NEW GRAPE ROOTSTOCKS WITH BROAD AND DURABLE NEMATODE RESISTANACE.

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Nematodes are becoming increasingly important pests in vineyards around the world, and these soil-borne pests can be particularly problematic in California vineyards. Two factors intensify the impact of nematodes – the high value of grapes and of vineyard land. These factors force growers to ignore the need to leave land fallow and to rotate crops, both of which reduce nematode build up and the delay the selection of adapted strains. Nematicides and fumigants help control nematodes, but the use of these pesticides has been greatly restricted and their future use in doubt because they must be persistent and penetrate deeply through the soil profile to be highly effective. In addition, grape rootstocks were bred to resist grape phylloxera (a devastating root-feeding aphid), and were not selected for nematode resistance. The grape rootstocks Freedom and Harmony were released by the USDA/Fresno to provide resistance to nematodes, but they do not resist grape phylloxera. More recently, McKenry at UC Riverside released two rootstocks, RS3 and RS9 (siblings of a Schwarzmann x Ramsey cross), designed to resist multiple nematode species and provide growers with nematode resistant rootstock alternatives. However, they have nematode resistance from a relatively narrow genetic base, which may promote the evolution of strains capable of feeding on them.

A breeding program was initiated in 1990 at UC Davis to provide a group of rootstocks with broad and durable resistance to the nematodes found in California vineyards. This program commenced with an evaluation of selections that remained from Lider's breeding efforts in the late 1960s and early 1970s. Lider had screened these selections against two root knot nematode species (*Meloidogyne incognita acrita* and *M. arenaria thamsei*), the lesion nematode (*Pratylenchus vulnus*), and the dagger nematode (*Xiphinema index*). In 1990, these selections were evaluated for their ability to root and for growth habits such as brushy growth, internode length and the degree of lateral shoot production. In 1993 and 1994, the best of these selections were crossed to species chosen for their ability to reduce scion vigor or improve the rooting of the progeny. The parentage and species composition of these crosses and one other used in 1989 (8909-05) are listed in Table 1. About 5,000 progeny were planted in the vineyard and their evaluation for nematode resistance was initiated in 1996.

The first phase of the selection process examined the progeny for their general vigor and horticultural characters such as internode length and the degree of lateral shoot formation. The best 1,000 progeny, selected from as many families as possible, were advanced to a rooting assay. Ten 2-node dormant cuttings of the best 1,000 were taken in December 1996 and tested for their ability to form roots. Rootstocks that root well generally graft well, thus this evaluation was a key indicator of their future success as rootstocks. One hundred of the progeny were selected, again with an effort to get a broad representation from as many families as possible.

The second phase of the selection process involved testing these 100 selections for resistance to *M. incognita* I3. This root-knot nematode isolate is capable of feeding on many rootstocks, but does not feed on rootstocks that derive their resistance from *V. champinii* (Freedom, Harmony, Dog Ridge and Ramsey). All of the nematode testing in this breeding program utilized potted plants under greenhouse conditions with optimized soils and irrigation techniques to promote nematode feeding. Resistance to this isolate of root-knot nematode was evaluated by assaying the number of galls on the roots after inoculation with 1,000 J2 larvae (the free-living infectious stage of this nematode). Resistance was also evaluated by extracting the J2 nematodes in the pots after the root galls were counted. Later phases of the root-knot nematode screening evaluated resistance by counting the number of egg masses formed using a technique developed in the Walker lab (Cousins and Walker 2001 Plant Disease 85:1052-1054). There were no root galls on 33 of the 100 selections and they were advanced to the next phase of screening.

