PENETRATION, DEVELOPMENT AND REPRODUCTION OF *MELOIDOGYNE ARENARIA* ON TWO NEW RESISTANT *VITIS* SPP.

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ABSTRACT

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Penetration, development and reproduction of a Harmony pathotype of *Meloidogyne arenaria* was studied on two resistant grape rootstocks, 10-23B and RS-3. Associated resistance mechanisms were examined microscopically. Cabernet Sauvignon was used as a susceptible control. The plants were inoculated with freshly hatched second-stage juveniles (J2). Significantly more J2 penetrated roots of Cabernet than 10-23B by 4 days after inoculation. Penetration was delayed by one week in roots of RS-3. Cabernet roots contained a greater number of nematodes coupled with faster development than that found in resistant rootstocks. Hypersensitive responses were expressed in the presence of penetrating J2 in resistant rootstocks only. This occurred along the root epidermis, among the cortical cells and along the differentiating vascular bundle. Cortical necrosis halted or delayed migration of J2 to vascular tissues and vascular necrosis prevented establishment of successful feeding sites which arrested further development of J2. Cell necrosis was associated with nematode starvation whenever it was in abundance. Thirty-five days after inoculation, the resistant rootstocks supported less than 4% of the adult females supported by susceptible grape. In 10-23B an additional defense mechanism became apparent. It involved starvation of globose juveniles through adult female stage without development of hypersensitive reaction. The final result was undersized adult females and lack of reproduction. Both resistant rootstocks exhibited resistance to *M. arenaria* pt. Harmony including mechanisms that delayed penetration and development compared to that of Cabernet.

Key words: development, grape rootstocks, hypersensitive reaction, *Meloidogyne arenaria* pt. Harmony, penetration, reproduction.

RESUMEN

Anwar, S. A. y M. V. McKenry. 2000. Penetración, desarrollo y reproducción de *Meloidogyne arenaria* en dos nuevos *Vitis* spp., portainjertos resistentes de parras. Nematrópica 30:9-17.

La penetración, desarrollo y reproducción de un patotipo Harmony de *Meloidogyne arenaria*, fueron estudiados en dos patrones de vid resistentes; 10-23B y RS-3. Los mecanismos de resistencia asociados fueron examinados microscópicamente. La Cabernet Sauvignon, se usó como control susceptible. Las plantas se inocularon con juveniles (J2) recién eclosionados. Más J2 penetraron a las raíces de Cabernet que en las de 10-23B, en el cuarto día después de la inoculación. La penetración ocurrió una semana más tarde en las raíces de RS-3. Las raíces de Cabernet presentaron un mayor número de nematodos, paralelo a un desarrollo más rápido de los mismos, comparado con las raíces del injerto. Solamente hubo respuestas de hipersensibilidad en raíces de los patrones penetrados por J2. Esto ocurrió a lo largo de la epidermis, entre las células corticales y a lo largo del tejido vascular de diferenciación. La necrosis cortical, interrumpió o demoró la migración de J2 a los tejidos vasculares, mientras la necrosis vascular, impidió el establecimiento exitoso de sitios de alimentación, perturbando así el desarrollo posterior de los J2. La necrosis celular estuvo asociada con inanición, siempre y cuando, ésta fuese abundante. Treinta y cinco días después de la inoculación, patrones sostuvieron menos del 4% de las hembras adultas en comparación de las raíces a pie franco de las parras susceptibles. En 10-23B, un mecanismo de defensa adicional se hizo aparente. Este involucró, la inanición de los juveniles globosos hasta el estado de hembras adultas, sin desarrollo de las reacciones

de hipersensibilidad. El resultado final fueron hembras adultas enanas y la pérdida de la reproducción. Ambos patrones mostraron resistencia a *M. arenaria* pt. Harmony, incluyendo mecanismos que demoraron la penetración y el desarrollo, comparables al de Cabernet sin injertar.

Palabras claves: desarrollo, patrones de parras, reacción de hipersensibilidad, *Meloidogyne arenari* pt Harmony, penetración, reproducción.

