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Walker

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(54) **NEMATODE RESISTANT GRAPEVINE
ROOTSTOCK PLANT '9365-85'**

(50) Latin Name: (*Vitis rufotomentosa* × (*V. champinii*
'Dog Ridge' × *V. riparia* 'Riparia
Gloire')) × *V. champinii* 'c9038'

Varietal Denomination: **9365-85**

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patent is extended or adjusted under 35
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(58) **Field of Classification Search** **Plt./205,**
Plt./207

See application file for complete search history.

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(57) **ABSTRACT**

Rootstock '9365-85' is a cross of 'L514-10' (*Vitis rufotomen-
tosaxV. champinii* 'Dog Ridge' × *V. riparia* 'Riparia
Gloire')) × *V. champinii* 'c9038'. This invention relates to a
new and distinct variety of grapevine rootstock plant with
resistance to a broad array of soil-borne nematodes including
root-knot, dagger, lesion and citrus nematodes. The variety is
moderately susceptible to ring nematode, but has strong resista-
nce to grape phylloxera.

2 Drawing Sheets

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Botanical/commercial classification: 'L514-10' (*Vitis
rufotomentosax(V. champinii* 'Dog Ridge' × *V. riparia*
'Riparia Gloire')) × *V. champinii* 'c9038'.

Variety Denomination: '9365-85'.

BACKGROUND OF THE INVENTION

Nematodes are important pests in vineyards around the
world, and these soil-borne pests can be particularly problem-
atic 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 steps of leaving land
fallow and rotating crops, both of which reduce nematode
build up and delay the selection of adapted strains. Nematoc-
ides 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 addi-
tion, 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 to provide resistance to nematodes,
but they are not resistant to grape phylloxera. More recently
two rootstocks, 'RS3' and 'RS9' (siblings of a 'Schwarz-
mann' × 'Ramsey' cross), were released, which were 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. Therefore, there is a need to develop nema-
tode resistant grape rootstock.

BRIEF SUMMARY OF THE INVENTION

This invention relates to a new and distinct variety of grape-
vine rootstock plant (grape rootstock) with resistance to a
broad array of soil-borne nematodes including root-knot,

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dagger, lesion and citrus nematodes. It is moderately suscep-
tible to ring nematode, but has strong resistance to grape
phylloxera. It is a cross of 'L514-10' (*Vitis rufotomentosax(V.
champinii* 'Dog Ridge' × *V. riparia* 'Riparia Gloire')) × *V.
champinii* 'c9038'. It is recommended for vineyard sites with
severe nematode infestations.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: number of ring nematodes recovered off rootstock
selections growing in 1 gal pots with three soils, known to
have severe and chronic nematode pressure. Results per soils
are means of three replicate pots.

FIG. 2: a photograph showing rootstock '9365-85'.

DETAILED DESCRIPTION OF THE INVENTION

A breeding program was initiated in 1990 at Davis, Calif.,
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 breeding efforts in the late 1960s and
early 1970s. These selections from the late 1960s and early
1970s were previously screened against two root knot nema-
tode species (*Meloidogyne incognita acrita* and *M. arenaria
thamsei*), the lesion nematode (*Pratylenchus vulnus*), and the
dagger nematode (*Xiphinema index*). In 1990, these selec-
tions 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 the '8905-05' and other
crosses 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 prog-
eny 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. In December of 1996, ten 2-node dormant cuttings of the best 1,000 were 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 the large number of families.

The second phase of the selection process involved testing these 100 selections for resistance to *M. incognita* 13. 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 by Cousins and Walker (Cousins and Walker 2001 Plant Disease 85:1052-1054). There were no root galls on 33 of the 100 selections and the 33 selections 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 from soils from a declining vineyard of 'Harmony' rootstock. 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 32° C. 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 28° C. in a wide range of species including tomato, pepper and plum. The 14 selections were also evaluated for resistance to lesion (*Pratylenchus vulmus*), citrus (*Tylenchulus semipenetrans*) and ring (*Mesocriconema xenoplax*) nematodes (Table 3). From this series of tests a number of six rootstock selections were made, including '9365-85'. A summary of the '9365-85' 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. 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, Calif. The six rootstock selections in large pots using soils with 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 (FIG. 1).

Each of the selections was repeatedly asexually reproduced to provide testing materials for nematode resistance evaluations. The cuttings were taken from the original seedling vine planted in Davis, Calif. On Jun. 7, 2002 the original seedlings were asexually reproduced to provide more cuttings for greenhouse and eventual field testing. The variety '9365-85' was first asexually reproduced for distribution in Davis, Calif. from woody and herbaceous cuttings.

Detailed Botanical Description

Rootstock '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). The

resistance of '9365-85' 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.

The following is a further description of rootstock '9365-85':

Shoot tips.—The variety has shoot tips which are enclosed in expanding leaves, typical and observed color - green (5GY 5/6), and sparsely covered with arachnose tomentum.

Young leaves.—The variety has young leaves with typical and observed color green (5GY 5/6), and typical and observed shape - three lobed glabrous leaves with fine arachnose tomentum and relatively long sharply pointed teeth.

Mature leaves.—The variety has a typical and observed size—medium sized (8.7 ×11 cm), typical and observed upper surface color - dark green upper (5GY 4/4) and typical and observed lower surface color - dark green (5GY 5/4), slightly 3-lobed, broad cuneiform-shaped leaves with medium-sized angular to convex teeth, and medium-sized petioles. The upper surface is glabrous, broadly rugose with some indented puckering near the petiole. The lower surface has scattered arachnose tomentum, fine bristles along the main vines and small tufted bristles (domatia) at the intersection of the main veins. The petiolar sinus is a narrow U to lyre shape.