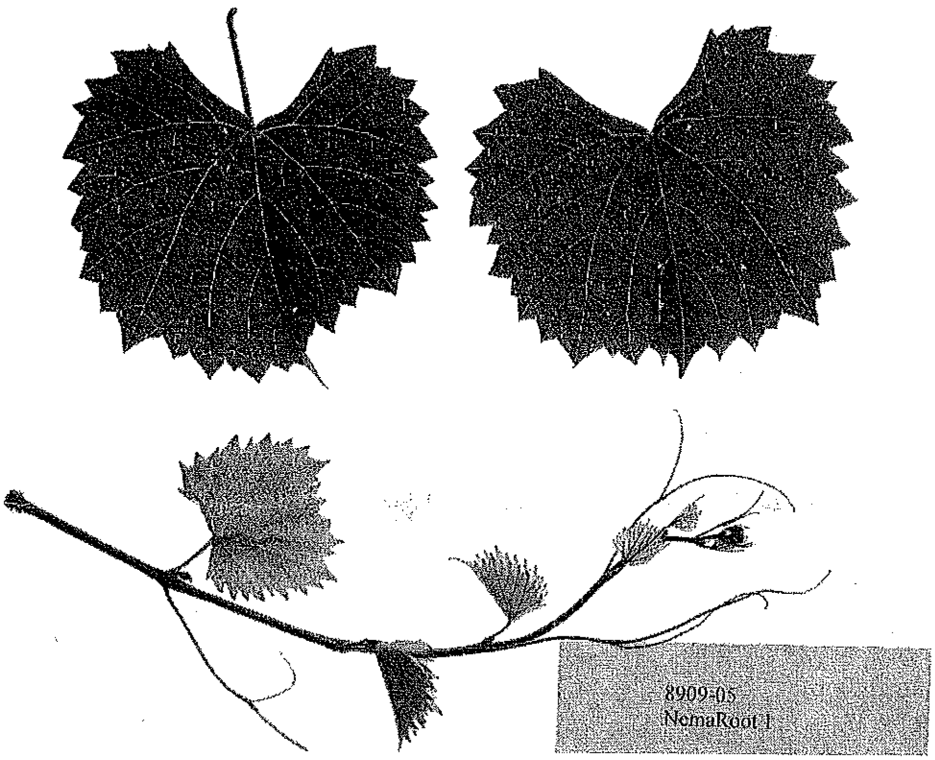
The third phase of the selection process tested the 33 root-knot nematode resistant selections against two aggressive strains of root-knot nematode and against the dagger nematode, *X. index*. The two aggressive strains were selected in the Walker lab from soils provided by McKenry, from a declining Harmony vineyard. Root-knot nematodes were extracted from this soil and the larvae were placed onto tomato plants with high susceptibility to root-knot nematodes. Two egg masses were collected from these infested plants and J2 larvae from each mass were put on a separate uninfested tomato plant to create new strains from a single egg mass (root-knot nematodes are parthenogenic). These new strains were multiplied on tomato and were then used to inoculate potted plants of Harmony rootstock to verify their ability to feed aggressively on this resistant rootstock. These two strains were named HarmA and HarmC. Later investigations determined that HarmA was a strain of *M. arenaria* while HarmC was a strain of *M. incognita*. The dagger nematodes were collected from several vineyards in the Napa Valley of California, which were known to have fanleaf degeneration.

The 33 selections were then inoculated with each of the three nematode strains independently. The root-knot nematode screens used 1,000 J2 larvae to inoculate plants growing in 1,000 cm3 plastic pots with a coarse sand / clay loam soil mix. The dagger nematode screens were done in the same pots and soils, but used 200 adult *X. index* as the inoculum. Fourteen selections did not produce egg masses when inoculated with the three root-knot nematode strains nor did they produce root tip galls after inoculation with *X. index* (Table 2).

In the fourth phase, the 14 selections were subjected to a series of tests. The first was a combined inoculum of all four nematodes at once to determine the impact of simultaneous nematode feeding on resistance (Table 3). They were also tested against the four nematodes over a range of temperatures, 24, 27, 30 and 32C. There was some erosion of resistance to HarmA (the most aggressive strain of root-knot nematode) at this temperature, but 6 of the selections performed very well (Table 4). Root-knot nematode resistance is known to fail at about 28C in a wide range of species including tomato, pepper and plum. The 14 selections were also evaluated for resistance to lesion (*Pratylenchus vulnus*), citrus (*Tylenchulus semipenetrans*) and ring (*Mesocriconema xenoplax*) nematodes (Table 3). From this series of tests six rootstock selections were made and five of these are proposed for release. A summary of their characteristics is presented below.