INTRODUCTION

In warmer regions root-knot nematodes (*Meloidogyne* spp.) are the most important root pests attacking grapevines. In California, nematodes can reduce vine yield by 25% (McKenry, 1981). Over the last four decades several rootstocks have been selected for their resistance to *Meloidogyne* spp. The primary source of this resistance has been *Vitis champinii* Munson, which may or may not be crossed with various intra-specific hybrids of these crosses, and resulted in *Meloidogyne* resistant rootstocks including cultivars Harmony and Freedom. Shortcomings of these two rootstocks have gradually become apparent. First, in addition to *Meloidogyne* spp., there are other soil pests damaging to grape. Second, in the sandiest soils more aggressive *Meloidogyne* pathotypes have gradually been selected. Third, these rootstocks can exhibit excessive vigor in better soils.

Host plant resistance restricts or prevents nematode reproduction by activating resistance mechanisms in response to nematode infection. By contrast, susceptible plants lack resistance or tolerance or both, making them good hosts for pathogen reproduction (Trudgill, 1992). Resistance that deters root-knot nematode can involve pre- or post-infection mechanisms (Huang, 1985). Pre-infection resistance may occur at the root surface or within the rhizosphere thereby influencing nematode penetration. Plant produced root exudates can also attract or repel root-knot nematodes. Post-infection resistance mechanisms can involve physiological processes within the roots which: 1) deter nematode feeding, 2) deter the establishment of feeding sites, 3) delay or prevent nematode development, or 4) inhibit reproduction (Trudgill, 1992).

Grape rootstocks 10-23B, *Vitis doanianna* Munson and RS-3 (*V. candicans* Engelmann × *V. rupestris* Scheele) × (*V. riparia* Michaux × *V. rupestris*) were selected from among 520 *Vitis* by Drs. David Ramming and M. V. McKenry. These grape rootstocks are not only resistant to aggressive pathotypes of *Meloidogyne* spp. but possess "broad nematode resistance" against *Xiphinema index* Thorne & Allen, *Pratylenchus vulnus* Allen & Jensen, *Tylenchulus semipenetrans* Cobb plus some protection against *Criconemella xenoplax* (Raski) Luc & Raski and *X. americanum* sensu stricto Cobb.

The objectives of this study were to evaluate root-knot nematode penetration, development, and reproduction in susceptible and resistant rootstocks. The nematode studied is a pathotype capable of breaking resistance in the most popular nematode-resistant grape rootstocks.

MATERIALS AND METHODS

The pathotype *M. arenaria* (Neal) Chitwood pt. Harmony was from a vineyard located near Livingston, California. Second stage juveniles (J2) were recovered from galled Harmony roots using Baermann funnels placed in a mist chamber for 5 days. Suspensions of J2 were prepared in tap water to enable the desired inoculum density to be added in 10 cm^3 of water per

plant. Two grape rootstocks, RS-3 and 10-23B, which possess resistance to root-knot nematode pathotypes, were compared to the susceptible Cabernet Sauvignon (*V. vinifera*) control. Plants were grown from shoot tip cuttings by placing them in a bed consisting of a 1-inch-thick layer of autoclaved sand layered over a 2-inch-thick layer of a peat-perlite mixture. Beds were irrigated by water mist of 30-second duration every 9 min within a greenhouse maintained at 30°C. Plants of uniform root and shoot size were selected and transplanted into deepots [Stewe and Sons, Inc. Corvallis, OR 97333-9461, USA] of 5 cm × 25 cm length filled with autoclaved sand. The deepots were immediately watered with Hoagland's solution and plants were allowed 7 days to heal injuries before nematode inoculation. Plants were fertilized with Hoagland's solution biweekly. Plants of each rootstock were inoculated with 500 J2 of *M. arenaria* pt. Harmony by injecting at 12-cm depth on two sides of the plant. The treatments were arranged in a completely randomized design on a greenhouse bench. Each treatment consisted of five replications with five harvest dates.

At 4, 13, 21, 27 and 35 days after inoculation (DAI), five inoculated plants of each rootstock were harvested. The roots were washed free of soil, blotted with paper, damp-dried, and weighed. The whole root system of each plant at each harvest was stained with acid fuschin (Byrd *et al.*, 1983). Each root system was spread in a film of glycerin between two glass plates (7.5 × 15 cm). Number of nematodes and stage of development within the roots were determined using a dissecting microscope. The numbers of nematodes in the stained root system recorded at each sampling were classified into four developmental stages (Jenkins *et al.*, 1995; Syndenham *et al.*, 1996): vermiform, non-swollen J2; swollen, sausage shaped J2; globose, sub-spherical juveniles exhibiting spiked tail [J_3 and J_4]; adult fully differentiated female with or without eggs.

