloxera was evaluated by randomly selecting two root-feeding females and counting eggs over the "egg-laying period" which varied between 10 and 26 d.

Results and discussion

Most of the phylloxera eggs hatched 1-3 d after inoculation. The motile crawlers (first instar) were observed probing on the root tip close to the meristematic zone. Numerous root hairs developed around the feeding sites on each test plant. The response to feeding varied among the *Vitis* genotypes tested: root tips of Riesling increased about 2-3 times in diameter and bent abruptly (often by 90 to 180°) at the site of feeding partially covering the insect in the crook (Fig. 2 a). As soon as the secondary branching of the swollen rootlet (nodosity) started, the new rootlets became infected by phylloxera of the second and third generation and they developed nodosities as described above.

SO 4 developed nodosities, but they were not as abruptly bent as those on Riesling. The color of the swollen rootlet changed from beige to light brown with clear brown and black layers encircling each feeding site (Fig. 2 b). Finally, the developing nodosity became necrotic and new roots developed immediately behind the former root tip. These new rootlets also became infested.

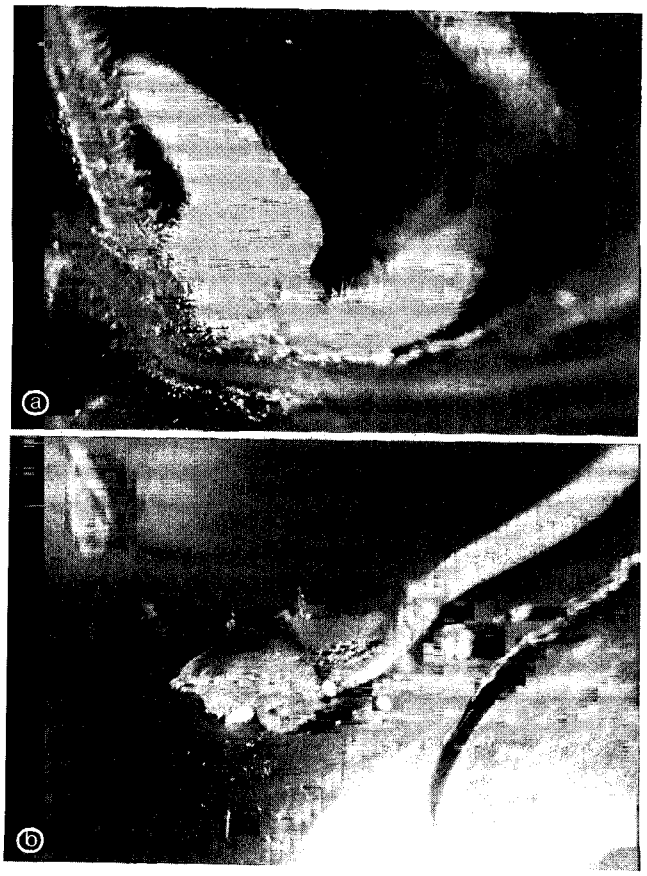

Fig. 2: Comparison of nodosities formed on *in vitro* roots of Riesling (**a**) and SO 4 (**b**).

Phylloxera attempted to feed on *V. riparia*, but did not establish feeding sites nor was reproduction evident. Brown circles were observed around these feeding sites and rounded swellings (not crocked and much smaller than on Riesling) developed. As these swellings developed their color changed from white/beige to dark brown followed by a black layer that restricted the area of infection. These blackened nodosities decayed. Very few of the roots which were formed behind these necrotic nodosities became infected by phylloxera.

In vitro phylloxera populations were established on Riesling and SO 4 and 4 or more generations could be observed. An average of 39 eggs per female were laid on Riesling, and 12 on SO 4 over a 4-week period. No reproduction was observed on *V. riparia*.

Phylloxera also infested the foliage of the tested plants and were observed reproducing on leaf galls, petioles, stems and callus. Differences in the degree of gall formation and necrotic responses were observed among the tested geno-

types. Several stages of phylloxera developed about 4 weeks after inoculation on Riesling. Nymphs (Fig. 3 a) and winged phylloxera (alatae) (Fig. 3 b) appeared crawling upwards to the lid of the magenta vessel. Alatae were observed to lay 2-3 eggs, which hatched after 2 d producing what appeared to be male phylloxera.

The phylloxera feeding symptoms induced on these *in vitro* roots were very similar to those observed under greenhouse or field conditions. The crocked appearance of nodosities which developed on susceptible plants and the limited swellings on resistant cultivars were described by DAVIDSON and NOUGARET (1921). KING *et al.* (1982) observed a brown ring on root tips of SO 4 as was noted on our cultured plants. They also observed the induction of lateral root proliferation on Kober 5 BB which we also observed on the closely related SO 4. The fecundity of phylloxera feeding on susceptible cultivars was studied by various authors. DAVIDSON and NOUGARET (1921) observed 45 eggs/adult over a 2-week period under field conditions. DE KLERK (1974) noticed 29 eggs/female in experiments performed in the greenhouse over a period of 12 d. We observed an average of 39 eggs/female over a 4-week period on Riesling.

