# The Course of Colonization of Two Different *Vitis* Genotypes by *Plasmopara viticola* Indicates Compatible and Incompatible Host–Pathogen Interactions

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Accepted for publication 2 February 2007.

## ABSTRACT

Unger, S., Büche, C., Boso, S. and Kassemeyer, H.-H. 2007. The course of the colonization of two different *Vitis* genotypes by *Plasmopara viticola* indicates compatible and incompatible host–pathogen interactions. Phytopathology 97:780-786.

The course of colonization of leaf mesophyll by the causal agent of grapevine downy mildew, *Plasmopara viticola*, in a susceptible and a resistant grapevine genotype was examined in order to characterize the development of the pathogen in compatible and incompatible host–pathogen interactions. Within a few hours after inoculation, the pathogen was established in the susceptible *Vitis vinifera* cv. Müller-Thurgau and formed primary hyphae with a first haustorium. No further development occurred in the following 10 to 18 h. The next step, in which the hyphae grew and branched to colonize the intercellular space of the host tissue, was observed 1.5 days after inoculation. After 3 days, the intercostal fields were entirely filled with mycelium and sporulation was abundant under favorable environmental conditions. The first infection steps were essentially the same in the resistant *V. rupestris*. However, the invasive growth of *P. viticola* was delayed, and further development ceased before the intercostal fields were filled with mycelium.

*Additional keyword*: resistance.

Downy mildew is among the most important diseases of grapevine, particularly in warm and humid climates. Its causal agent, *Plasmopara viticola* (Berk. & M. A. Curtis) Berl. & De Toni, belongs to the family Peronosporomycetes (6,8) and is a biotrophic pathogen adapted to members of the family Vitaceae, especially to the subgenus *Euvitis*. *P. viticola* is endemic to southeastern North America, and is found there on several wild *Vitis* spp., such as *Vitis aestivalis*. Since the first collection of *P. viticola* on wild *Vitis* spp. by Schweinitz (9) in 1838, and particularly after the first occurrence of the grapevine downy mildew disease in Europe in the late 19th century, considerable attention has been paid to this pathogen (14,22). The classical cultivars of *V. vinifera* all are highly susceptible to *P. viticola,* resulting in severe epidemics under favorable conditions. In comparison, North American *Vitis* spp. typically express significant resistance to this disease (1,21). Hybrids between *V. vinifera* and American species, including those resulting from multiple backcrossings with European cultivars, exhibit a variable range of intermediate resistance to downy mildew (20).

The time course of the disease cycle, from infection to the appearance of symptoms and sporulation, has received in-depth investigation (2,16,17). During the growing season, *P. viticola* spreads by asexually formed zoosporangia, which are released in large quantities under favorable conditions. Zoosporangia attach to the host surface (11) and release four to eight zoospores in the presence of free water. The pathogen infects through the stomata, bypassing preformed barriers on the host surface such as the cuticle and the epidermal cell wall. For this, the motile zoospores attach specifically to the stomata after a swarming phase (10).

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doi:10.1094/PHYTO-97-7-0780


Immediately, they encyst by forming a cell wall and subsequently develop a penetration peg, which grows through the stomatal pore (10,13,18). Under optimal conditions at 22 to 24°C, release of zoospores and targeting of the stomata occur within 2 h. Subsequently, *P. viticola* establishes in the substomatal cavity and colonizes the host tissue during the incubation period. The relationship between incubation period and temperature has been described in detail by Müller and Sleumer (15). At the end of the incubation period, the first symptoms occur on infected leaves, inflorescences, and berries. Sporulation occurs thereafter once the relative humidity exceeds 92% at night (3). Under such conditions, sporangiophores emerge from the stomata within 7 h, after which they branch and form sporangia at their tips (19).

To date, only a few details are known about the growth and development of *P. viticola* in host tissues during the incubation period. Particularly, the colonization of the intercellular space and the development of *P. viticola* within the mesophyll of infected leaves have not yet been described. Hence, it was the objective of our study to characterize the spatial and temporal development of the pathogen within such tissue of both susceptible and resistant genotypes. Characterization of pathogen development in resistant grapevines should provide insight into the nature of the defense mechanisms of the host as it affects the pathogen's growth and further development following initial penetration.

## MATERIALS AND METHODS

**Pathogen and plants.** A mixed culture of *P. viticola* was obtained from a natural field population near Freiburg (Germany) and maintained on susceptible *V. vinifera* cv. Müller-Thurgau in a greenhouse. Periodically, the abaxial surfaces of young leaves were inoculated with a aqueous suspension containing $\approx 2 \times 10^4$ sporangia $ml^{-1}$. The inoculated plants were kept overnight under high relative humidity (RH) (RH > 96%) at 24°C. After incubation for 5 to 6 days under ambient

greenhouse conditions, the plants again were maintained overnight under moist conditions to induce sporulation. The sporangia that were formed were harvested and used for the next inoculation series.

Cuttings of *V. rupestris* Michx. and *V. vinifera* L. cv. Müller-Thurgau (21,24) were cultivated in a greenhouse. The American wild species *V. rupestris* represents a resistant grapevine genotype, whereas *V. vinifera* cv. Müller-Thurgau is considered highly susceptible.