Infection sites were scored for host cell necrosis and appearance of juveniles. Necrosis was recorded when several deformed and brown cells appeared close to a nematode head. The appearance of a clear area in the intestine indicated starvation and severe reduction in body diameter and coiling indicated degradation and death of the nematode (Van Gundy *et al.*, 1967). Data were expressed as the percentage of juveniles associated with host-cell necrosis in relation to the total number of nematodes observed within roots.

Root systems of plants harvested 21, 27 and 35 DAI were stained with Phloxine B (Holbrook *et al.*, 1983) before staining with acid fuschin to assess the presence of egg masses. The root systems were rated for galling and egg mass presence on a 0 to 5 scale (Taylor and Sassar, 1978), where 0 = no gall or egg mass, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = >100 galls or egg masses per root. At 27 and 35 DAI an additional five plants of each cultivar were harvested with five receiving stains and the other five incubated in a mist chamber for 5 days to hatch the eggs. Number of eggs per female was determined after dissolving the matrix of handpicked egg masses in 2% NaOCl (Hussey and Barker, 1973).

The data were subjected to analysis of variance with the procedure of SAS. Significant differences in means of nematode development were separated using Duncan's Multiple Range Test.

RESULTS

Penetration, development and reproduction of *M. arenaria* pt. Harmony differed among the three grape cultivars. At 4 DAI, a greater ($P = 0.01$) number of J2 penetrated susceptible Cabernet roots com-

pared to the resistant 10-23B roots. Nematodes were not present in resistant RS-3 roots at 4 DAI (Fig. 1, 4-DAI). The infection sites of J2 on susceptible Cabernet were swollen, whereas there were no swollen infection sites in resistant 10-23B. The J2 were found clustered within the root apex of resistant 10-23B, but a few had also migrated upward into the developing vascular cylinder.

At 13 DAI more ($P = 0.01$) nematodes had reached the swollen stage of development on susceptible Cabernet roots than on either of the resistant rootstocks (Fig. 1, 13-DAI). Roots of resistant RS-3 were penetrated by J2 but only 23% had advanced to a swollen stage of development compared to 37% and 94% on resistant 10-23B and susceptible Cabernet, respectively. The number of vermiform and swollen J2 was equal in the roots of both resistant RS-3 and 10-23B rootstocks. Galls were visible on the root system of susceptible Cabernet and resistant 10-23B, with a mean gall index of 3.0 and 2.0, respectively. There were no galls on the roots of RS-3. However, infection sites of J2 on roots of both resistant rootstocks were swollen. The root tissues of resistant RS-3 exhibited a hypersensitive reaction in response to 46% of the penetrated juveniles (Fig. 2). The entombed nematodes were starved, shrunken or dead.

By 21 DAI, a greater ($P = 0.01$) number of juveniles advanced to globose and adult female stages on the roots of susceptible Cabernet, compared to either resistant rootstock (Fig. 3, 21-DAI). At this time the gall index on roots of susceptible Cabernet

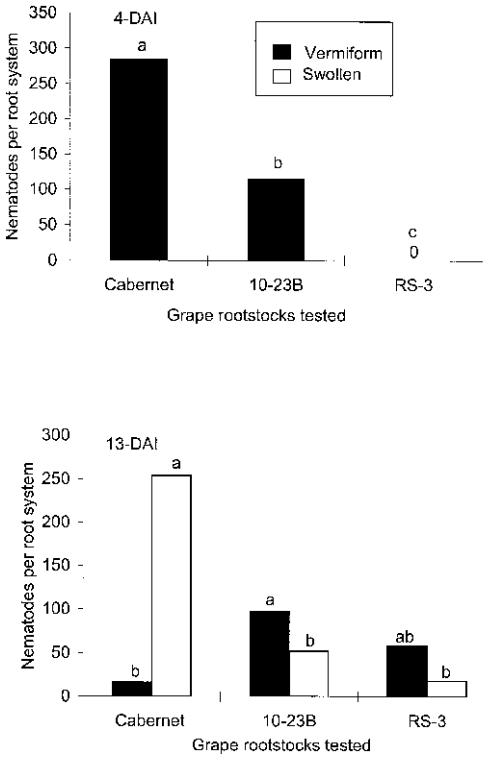

Fig. 1. Penetration and development of *Meloidogyne arenaria* pt. Harmony in roots of susceptible Cabernet and resistant 10-23B and RS-3 at 4 days after inoculation (DAI) and at 13 DAI. Bars are means of five replicates. Bars of same pattern with different letters differ significantly ($P = 0.05$).