Grape phylloxera are capable of feeding and producing galls (nodosities) on the young roots of virtually all grape rootstocks and species. In order to gauge the phylloxera hosting ability of the final six selections, they were tested for the ability to support phylloxera on nodosities and compared to a set of commercial rootstocks. Three sets of young root pieces from each selection were inoculated with 10 phylloxera eggs collected from 101-14 Mgt rootstock roots. Over a 21-day period, the number of eggs and juveniles that were produced were summed and divided by 10 (the original inoculum) to produce the average rate of increase. Table 5 presents these results for the six selections and five rootstocks. Nodosity galling on young roots does not appear to damage grapevines, only feeding and galling on mature roots (tuberosities) leads to vine death. The high rate of phylloxera feeding, galling and reproduction on 101-14 Mgt (7.98 average rate of increase) has been observed in past tests. Most of the values were very low, although this 101-14 Mgt strains is well adapted to 9407-14. Three of the selections were very resistant: 8909-05, 9363-16 and 9449-27. The low values for AXR#1 demonstrate that the results of this test do not reflect field level or tuberosity level feeding and damage, and that phylloxera adapt independently to rootstock hosts.

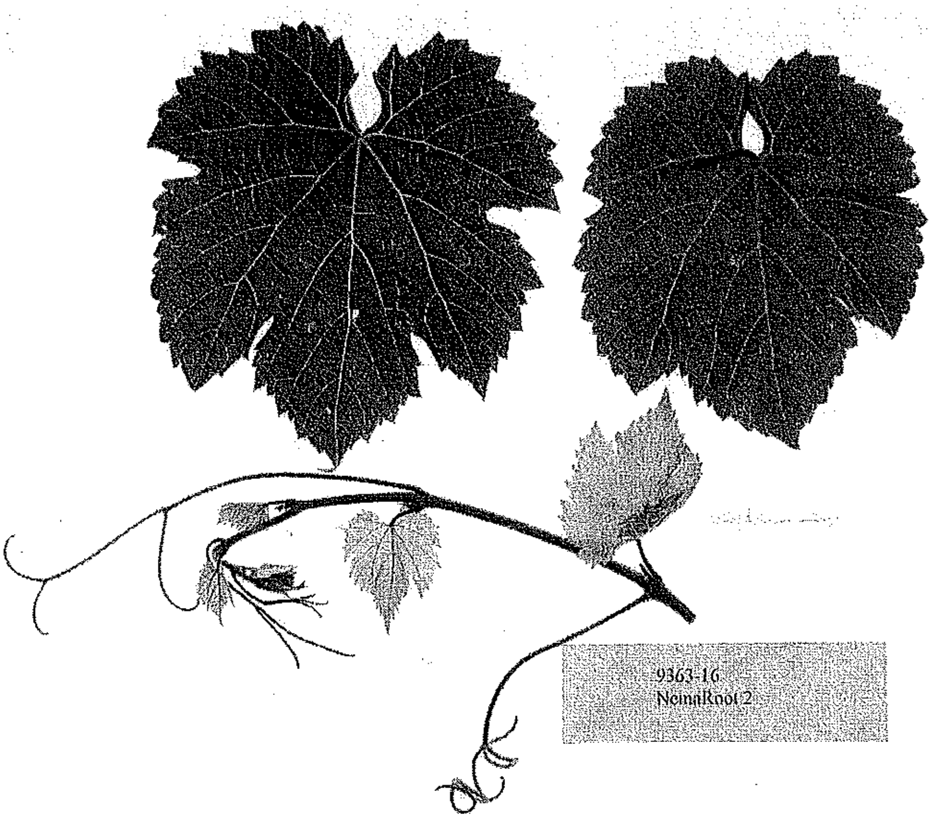
It will take years to determine which sites each of these rootstock selections are best suited to, but they have unparalleled levels of resistance to nematodes and should excel in sites with single and mixed nematode species infestations. Four of the selections (9365-43, 9365-85, 9407-14 and 9449-27) were grafted to Fiesta Seedless and planted in a Fresno rootstock trial in 2004. That year two of these selections (9365-43 and 9365-85) were also included in a Chardonnay rootstock trial in Santa Maria. We also tested the six rootstock selections in large pots using soils from the Gallo Livingston Ranch where nematode pressure from root-knot, lesion, ring and *Xiphinema americanum*, is known to be severe and chronic. This test was conducted to evaluate these selections under "field conditions" using infested soil without added inoculations. All of the selections performed very well against root-knot nematodes (Table 6) and two, 9407-14 and 8909-05, also performed very well against ring nematode (Figure 1).