**Inoculation experiments.** For experiments with intact cuttings, the third to sixth unfolded leaves, counted from the apex, were inoculated by spraying the abaxial surface of the leaf with an aqueous suspension containing $2 \times 10^4$ sporangia $ml^{-1}$ (19). The inoculated plants were incubated in a growth chamber under long-day conditions (16 h of white light, photosynthetic photon fluent rate [PPFR] 300 µmol $m^{-2}$ $s^{-1}$ over the wave band 400 to 700 nm, 8 h of darkness) at 25°C and >95% RH. Additional experiments were carried out with detached leaves. Detached leaves, always obtained from the same position on the shoot (fifth to sixth unfolded leaf), were surface sterilized with 70% (vol/vol) ethanol and subsequently rinsed in deionized water. Leaves with peduncles were placed in water-filled test tubes, the opening was sealed with parafilm, and the tubes were placed in a transparent plastic box. The abaxial surface of each leaf was inoculated with 30 or more 100-µl droplets of a sporangial suspension ($2 \times 10^4$ sporangia $ml^{-1}$) and the plastic boxes were transferred to a growth chamber maintained under the conditions described above.

**Microscopic analysis.** For microscopic analysis, leaves were harvested at distinct intervals from 6 to 96 h postinoculation (hpi) and the lesions were excised. Samples were cleared in 1 M KOH, autoclaved for 15 min (121°C), and washed three times with deionized water. For epifluorescence microscopy, the samples were stained with 0.05% aniline blue in 0.067 M $K_2HPO_4$ (pH 9) (23). Examination was performed with a Zeiss Axiophot (Carl Zeiss GmbH, Gottingen, Germany) equipped with an epifluorescence facility (excitation at 395 to 440 nm; beamsplitter FT 460 nm, longpass emission filter 470 nm) and Plan-Neofluar objectives (Carl Zeiss). The imaging analysis was accomplished with a Zeiss AxioCam digital camera and the Zeiss AxioVision software.

**Comparison of disease severity and incidence.** Five plants per *Vitis* genotype were inoculated as described above. The inoculation experiment was performed in four repetitions. Disease incidence on intact cuttings was quantified 96 hpi by determining the proportion of leaves showing symptoms. To score disease severity, we estimated visually the proportion of the leaf area exhibiting (i) "oil spots" (chlorotic spots on the adaxial leaf surface), (ii) sporulation from the abaxial surface, and (iii) necrosis. The disease severity was calculated as the percentage of the leaf area covered with visible symptoms on leaves with lesions; disease severity and disease incidence were regarded as independent parameters.

**Characterization of the development of *P. viticola*.** To determine the colonization of the mesophyll in detached leaves of *V. vinifera* cv. Müller-Thurgau and *V. rupestris*, 30 inoculation sites from three true replicate leaves of each genotype were randomly selected in distinct intervals from 6 to 96 hpi. The frequency of each developmental stage of *P. viticola* in the leaf was assessed microscopically. Developmental stages were defined as follows: S1, substomatal vesicle; S2, substomatal vesicle with primary hypha; S3, long primary hypha; S4, very long and branched hypha; S5, loose mycelium; and S6, intercostal field completely filled with mycelium. The length of unbranched hyphae (S2 and S3) was measured by means of the AxioVision (Zeiss) digital imaging system. From long primary hyphae (S3) and very long branched hyphae (S4), the total number of haustoria of each hypha was assessed.

**Statistical analysis.** The variables (i) disease incidence and severity, (ii) length of hyphae, and (iii) frequency of haustoria were analyzed by an analysis of variance). Fisher's protected test (least significant difference method) was used to check for significant differences among the genotypes at the ≤0.05 probability level with respect to disease incidence and severity, relative frequency of developmental stages, length of hyphae, and frequency of haustoria. All calculations were performed using SAS system software (version 9; SAS Institute, Cary, NC).

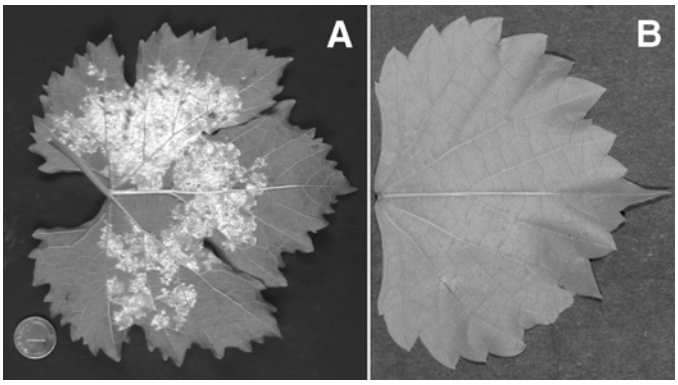


**Fig. 1.** Expression of symptoms of *Plasmopara viticola* on susceptible and resistant *Vitis* genotypes 96 h postinoculation. **A,** *Vitis vinifera* cv. Müller-Thurgau, showing profuse sporulation. **B,** *V. rupestris* with local necrotic lesions.

TABLE 1. Analysis of variance of disease incidence (mean % of plants with visible symptoms) and disease severity (mean % leaf area with visible symptoms on leaves with lesions) on intact cuttings of susceptible (*Vitis vinifera* cv. Müller-Thurgau) and resistant (*Vitis rupestris)* grapevine genotypes[a]

| | | Disease incidence | | | Disease severity | | |
|---|---|---|---|---|---|---|---|
| | df[b] | Sporulation | Necrosis | Oil spots | Sporulation | Necrosis | Oil spots |
| Source of variation | | | | | | | |
| Genotypes | 1 | 44,291.68 ***[c] | 1,028.19 *** | 42,290.30 *** | 1,500.62 *** | 93.02 *** | 3,330.62 *** |
| Repetition | 3 | 329.65 ns[d] | 698.56 ns | 131.34 ns | 15.62 ns | 0.42 ns | 22.29 ns |
| Genotype*repetition | 3 | 329.65 ns | 2,750.12 ns | 131.34 ns | 15.60 ns | .62 ns | 22.29 ns |
| Error | 32 | 49.05 | 55.26 | 28.75 | 13.43 | 1.23 | 16.56 |
| Mean | | | | | | | |
| cv. Müller-Thurgau | | 66.55 A[e] | 29.25 A | 65.03 A | 12.52 A | 0.90 A | 18.25 A |
| *Vitis rupestris* | | 0.00 B | 39.39 B | 0.00 B | 0.00 B | 3.95 B | 0.00 B |
| LSD (0.05) | | 4.51 | 4.78 | 3.45 | 2.36 | 0.71 | 2.62 |