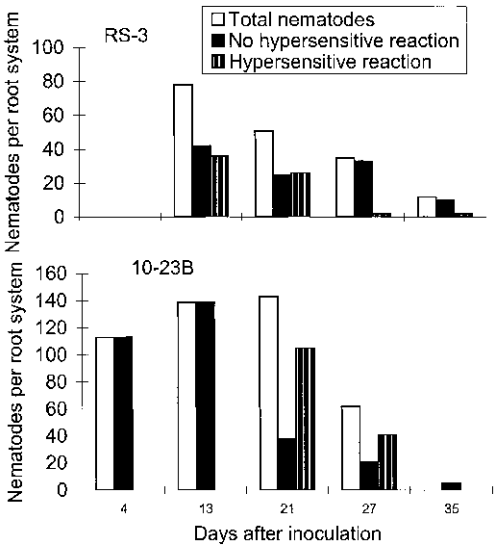

Fig. 2. Total number of *Meloidogyne arenaria* pt. Harmony in roots of resistant grape rootstocks over a period of 35 days and number of nematode-induced hypersensitive reactions.

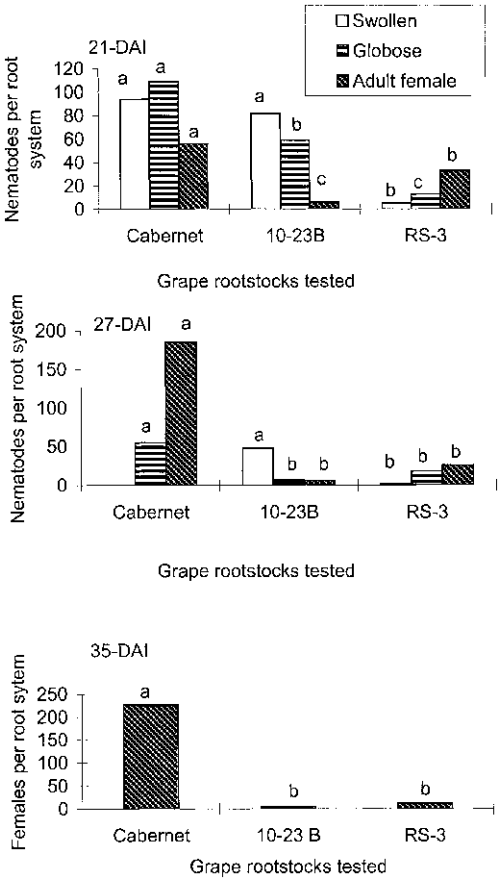

Fig. 3. Penetration and development of *Meloidogyne arenaria* pt. Harmony in roots of susceptible Cabernet and resistant 10-23B and RS-3 at 21, 27 DAI and 35 days after inoculation (DAI). Bars are means of five replicates. Bars of same pattern with different letters differ significantly ($P = 0.05$).

and resistant 10-23B had increased to 4.0. Resistant RS-3 roots showed a galling index of 3.0. Galls were largest on Cabernet roots, intermediate on roots of resistant 10-23B and very small on roots of resistant RS-3. Egg masses were visible on roots of susceptible Cabernet, which had a 5.0 egg mass index, whereas egg masses were absent on the roots of both resistant rootstocks. The root tissues of resistant rootstocks exhibited vascular hypersensitive reaction associated with 73% and 50% of the total nematodes present within 10-23B and RS-3, respectively. The observed necrotic tissues always surrounded these nematodes during their vermiform J2 stage, thus arresting their development to J3 (Fig. 2).