8909-05 NemaRoot 1

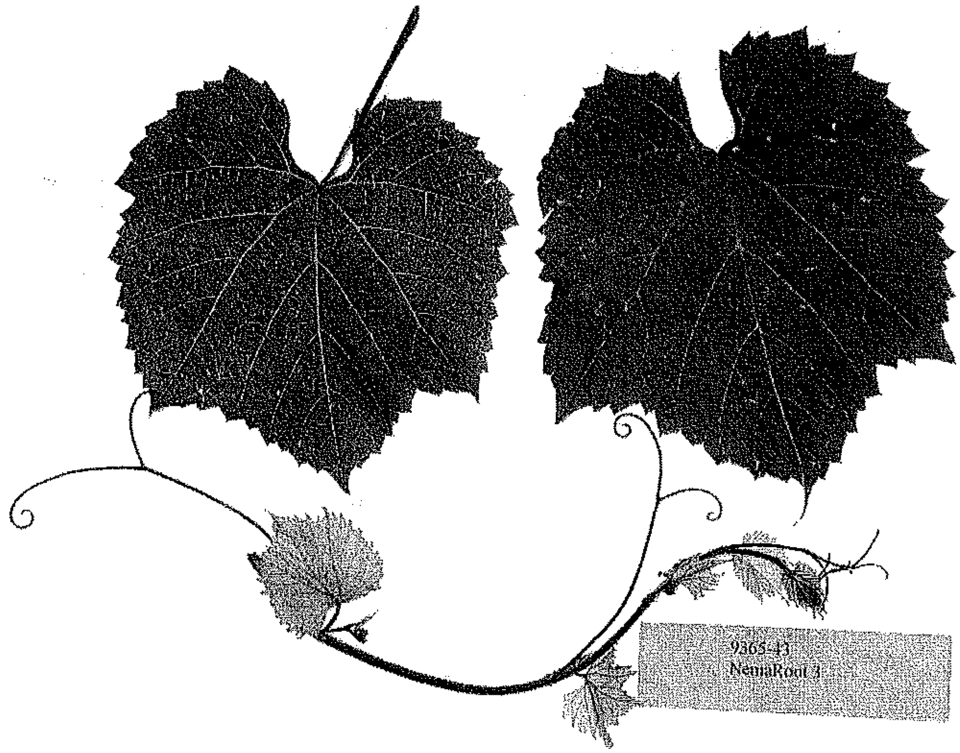
The most resistant selection of the group is 8909-05, a *V. rupestris* x *M. rotundifolia* hybrid, and is one of the rare sources of resistance to ring nematode. This plant has extremely strong and broad nematode resistance. 8909-05 may also possess the same ability to control fanleaf degeneration that O39-16 does. O39-16 has strong resistance to *Xiphinema index*, the dagger nematode that vectors grapevine fanleaf virus (GFLV), the causal agent of fanleaf degeneration. However, *X. index* is still able to vector GFLV into O39-16 roots while probing for feeding sites, and the virus moves into the scion where it replicates and spreads. However, fanleaf infection does not result in disease when scions are grafted onto O39-16. GFLV infects other *X. index* resistant rootstock / scion combinations, but only O39-16 induces fanleaf tolerance to the scions grafted on it. O39-16 is a *V. vinifera* x *M. rotundifolia* hybrid and its ability to induce fanleaf tolerance is probably a result of its *M. rotundifolia* parentage. 8909-05 may also posses this ability, and it is a member of a group of *Vitis species* x *M. rotundifolia* selections currently being tested for their ability to induce fanleaf tolerance.