[a] Mean values were obtained by Fisher's protected test using the least significant difference (LSD) method at the ≤0.05 probability level.
[b] df = degrees of freedom.
[c] *** = significant at the 0.001 probability level.
[d] ns = not significant.
[e] Means with the same letter are not significantly different.

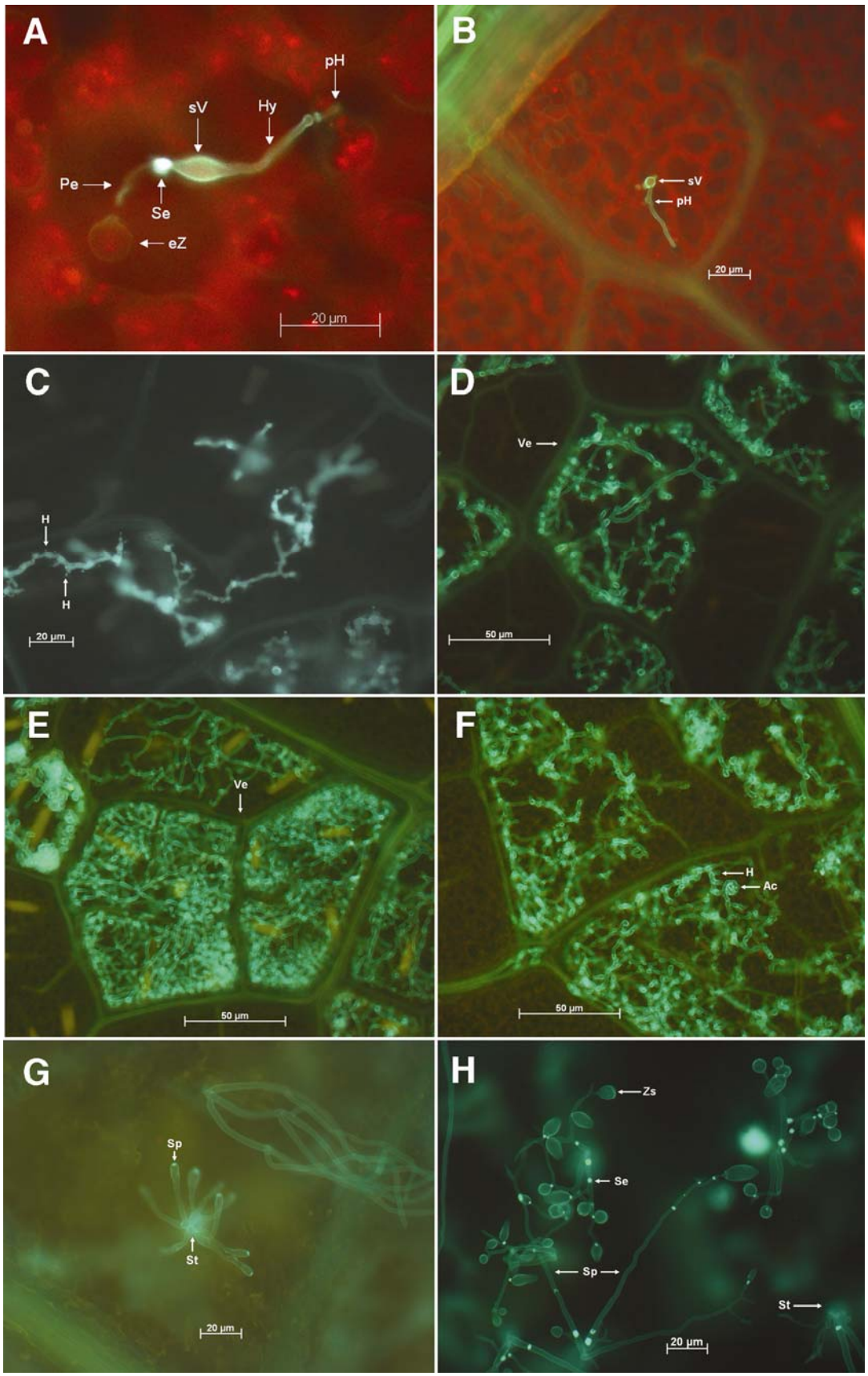


**Fig. 2.** Course of colonization of the intercellular space in the susceptible *Vitis vinifera* cv. Müller-Thurgau by *Plasmopara viticola* as visualized with epifluorescence microscopy after staining with aniline blue. **A,** S2, substomatal vesicle (sV) with primary hypha (Hy) and primary haustorium (pH). Also shown are the septum (Se) between the empty penetration peg (Pe) and the substomatal vesicle. The empty encysted zoospore (eZ) on the leaf surface is still visible 6 h postinoculation (hpi). **B,** Elongated hypha invading the intercellular space 24 hpi. **C,** Long hyphae branching and spreading within the mesophyll; many haustoria (H) are already formed at 48 hpi. **D,** Loose mycelium in the intercostal fields, limited by the veins (Ve) at 66 hpi. **E,** intercostal fields filled with mycelium at 96 hpi. **F,** Accumulation of hyphae (Ac) underneath a stoma at 72 hpi. **G,** Initials of sporangiophores (Sp) emerging from a stoma (St). **H,** Sporangiophores with mature zoosporangia (Zs) at the tip.