By 27 DAI inoculation, significantly ($P = 0.01$) more juveniles had progressed to adult female stage on roots of susceptible Cabernet compared to those on resistant rootstocks. The swollen development stage was more persistent in resistant 10-23B roots with 77% of the nematodes still present in this stage compared with 0% and 6% in susceptible Cabernet and resistant RS-3, respectively (Fig. 3, 27-DAI). The gall and egg mass indices were 5.0 on roots of susceptible Cabernet and 3.0 on resistant RS-3 roots, whereas on the resistant 10-23B the gall index reduced to 2.0. Most of the galls on the roots of 10-23B were either empty or contained fragments of nematodes or under-developed juveniles.

Numbers of J2 hatched per root system and females per gram of root were greater ($P = 0.01$) for susceptible Cabernet roots than for resistant 10-23B or RS-3 roots (Table 1). The hypersensitive reaction persisted and was 66% and 6% on roots of resistant 10-23B and RS-3, respectively (Fig. 2).

At the 35-day sampling, more ($P = 0.01$) juveniles (45.4%) had developed to adult females on roots of susceptible Cabernet compared to roots of resistant rootstocks RS-3 (1%) and 10-23B (2%). All the juveniles had advanced to the adult stage with or without egg masses in all three grape rootstocks (Fig. 3, 35-DAI). There was no change in gall and egg mass indices at this stage. However, the galls and adult females within Cabernet roots were largest in size, intermediate in size on roots of resistant 10-23B and very small on the roots of resistant RS-3. The number of J2 hatched per root system and females per gram of root on susceptible Cabernet were also higher at the 35-day sampling, but the

Table 1. Number[y] of *M. arenaria* pt. Harmony females, eggs and hatched juveniles on roots of susceptible Cabernet, and resistant 10-23B and RS-3 grape rootstocks.

Days after inoculation	Females per gram of root			Hatched J2 per gram of root			Eggs per female		
	Cabernet	10-23B	RS-3	Cabernet	10-23B	RS-3	Cabernet	10-23B	RS-3
27	91 a[z]	14 b	9 b	101 a	0.71 b	14 b	—	—	—
35	108 a	1 b	3 b	810 a	1.40 b	81 b	107 b	0 c	157 a

[y]Data are means of five replicates for females and hatched J2 whereas eggs per female are means of 20 egg masses.

[z]Within a row, means for female and hatched J2 not followed by same letter differ significantly ($P = 0.01$), whereas means for eggs per female differ significantly ($P = 0.05$) according to Duncan's Multiple Range Test.

fecundity (eggs per egg mass) was higher ($P = 0.05$) in roots of resistant RS-3 than in susceptible Cabernet (Table 1). No egg masses occurred on roots of 10-23B. Empty galls containing fragments of nematodes or under-developed juveniles were found in 10-23B rootstock 35 DAI. At 35 days there were only two under-developed juveniles remaining in RS-3, both having an associated hypersensitive reaction, whereas all under-developed juveniles on the roots of 10-23B had died.

DISCUSSION

The observed host-parasite interactions resulted in important changes for both organisms. Compared to the susceptible rootstock where the nematodes developed, reproduced and induced gall formation, the resistant rootstocks RS-3 and 10-23B defend themselves against nematode infection by epidermal, cortical and vascular necrosis leading to reduced penetration followed by limited development and reproduction.

The resistance response to *M. arenaria* pt. Harmony in the resistant grape rootstocks was expressed at the epidermal level by delayed J2 penetration and differential penetration compared with the susceptible grape rootstock. Failure of J2 to penetrate roots of resistant RS-3 indicates the existence of physical or chemical root barriers preventing penetration. Delayed penetration of a resistant cotton cultivar has also been reported (Anwar *et al.*, 1994). Differential penetration by nematodes of roots of resistant and susceptible cultivators of soybean (Dropkin and Nelson, 1960), cotton and grape rootstocks (Ferris *et al.*, 1982) have been reported. The reduced number of J2 in resistant compared to susceptible cultivars indicates that resistance is largely attributable to reduced penetration by J2.

Juvenile infection sites occurring in susceptible Cabernet roots resulted in root tip swelling by 4 DAI, whereas the swelling of infection sites in roots of resistant rootstocks was delayed. This might be related to the behavior of J2 in roots. In Cabernet, the J2 were observed to migrate to the developing vascular cylinder, insert their heads within vascular tissues and orient parallel to the long axis of the root. In resistant cultivars the J2 remained clustered at the root apex. The induction of differential biochemical changes in susceptible and resistant cultivars is related to establishment of parasitic relationships by J2 (Potenza *et al.*, 1996).