The appearance of 8909-05 was originally thought to be "too *rotundifolia*-like" to allow it to root well and because of this it was not included in either the Fresno or Santa Maria trial. However, it has rooted and grafted at 80% success from dormant cuttings. This last winter was an exception; it did not root or graft at all from dormant cuttings. These cuttings were taken in early February after a very cold mid January (lows of 23F, -5C). O39-16 cuttings, taken at the same time also failed to root and graft. Mother vines or 8909-05 have sterile flowers, moderately long shoots with shorter internodes and more laterals than the other 5 selections. Preliminary rooting tests found it to have moderately deep rooting angles.

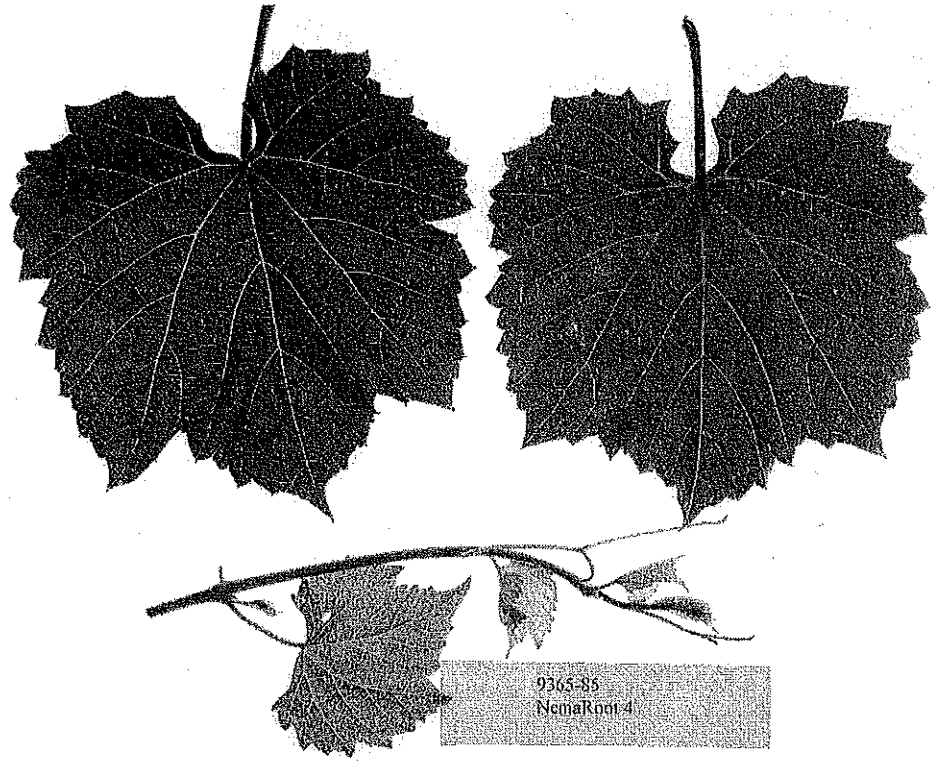


9363-16 NemaRoot 2
9363-16 acquires its nematode resistance from *V. rufotomentosa* (highly resistant to *X. index*) and *V. champinii* 'Dog Ridge' (strong resistance to root-knot and dagger nematodes) and roots and grafts easily because of its *V. riparia* parentage. It has slightly lobed leaves, acquired from *V. rufotomentosa*, that are relatively glabrous, acquired from *V. riparia*. 9363-16 is a good mothervine with staminate flowers, longs shoots, long internodes and few laterals. Preliminary propagation tests have found it to have a relatively shallow rooting angle. It is susceptible to citrus and ring nematodes, but has excellent resistance to root-knot and dagger nematodes (Table 3).