## RESULTS

**Expression of symptoms of *P. viticola* on different *Vitis* genotypes.** Significant differences were found between cv. Müller-Thurgau and *V. rupestris* for all variables. The genotype–repetition interaction was not significant (Table 1). The development of *P. viticola* in the susceptible cv. Müller-Thurgau caused macroscopically visible symptoms such as oil spots on the adaxial and sporulation on the abaxial leaf surface within 3 days after inoculation (Fig. 1A). The frequency of sporulation and oil spots, designated as disease incidence, was ≈65% (Table 1). Disease severity, representing the proportion of leaf area surface with symptoms on leaves with lesions, was 12.52% for sporulation and 18.25% for oil spots (Table 1); that is, no sporulation occurred on ≈6% of the lesions with oil spots. On leaves of *V. rupestris*, neither oil spots nor sporulation, but small local necrotic lesions were visible (Fig. 1B). Necrosis with a severity of 0.9% was found on cv. Müller-Thurgau on 29.25% of the leaves, but exclusively on sporulating lesions. *V. rupestris* showed a significantly higher frequency (39.39%) and severity (3.95%) of local necrotic lesions (Table 1).

**Characterization of the development of *P. viticola* in the susceptible *V. vinifera* cv. Müller-Thurgau.** At the first sampling time (6 hpi), the pathogen had penetrated the stomata and had reached the substomatal cavity, where substomatal vesicles with a primary hypha appeared (S2) (Fig. 2A). The primary hyphae appeared to form rapidly after penetration, because no substomatal vesicles without hyphae could be detected 6 hpi (S1) (Fig. 3A). The formation of primary haustoria was completed within 6 h (Fig. 2A). Further growth of the hyphae was delayed for some time, and the frequency of lesions at this developmental stage remained ≈100% through 18 hpi (Fig. 3B). Longitudinal growth of the primary hypha resumed in the following 6 h, and elongated hyphae were found to invade the intercellular space of the meso-

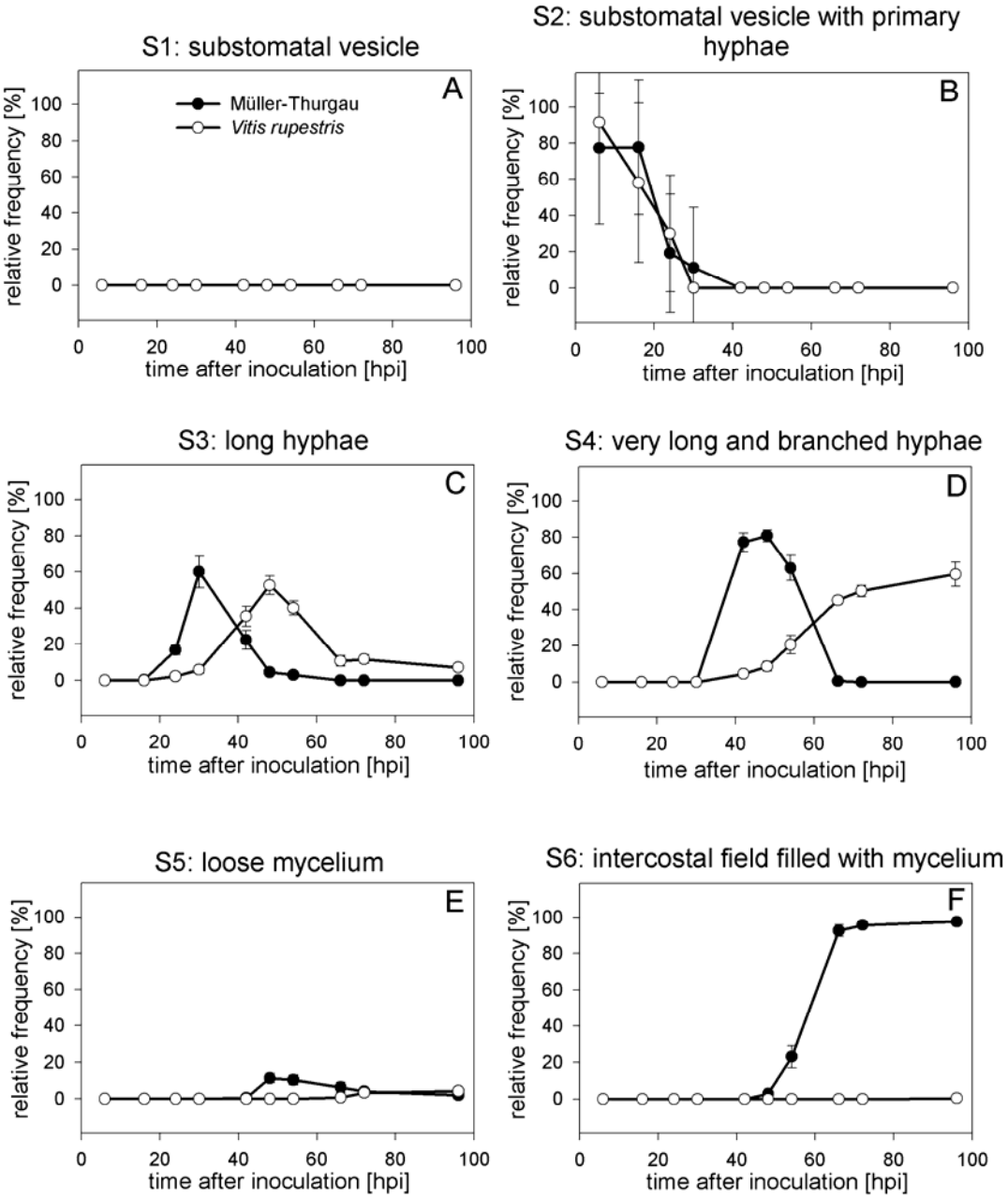


**Fig. 3.** Relative frequencies of individual stages of *Plasmopara viticola* over time during colonization of the mesophyll of the two *Vitis* genotypes. Each data point is the mean of 30 infection sites from three true replicate leaves, taken from *Vitis vinifera* cv. Müller-Thurgau and *V. rupestris*; bars indicate the standard deviation, displayed with SigmaPlot (Systat Software Inc., Erkrath, Germany).