The higher number of vermiform J2 at 13 days present in roots of resistant root-

stocks compared to susceptible Cabernet was due to faster development of J2 in susceptible roots compared to roots of resistant rootstocks. This delayed nematode development indicates the involvement of post-infectional biochemical defense mechanisms (hypersensitive response). By contrast, roots of susceptible Cabernet did not exhibit hypersensitive reaction, nematode development was normal, and there appeared to be no biochemical defense mechanisms present.

The smaller number of adult females in RS-3 and 10-23B roots compared to susceptible Cabernet roots might be related to the number of juveniles per feeding site and the intensity of hypersensitive reaction. We observed very strong necrosis of feeding sites parasitized by few compared to little necrosis when there were multiple juvenile infections per feeding site. Once root-knot nematodes have reached the globose stage they can develop to adult females in these grape examples. The development of *M. arenaria* juveniles in roots of resistant grape rootstocks is comparable to development of *M. incognita* in roots of resistant cotton genotypes (Jenkins *et al.*, 1995) and of resistant kidney bean (Syndenham *et al.*, 1996). We observed no hypersensitive reaction associated with feeding sites of globose or adult females; still the females were undersized in both resistant rootstocks and no reproduction occurred in roots of 10-23B. This suggests the occurrence of an additional mechanism leading to undersized females and limited reproduction.

The resistance response in 10-23B rootstock was more effective compared with that in roots of RS-3. Significantly fewer nematodes developed beyond swollen stage, the gall index was lower and no egg masses developed on this rootstock. This suggests that 10-23B rootstock possesses very active defense mechanisms including hypersensitive reactions which limit nematode development as well as reproduction. We observed very strong vascular hypersensitive reaction in roots of 10-23B. The presence of empty galls containing underdeveloped juveniles or few fragments of nematodes indicates that nematodes were able to initiate the development of giant cells but further development of giant cells was inhibited. Similar mechanisms of resistance have been observed in resistant cotton cultivars infected with *M. incognita* (McClure *et al.*, 1974).

The resistant plant responds to nematode infection by producing necrosis around the nematode or its feeding sites preventing further development of this soon to become sedentary organism. The degree of hypersensitivity, its time of initiation, and the eventual fate of juveniles depends upon the host parasite combination, type of plants, tissues infected and may differ in plants of the same crop (Canto-Saenz and Brodie, 1987). With *Tylenchulus semipenetrans*, hypersensitive reaction in citrus took up to 2 weeks to develop (Kaplan, 1981), whereas the hypersensitive reaction to *M. incognita* was visible in resistant tomato within 2 days (Dean and Struble, 1953; Paulson and Webster, 1972), in resistant tobacco after 7 days though even more apparent after 10 and 17 days, and in sweet potato after several days (Powell, 1962). We observed epidermal hypersensitive reaction in response to epidermal penetration around the head of juveniles. These nematodes starved soon after penetration. There was also a cortical hypersensitive reaction entombing, shrinking and killing the nematodes or delaying the movement to reach vascular tissues to establish feeding sites during the first week after inoculation. Hypersensitive reactions were also common in response to vermiform J2 present along the developing vascular system. The presence of greater numbers of globose stage females in resis-

tant 10-23 and RS-3 roots compared to the susceptible Cabernet roots appears to be associated with arrested development.

Although roots of resistant RS-3 supported less nematode development compared to susceptible Cabernet, the fecundity rate (eggs per egg mass) was high enough to be concerned about the durability of such resistance mechanisms. Similar observations have been made for *Meloidogyne* spp. on resistant alyce clover (Powers *et al.*, 1992). Ferris *et al.* (1984) found that *M. arenaria* produced more eggs per female on susceptible compared to resistant grape rootstocks. These differences appear to be associated with different rootstocks used.

This research has demonstrated that resistant rootstocks express their genetic resistance at penetration, development and reproduction during root-knot nematode life cycle. The development of a hypersensitive reaction associated with slower rates of female development resulted in undersized adult females. There was also a mechanism during reproduction that limited formation of egg mass thereby limiting reproduction. Our results provide an indication of diverse mechanisms of resistance at work in the root tips of these two promising grape rootstocks. Both rootstocks offer improvements over commercially available rootstocks but the durability of their resistance remains unclear.

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