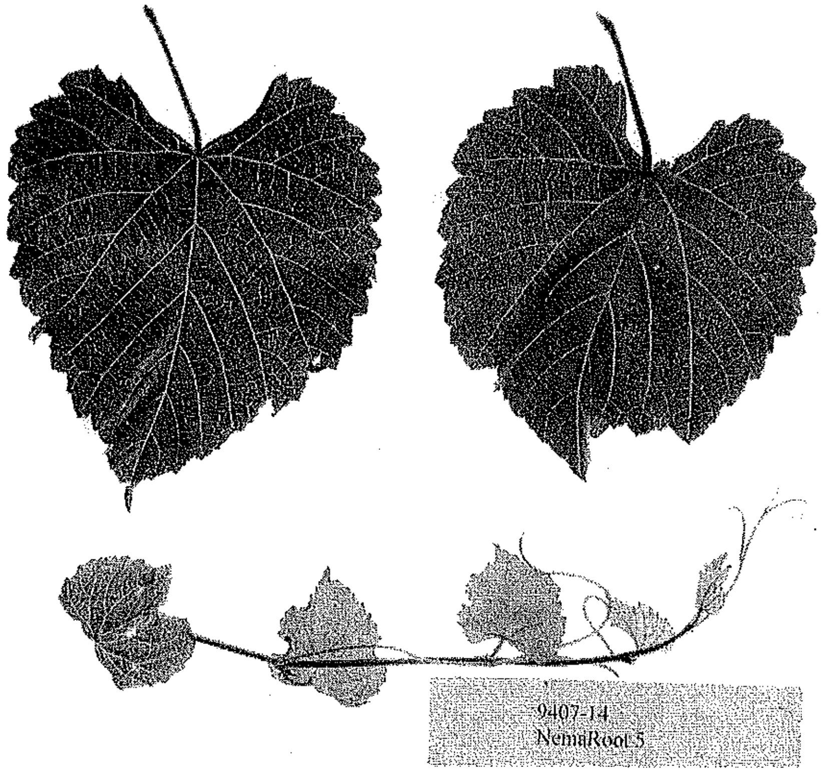
9365-43 NemaRoot 3 and 9365-85 NemaRoot 4
These two selections are siblings. They derive their strong nematode resistance from *V. rufotomentosa*, *V. champinii* 'Dog Ridge' and from c9038 a form of *V. champinii* that appears to intergrade with *V. monticola*. *Vitis monticola* is an unusual species that grows on very dry, gravelly or rocky limestone sites. *Vitis riparia* was used in the cross to impart good rooting and grafting abilities.



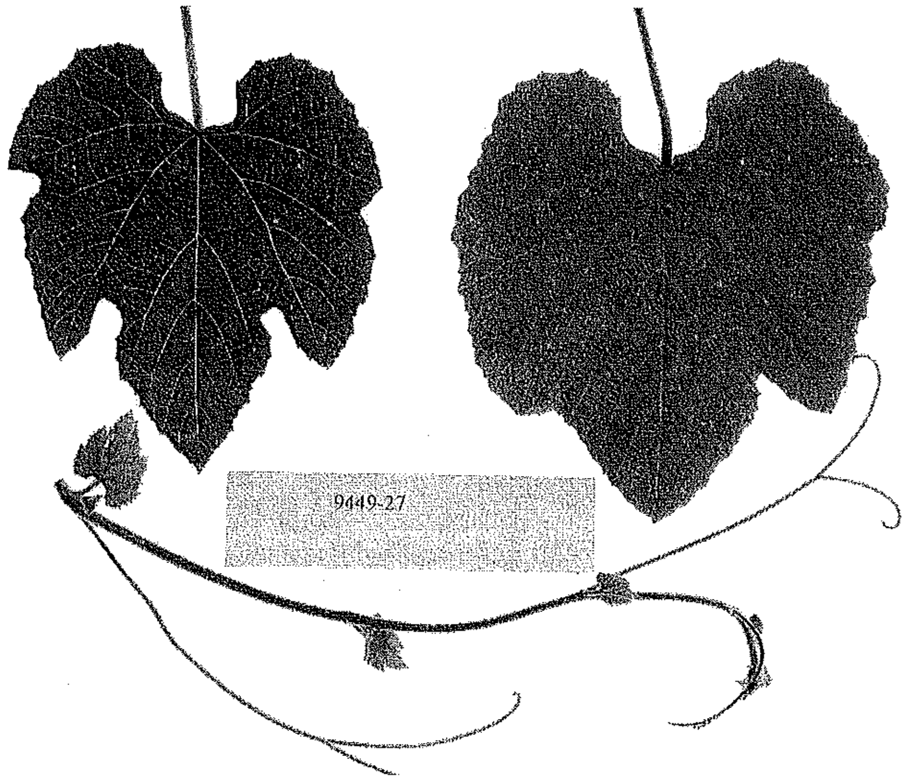
9365-43is a pistillate flowered vine with leaves that appear *V. champinii*-like with shorter teeth, thicker texture and limited lobing. It produces mothervines with moderate vigor, long straight canes with moderately long internodes and a moderate number of lateral shoots. 9365-43 had excellent nematode resistance in the combined testing, resists citrus and lesion nematodes, and is moderately susceptible to ring nematodes (Table 3). Preliminary propagation tests found it to have moderately deep rooting angles.