phyll by 24 hpi (Fig. 2B); by 30 hpi, ≈60% of the examined lesions had reached this stage (S3) (Fig. 3C). The development of the pathogen advanced rapidly after this point. Following formation of several haustoria (Table 2), the long hyphae branched and spread into the intercellular space of the mesophyll (Fig. 2C). Branched hyphae (S4) showing numerous well-developed haustoria became dominant between 42 and 48 hpi (Fig. 3D). After 48 hpi, these hyphae formed a loose mycelium (S5) (Fig. 2D), the frequency of which never exceeded 20% at any single time (Fig. 3E). The mycelium grew apoplastically but haustoria penetrated the cell walls and were formed continuously through this phase of development. The shape of the branched hyphae varied depending on the topology of the intercellular space. The veins of fully expanded leaves demarcating the intercostal fields limited the extension of the mycelium. The frequency of intercostal fields completely filled with mycelium (S6) (Figs. 2E and 4A) increased rapidly, reaching nearly 100% by 66 hpi (Fig. 3F). At the last sampling time (96 hpi), all previous developmental stages had been completed. At this stage, hyphae accumulated in the substomatal cavities (Fig. 2F), forming secondary vesicles, and sporangiophore initials emerged from the stomata (Fig. 2G). Exposed to favorable humidity conditions overnight, they grew and, after branching, formed sporangiophores with numerous well-developed sporangia at the tips (Fig. 2H).

**Characterization of the development of *P. viticola* in the resistant *V. rupestris*.** Although initial stages of *P. viticola* development in resistant *V. rupestris* were similar to those in the susceptible cv. Müller-Thurgau, subsequent development was retarded and only rarely was completed. In the resistant genotype, the pathogen developed a substomatal vesicle with a primary hypha (S2) in all lesions by 6 hpi (Fig. 3B). The S2 stage proceeded into the next stage (S3) more slowly than in the susceptible cv. Müller-Thurgau. The long hyphae began to branch (S4) at 30 hpi, and the frequency of lesions at this stage continued to increase thereafter, reaching a maximum of 60% at 96 hpi. (Figs. 3D and 4B). In most cases, further development of *P. viticola* ceased and only a few lesions progressed beyond this stage. Lesions with loose mycelium (S5) occurred at 66 hpi, but only at a very low frequency (Fig. 3E), and intercostal fields filled with mycelium (S6) were not observed (Fig. 3F). Only a few very long, thin, unbranched, and sterile hyphae emerged from the stomata (Fig. 5). The different time course of infection in the two genotypes was evident in significant differences at the ≤0.05 probability level between the genotypes in some developmental stages and at certain times (S3 at 30 hpi, S4 at 42 and 48 hpi, S5 at 54 hpi, and S6 at 72 and 96 hpi) (Table 2).

**Hyphal length and frequency of haustoria in susceptible and resistant genotypes.** The length of primary hyphae (S2) and long hyphae (S3) were significantly different at the 0.001 probability level between the two genotypes (Table 3). After 24 hpi, *P. viticola* progressed rapidly to the branching stage in the susceptible genotype and single hyphae were not detected. In the resistant genotype, most hyphae remained without branching up to 48 hpi. Thereafter, unbranched hyphae grew slowly and reached their final length at 96 hpi. In cv. Müller-Thurgau, the mean num-

TABLE 2. Relative frequency (mean %) for each developmental stage and hours postinoculation (hpi) of *Plasmopara viticola* in susceptible (*Vitis vinifera* cv. Müller-Thurgau) and resistant (*V. rupestris*) grapevine genotypes[a]

| | | % Frequency | | |
|---|---|---|---|---|
| Stage | Time (hpi) | cv. Müller-Thurgau | *V. rupestris* | LSD (0.05) |
| S1 | 6 | 0.00[b] A | 0.98 A | 1.44 |
| S2 | 6 | 76.67 A | 99.01 A | 30.83 |
| | 16 | 95.92 A | 100.00 A | 7.30 |
| | 24 | 16.25 A | 23.31 A | 19.29 |
| | 30 | 1.11 A | 0.00 A | 1.66 |
| | 42 | 0.00 A | 0.00 A | 0.00 |
| | 48 | 0.74 A | 1.11 A | 2.29 |
| S3 | 6 | 11.11 A | 0.00 A | 23.55 |
| | 16 | 4.07 A | 0.00 A | 15.19 |
| | 24 | 82.12 A | 76.26 A | 18.87 |
| | 30 | 97.23 B | 100.00 A | 1.77 |
| | 42 | 3.28 A | 20.11 A | 2.60 |
| | 48 | 11.11 A | 17.12 A | 17.18 |
| | 54 | 0.00 A | 0.74 A | 1.57 |
| S4 | 24 | 1.47 A | 0.36 A | 1.88 |
| | 30 | 0.73 A | 0.00 A | 1.02 |
| | 42 | 61.67 A | 65.78 B | 3.06 |
| | 48 | 38.52 B | 49.26 A | 7.28 |
| | 54 | 70.00 A | 42.22 A | 65.23 |
| | 72 | 0.74 A | 3.33 A | 4.04 |
| S5 | 42 | 35.34 A | 13.52 A | 26.87 |
| | 48 | 54.07 A | 32.51 A | 30.74 |
| | 54 | 8.88 A | 1.47 B | 4.08 |
| | 72 | 47.76 A | 64.35 A | 21.16 |
| | 96 | 8.52 A | 18.90 A | 10.34 |
| S6 | 48 | 1.47 A | 0.00 A | 2.36 |
| | 54 | 4.44 A | 0.00 A | 9.42 |
| | 72 | 51.50 A | 32.30 B | 10.40 |
| | 96 | 91.47 A | 69.62 B | 20.51 |