The alatae that were produced on our cultured plants lived 2-3 d. DAVIDSON and NOUGARET (1921) reported a life span of 3 d under field conditions. DE KLERK (1974) observed a life span of 1-2 d under greenhouse conditions. DE KLERK (1974) observed the production of sexual eggs from *in vitro* cultures, but these eggs did not hatch to produce sexual phylloxera.

In addition to producing significant and typical feeding symptoms, an *in vitro* culture system should facilitate and accelerate the evaluation of plant reactions to phylloxera. The system described here requires about 4 weeks to prepare the *in vitro* plants for inoculation with phylloxera. Once infested feeding symptoms can be observed after 3 to 4 d, and the cycle from egg to egg was as short as 16 d. Riesling plants declined and the root system decayed about 3 months after infestation. The leaves of SO 4 plants were attacked and had numerous galls, but the root system was much less damaged than Riesling and new roots branched out and were less severely attacked than those initially fed upon. The *V. riparia* plants had an intact root system and relatively few symptoms on the leaves.

Different factors affect the length of time during which the *in vitro* interactions of phylloxera and grape plants can be observed. Fungal contamination, caused by insufficient surface sterilization or work space contamination, greatly reduces the longevity of the system. In addition, the direct comparison of *in vitro* results with results obtained under field conditions should be done with care, though the *in vitro* infested plants developed similar symptoms.

This *in vitro* system for co-culturing grape with phylloxera readily allows the observation and quantification of phylloxera resistance. Such resistance might range from antibiosis to reduced nourishment resulting in decreased fecundity. Field trials allow long-term investigations of phylloxera effects on the whole vine, but the root/phylloxera interaction is very difficult to observe. Furthermore, results of field trials should be carefully evaluated taking into consideration the difficulty of uniform inoculation, symptom evaluation and the uncertainty of environmental conditions.

This method of culturing grape with phylloxera allows evaluation of the host/pest interaction under controlled environmental conditions. Its primary advantage over previous methods is this system's capacity for continuous observation without disrupting the host/parasite interaction, while excluding secondary interactions with soil organisms as well as suppressive soil effects. This system also provides a rapid and space efficient means of evaluating phylloxera resistance that is seasonally independent.

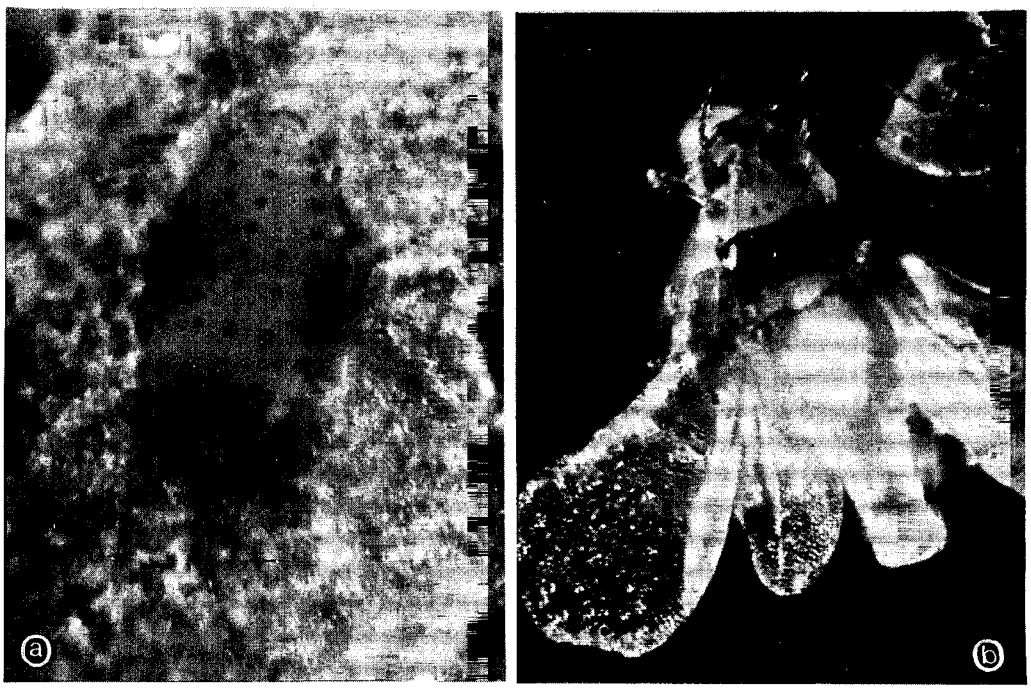

Fig. 3: **a** – Nymph and **b** – winged phylloxera (alata), formed in a culture vessel containing *V. vinifera*, var. Riesling.

Based on these attributes different use for this dual culture system is possible:

- evaluating new rootstock seedlings for phylloxera resistance
- distinguishing phylloxera biotypes on a living plant under favorable conditions
- propagating and maintaining phylloxera under quarantine conditions
- better observation and evaluation of the phylloxera life cycle including both asexual and sexual forms
- rapidly increasing phylloxera for experimentation
- the study of phylloxera genetics including mutation and selection effects with different hosts and environments.

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