9365-85 is a staminate flowered vine with leaves that appear more like *V. riparia* with longer teeth, more prominent lobing, and thinner more puckered texture. Its resistance to root-knot and dagger nematodes in the combined testing was very good, and it resists citrus and lesion nematode, but is susceptible to ring nematode (Table 3). 9365-85's resistance to *M. arenaria* HarmA was the most severely impacted by higher temperatures compared with the other five selections, although it is not statistically different from 9365-43 and 9363-16 (Table 4). 9365-85 is an excellent mother vine with long canes, good internode lengths and few lateral shoots. Preliminary propagation results indicate it has moderately deep rooting angles.



9407-14 NemaRoot 5
This selection derives its resistance from *V. champinii* 'Ramsey' and from c9021 a form of *V. champinii* that appears to intergrade with *V. berlandieri*. *Vitis riparia* was used to improve the rooting and grafting of cuttings. 9407-14 had excellent root-knot and dagger nematode resistance in the combined testing, resisted citrus and lesion nematodes, and supported a low number of ring nematodes (Table 3). It is a staminate flowered vine with moderate growth, but long canes, good internode lengths and few laterals. 9407-14 has glossy leaves with short rounded teeth and slightly puckered surface, and the leaves are mildly susceptible to powdery mildew in a no-spray block at the UCD campus. Preliminary propagation results indicate that 9407-14 has a deep rooting angle. This selection supported the highest level of nodosity based phylloxera, but research to date has concluded that high nodosity level feeding does not cause vine damage.



9449-27

The final selection, 9449-27, is not being released at this date, but will continue in rootstock trials. It is a cross of *V. rutomentosa* and *V. cinerea*, and the does not root or graft easily. It has been used as a parent to broaden the genetic base of nematode resistance.

Table 1. Parentage of the six nematode resistant grape rootstock selections to be released from UC Davis.

Selection	Parentage
8909-05	*V. rupestris* x *M. rotundifolia*
9363-16	(*V. rufotomentosa* x (*V. champinii* 'Dog Ridge' x Riparia Gloire)) x Riparia Gloire
9365-43	(*V. rufotomentosa* x (*V. champinii* 'Dog Ridge' x Riparia Gloire)) x *V. champinii* c9038 (probably *V. candicans* x *V. monticola*)
9365-85	(*V. rufotomentosa* x (*V. champinii* 'Dog Ridge' x Riparia Gloire)) x *V. champinii* c9038 (probably *V. candicans* x *V. monticola*)
9407-14	(*V. champinii* 'Ramsey' x Riparia Gloire) x *V. champinii* c9021 (probably *V. candicans* x *V. berlandieri*)
9449-27	*V. rufotomentosa* x *V. cinerea* c9008

Table 2. Selections with broad resistance to four nematodes when inoculated individually.