[a] Mean values were obtained by Fisher's protected test using the least significant difference (LSD) method at the ≤0.05 probability level. $n = 9$.
[b] Means with the same letter are not significantly different.

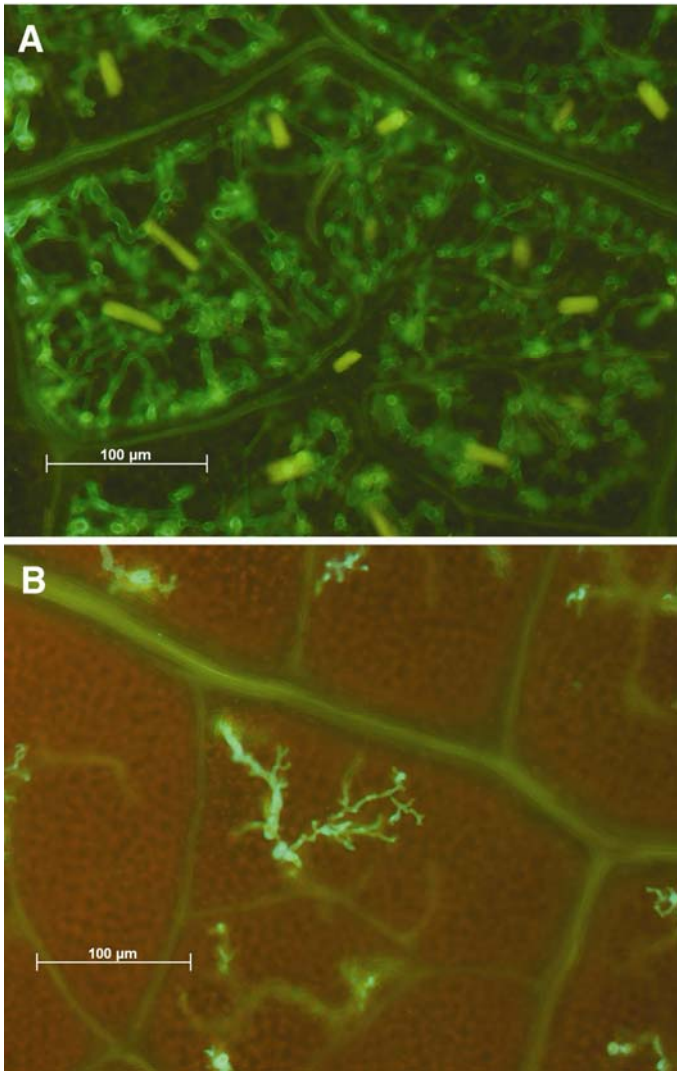


**Fig. 4.** Epifluorescence microscopy of the mycelium 96 h postinoculation. **A,** *Vitis vinifera* cv. Müller-Thurgau; intercostal fields filled with mycelium immediately before sporulation. **B,** *Vitis rupestris*; most of the hyphae ceased, only weakly developed mycelium is visible.

ber of haustoria on primary hyphae (S2) and long hyphae was not significantly different from *V. rupestris* (Table 4). After 24 hpi, the branched hyphae in the susceptible genotype formed haustoria with a high frequency, the amount of which could not be assessed correctly. In the resistant genotype, the frequency of haustoria increased only slowly, and the long hyphae (S3) had a mean number of 6.23 haustoria at 96 hpi.

## DISCUSSION

Compatible host–pathogen interactions are characterized by the manifestation of severe symptoms on the host plant and the ability of the pathogen to finish the infection cycle and to propagate (12). According to this definition, the progression of *P. viticola* lesions through the various developmental stages culminating in the expression of oil spots and the formation of fully developed sporangiophores with sporangia in *V. vinifera* cv. Müller-Thurgau represents a compatible interaction. In contrast, the absence of oil spots and sporulation and the occurrence of local, necrotic lesions may be attributed to an incompatible interaction between *P. viticola* and *V. rupestris*.

Although microscopical studies of the infection process of *P. viticola* have been provided by Kiefer et al. (10) and Langcake and Lovell (13), ours is the first report presenting the entire infection cycle and the course of the host mesophyll colonization with respect to a compatible versus an incompatible interaction. In *V. vinifera* and *V. rupestris*, Langcake and Lovell (13) found the first substomatal vesicle within 3.5 hpi. Kiefer et al. (10) described the targeted adhesion of zoospores to the stomata, followed by encystment and formation of an infection hypha, and found the same course of penetration and development of a substomatal vesicle. Our findings of a substomatal vesicle to be present within a few hours after inoculation in both susceptible and resistant genotypes indicates that *P. viticola* successfully penetrates the different genotypes and confirm the former results. Compared with development during the initial stage, *P. viticola* developed slowly in the period between S2 and S3 in both resistant and susceptible genotypes. The rapid movement through the subsequent developmental stages of *P. viticola* in cv. Müller-Thurgau reflects continuous progress in a susceptible host. In contrast, only in some lesions of the resistant genotype did *P. viticola* progress beyond S4; usually, further development mostly stopped in the stage of the primary hyphae (S2) and during the branching of hyphae. For example, in all lesions of the susceptible cv. Müller-Thurgau, pathogen development advanced quickly after 18 hpi whereas, in the resistant genotype, the primary hyphae persisted longer and, in 30 to 40% of the lesions, the development of *P. viticola* ceased at this stage. In the susceptible cv. Müller-Thurgau in nearly 100% of the lesions, the intercostals fields were filled with mycelium 66 hpi; whereas, in *V. rupestris*, this stage was not reached at all. The statistical analysis confirmed this different developmental pattern of *P. viticola* in the two genotypes.

In resistant *Vitis* genotypes, a transcript accumulation of putative defense genes and an increase of peroxidase activity have been described within 48 hpi with *P. viticola* (4,5,7,11). Thus, in the resistant genotypes, the significantly reduced growth of hyphae and the high frequency of lesions with ceased development of the pathogen in the early stage of colonization may be due to a resistance response.

The limitation of the mycelium by the veins in fully expanded leaves indicates that the hyphae cannot overcome the sclerenchymatic borderline. As soon as the mycelium expands to completely fill the intercellular space of a susceptible genotype 96 hpi, secondary substomatal vesicles with initials of sporangiophores are formed abundantly, and the first symptoms occur. These findings correspond well with the data on the incubation period originally described for *V. vinifera* cultivars by Müller and Sleumer in 1936 (15), who found that the incubation period under optimal conditions is 96 h. According to Rumbolz et al. (19), sporulation is regulated by the photoperiod. Our results suggest also that the abundance of hyphae in the mesophyll may trigger the sporulation. Altogether, the data on the frequency of the developmental stages and the course of colonization of *P. viticola* in resistant and

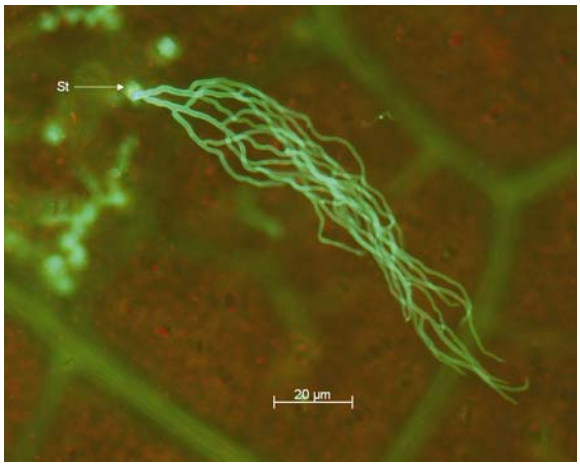


**Fig. 5.** Long, unbranched, sterile hyphae emerging from the stoma (St) at 66 h postinoculation on the resistant *Vitis rupestris*; epifluorescence micrograph after staining with aniline blue.

TABLE 3. Analysis of variance of the length of hyphae and frequency of haustoria per hypha of primary hyphae (S2) and long hyphae (S3) of *Plasmopara viticola* in susceptible (*Vitis vinifera* cv. Müller-Thurgau) and resistant (*Vitis rupestris*) grapevine genotypes[a]

| | | Mean square | |
|---|---|---|---|
| Source of variation | df[b] | Hyphae length | Frequency of haustoria |
| Genotypes | 1 | 76,377.26 ***[c] | 8.53 **[c] |
| Stage | 1 | 79,260.85 *** | 149.63 *** |
| Genotypes*stage | 1 | 35,284.41 *** | 0.83 ns[d] |
| Error | 116 | 484.31 | 0.77 |

[a] Mean values were obtained by Fisher's protected test using the least significant difference method at the ≤0.05 probability level.
[b] df = degrees of freedom.
[c] ** and *** indicate significant at the 0.01 and 0.001 probability levels, respectively.
[d] ns = not significant.

TABLE 4. Mean and standard deviations (SD) for hyphae length and number of haustoria in susceptible (*Vitis vinifera* cv. Müller-Thurgau) and resistant (*V. rupestris*) grapevine genotypes[a]

| | | Time | cv. Müller-Thurgau | | *V. rupestris* | |
|---|---|---|---|---|---|---|
| Parameter | Stage | (hpi)[b] | Mean | SD | Mean | SD |
| Hyphae length (µm) | S2 | 24 | 39.38 | 8.52 | 23.22 | 5.24 |
| | | 48 | …[c] | … | 26.56 | 16.72 |
| | | 96 | … | … | 28.91 | 10.33 |
| | S3 | 24 | 125.08 | 34.74 | 40.33 | 25.09 |
| | | 48 | … | … | 238.05 | 146.55 |
| | | 96 | … | … | 296.76 | 173.05 |
| Frequency of haustoria (%) | S2 | 24 | 1.63 | 0.55 | 1.26 | 0.73 |
| | | 48 | … | … | 1.36 | 0.83 |
| | | 96 | … | … | 1.56 | 0.50 |
| | S3 | 24 | 4.03 | 1.40 | 3.33 | 0.54 |
| | | 48 | … | … | 6.06 | 3.40 |
| | | 96 | … | … | 6.23 | 3.88 |

[a] Mean values were obtained by Fisher's protected test using the least significant difference method at the ≤0.05 probability level. $n = 9$.
[b] Hours postinoculation.
[c] … Indicates that in cv. Müller-Thurgau, no lesions at S2 and S3 were found 48 and 96 hpi due to the fast development of Plasmopara viticola in the susceptible genotype.

susceptible *Vitis* genotypes presented here provide a background for further investigations of host–pathogen interactions in grapevine.