Selection	*X. index*	*M. incognita* I3	*M. arenaria* - HarmA	*M. incognita* - HarmC
8909-05	R	R	R	R
9317-06	R	R	R	R
9332-43	R	R	R	R
9344-03	R	R	R	R
9363-16	R	R	R	R
9365-43	R	R	R	R-
9365-62	R	R	R	R
9365-85	R-	R	R	R
9403-35	R	R	R-	R-
9403-107	R	R	R	R
9407-14	R	R	R	R
9449-23	R	R	R	R
9449-25	R	R	R	R
9449-27	R	R	R	R
Control Group:				
1616C	S	R	R-	R-
Harmony	S	R	S	S
Colombard	S	S	S	S

R = Resistant, no gall symptoms or egg masses observed
R- = Trace infection
S = Susceptible, symptoms present, nematode reproduction supported

Table 3. Summary results for 14 nematode resistant selections when tested against combined inoculum with three root-knot nematode (RKN) strains and dagger nematode (Xi). Results of testing against citrus, lesion and ring are also reported.

Genotypes	Xi Galls in Combined Testing		RKN Egg Masses in Combined Testing		Citrus		Lesion		Ring	
8909-05	R	0	R	O	R	<100	R	<10	R	<1,000
9317-06	MS	<1	S	<5	R	<100	R	<50	HS	<20,000
9332-43	S	<5	S	<5	R	<100	R	<50	S	<10,000
9344-03	S	<5	MS	<1	S	>400	S	<200	S	<10,000
9363-16	R	0	R	0	S	>400	R	<50	S	<10,000
9365-43	R	0	R-	<1	R	<100	R	<50	MS	<5,000
9365-62	MS	<1	S	<5	R	<100	R	<50	MS	<5,000
9365-85	MS	<1	R-	<1	R	<100	R	<50	S	<10,000
9403-107	R	0	S	<5	R	<100	R	<50	MS	<5,000
9403-35	S	<5	S	<5			R	<50	MS	<5,000
9407-14	R	0	R	0	R	<100	R	<50	LS	<3,000
9449-23	MS	<1	R	0	R	<100	R	<50	S	<10,000
9449-25	MS	<1	R	0	R	<100	R	<50	S	<10,000
9449-27	MS	<1	R-	<1	R	<100	R	<50	S	<10,000
1616C	S	>50	S	<5	S	>400	S	<200	HS	<20,000
Freedom	S	>10	S	<10	R	<100	R	<50	HS	<20,000
Colombard	S	>100	S	>100	S	<1000	S	<300	HS	<30,000
Harmony	S	>10	S	<50	S	<1000	S	<200	HS	<30,000
St. George	S	<100	S	<50	S	>400	R	<50	S	<10,000

Table 4. Number of egg masses per potted plant and per gram of root after inoculation with 1,000 *M. arenaria* HarmA nematodes and testing at 32C.

Genotype	Egg Mass/Plant	Egg Mass/g Root
Colombard	442.50 a	155.86 a
Harmony	156.00 b	123.28 b
9365-85	32.25 c	16.49 c
9365-43	7.25 c	6.04 cd
9363-16	6.75 c	5.74 cd
9449-27	0.25 c	0.22 d
9317-06	0.00 c	0 d
8909-05	0.00 c	0 d

Table 5. The average rate of population increase of phylloxera on nodosities formed on young root tips of advanced rootstock selections and a set of standard rootstocks. The phylloxera were selected from a vineyard planted on 101-14 Mgt rootstock.

8909-05	0.59
9363-16	0.89
9365-43	1.86
9365-85	1.99
9407-14	9.83
9449-27	0.30
1103P	2.10
101-14 Mgt	7.98
Teleki 5C	1.38
AxR#1	2.15
O39-16	0.43

Table 6. Number of root-knot nematode egg masses recovered from rootstock selections growing in 1 gal pots with soils collected from sites at the Gallo Livingston vineyards. This vineyard is known to have severe and chronic nematode pressure. Results per soils are means of three replicate pots.

Selection	Soil 1	Soil 2	Soil 3
Colombard	69.02	89.8	74.6
Harmony	31.2	0	2.8
St. George	9.8	26.2	16.0
9365-85	1	0	1.4
9407-14	0	0	0
9363-16	0	0	1.4
9365-43	0	0	0.2
9449-27	0	0	0.8
8909-05	0	0	0

Figure 1. Number of ring nematodes recovered off rootstock selections growing in 1 gal pots with three soils collected from the Gallo Livingston Ranch, known to have severe and chronic nematode pressure. Results per soils are means of three replicate pots.