## ACKNOWLEDGMENTS

We thank W. Wilcox, V. Steinmetz, and M. Fischer for their helpful comments and discussions on this article.

### LITERATURE CITED

1. Alleweldt, G., and Possingham, J. V. 1988. Progress in grapevine breeding. Theor. Appl. Genet. 75:669-673.
2. Arens, K. 1929. Untersuchungen über Keimung und Zytologie der Oosporen von *Plasmopara viticola* (Berl. et De Toni). Jahrb. Wisse. Bot. 70:75-92.
3. Blaeser, M., and Weltzien, H. C. 1978. Die Bedeutung von Sporangienbildung, -ausbreitung und -keimung für die Epidemiebildung von *Plasmopara viticola*. J. Plant Dis. Prot. 85:155-161.
4. Busam G., Junghanns K. T., Kneusel R. E., Kassemeyer H.-H., and Matern, U. 1997. Characterization and expression of Caffeoyl-coenzyme A 3-*O*-methyltransferase proposed for the induction for the induced resistance response of *Vitis vinifera* L. Plant Physiol. 115:1039-1048.
5. Busam, G., Kassemeyer H. H., and Matern, U. 1997. Differential expression of chitinases in *Vitis vinifera* L. responding to systemic acquired resistance activators or fungal challenge. Plant Physiol. 115:1029-1038.
6. Cavalier-Smith, T. 1998. A revised six-kingdom system of life. Biol. Rev. Cambridge Philos. Soc. 73:203-266.
7. Dai, G. H., Andary, C., Mondolot-Cosson, L., and Boubals, D. 1995. Histochemical studies on the interaction between three species of grapevine, *Vitis vinifera, V. rupestris* and *V. rotundifolia* and the downy mildew fungus, *Plasmopara viticola*. Physiol. Mol. Plant Pathol. 46:177-188.
8. Dick, M. W. 2002. Evolution of the downy mildews. Pages 1-57 in: Advances in Downy Mildew Research. P. T. N. Spencer-Phillips, U. Gisi, and A. Lebeda, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
9. Farlow, W. G. 1883. Enumeration of the Peronosporaceae of the United States. Bot. Gaz. 8:305-315, 327-337.
10. Kiefer, B., Riemann, M., Büche, C., Kassemeyer, H.-H., and Nick, P. 2002. The host guides morphogenesis and stomatal targeting in the grapevine pathogen *Plasmopara viticola*. Planta 215:387-393.
11. Kortekamp, A., Wind, R., and Zyprian, E. 1998. Investigation of *Plasmopara viticola* with susceptible and resistant grapevine cultivars. J. Plant Dis. Prot. 105:475-488.
12. Kranz, J. 2003. Comparative Epidemiology of Plant Diseases. Springer-Verlag, Berlin, Heidelberg, New York.
13. Langcake, P., and Lovell, P. A. 1980. Light and electron microscopical studies of the infection of *Vitis* ssp. by *Plasmopara viticola*, the downy mildew pathogen. Vitis 19:321-337.
14. Millardet, A. 1883. Sur le rôle des spores d'hiver du mildiou (*P. viticola*) dans la reinvasion par ce parasite. Mem. Soc. Sci. Bordeaux 5:24-27.
15. Müller, K., and Sleumer, H. 1936. Biologische Untersuchungen über die Peronospora-Krankheit des Weinstocks unter besonderer Berücksichtigung ihrer Bekämpfung nach der Inkubationskalendermethode. Landwirtsch. Jahrb. 79:509-576.
16. Müller-Thurgau, H. 1911. Infektion der Weinrebe durch *Plasmopara viticola.* Zentralbl. Bakteriol. II. 69:683-695.
17. Ravaz, L., and Verge, G. 1911. Sur le mode de contamination de la vigne par le *Plasmopara viticola*. Compt. Rend. Acad. De France 135:18-36.
18. Riemann, M., Büche, C., Kassemeyer, H.-H., and Nick, P. 2002. Cytoskeletal response during early development of the downy mildew of grapevine (*Plasmopara viticola*). Protoplasma 219:13-22.
19. Rumbolz, J., Wirtz, S., Kassemeyer, H.-H., Guggenheim, R., Schäfer, E., and Büche, C. 2002. Sporulation of *Plasmopara viticola*: differentiation and light regulation. Plant Biol. 4:413-422.
20. Spring, J.-L. 2001. Premieres experiences avec les cepages interspecifiques "Merzling", "Johanniter", "Bronner" et "Solaris" en Suisse romande. Rev. Suisse Vitic. Arboric. Hortic. 33:57-64.
21. Staudt, G., and Kassemeyer, H.-H. 1995. Evaluation of downy mildew resistance in various accessions of wild *Vitis* species. Vitis 34:225-228.
22. Viala, P. 1887. Les Maladies de la Vigne. Bibliothèque du Progrès Agricole et Viticole, Montpellier, Paris.
23. Williamson, B., Breese, W. A., and Shattock, R. C. 1995. A histological study of downy mildew (*Peronospora rubi*) infection of leaves, flowers and developing fruits of Tummelberry and other *Rubus.* Mycol. Res. 99:1311-1316.
24. Zander, R., Erhard, W., Götz, E., Bödeker, N., and Seybold, S. 2002. Dictionary of Plant Names, 17th ed. Ulmer Verlag, Suttgart, Germany.