Petioles.—typical and observed length 4 to 5 cm, typical and observed diameter—3 to 4 mm, and color is similar to the upper leaf surface (5GY 4/4).

Canes.—*typical and observed color.*—dark brown (5YR 5/6), typical and observed internodes length—15 to 20 cm, and relatively limited lateral development, nodes have short pointed buds. Canes are oval in cross-section, have thin diaphragms and medium sized pith.

Flowers.—typical and observed flowers are staminate and do not produce berries.

TABLE 1

Parentage of the five nematode resistant grape rootstock selections.	
Selection	Parentage
8909-05	<i>V. rupestris</i> × <i>M. rotundifolia</i>
9363-16	(<i>V. rufotomentosa</i> × (<i>V. champinii</i> 'Dog Ridge' × Riparia Gloire)) × Riparia Gloire
9365-43	(<i>V. rufotomentosa</i> × (<i>V. champinii</i> 'Dog Ridge' × Riparia Gloire)) × <i>V. champinii</i> c9038 (probably <i>V. candicans</i> × <i>V. monticola</i>)
9365-85	(<i>V. rufotomentosa</i> × (<i>V. champinii</i> 'Dog Ridge' × Riparia Gloire)) × <i>V. champinii</i> c9038 (probably <i>V. candicans</i> × <i>V. monticola</i>)
9407-14	(<i>V. champinii</i> 'Ramsey' × Riparia Gloire) × <i>V. champinii</i> c9021 (probably <i>V. candicans</i> × <i>V. berlandieri</i>)

TABLE 2

Selections with broad resistance to four nematodes when inoculated individually.

Selection	<i>X. index</i>	<i>M. incognita</i> I3	<i>M. arenaria</i> -HarmA	<i>M. incognita</i> -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	
8909-05	R	0	R	0
9317-06	MS	<1	S	<5
9332-43	S	<5	S	<5
9344-03	S	<5	MS	<1
9363-16	R	0	R	0
9365-43	R	0	R-	<1
9365-62	MS	<1	S	<5
9365-85	MS	<1	R-	<1
9403-107	R	0	S	<5
9403-35	S	<5	S	<5
9407-14	R	0	R	0
9449-23	MS	<1	R	0
9449-25	MS	<1	R	0
9449-27	MS	<1	R-	<1
1616C	S	>50	S	<5
Freedom	S	>10	S	<10
Colombard	S	>100	S	>100
Harmony	S	>10	S	<50
St. George	S	>100	S	<50

Genotypes	Citrus		Lesion		Ring	
8909-05	R	<100	R	<10	R	<1,000
9317-06	R	<100	R	<50	HS	<20,000
9332-43	R	<100	R	<50	S	<10,000
9344-03	S	>400	S	<200	S	<10,000
9363-16	S	>400	R	<50	S	<10,000
9365-43	R	<100	R	<50	MS	<5,000
9365-62	R	<100	R	<50	MS	<5,000
9365-85	R	<100	R	<50	S	<10,000
9403-107	R	<100	R	<50	MS	<5,000
9403-35	R	<100	R	<50	MS	<5,000
9407-14	R	<100	R	<50	LS	<3,000
9449-23	R	<100	R	<50	S	<10,000
9449-25	R	<100	R	<50	S	<10,000

TABLE 3-continued

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.

9449-27	R	<100	R	<50	S	<10,000
1616C	S	>400	S	<200	HS	<20,000
Freedom	R	<100	R	<50	HS	<20,000
Colombard	S	<1000	S	<300	HS	<30,000
Harmony	S	<1000	S	<200	HS	<30,000
St. George	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* HammA nematodes and testing at 32° C.

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

TABLE 5-continued

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.

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 a vineyard site. 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

What is claimed is:

1. A novel and distinct variety of grapevine rootstock plant designated '9365-85' having the characteristics described and illustrated herein.

* * * * *

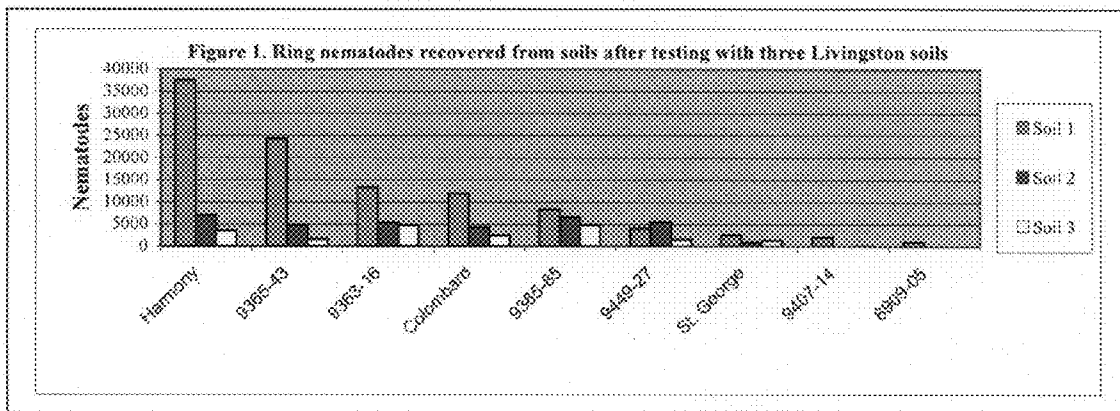


Figure 1

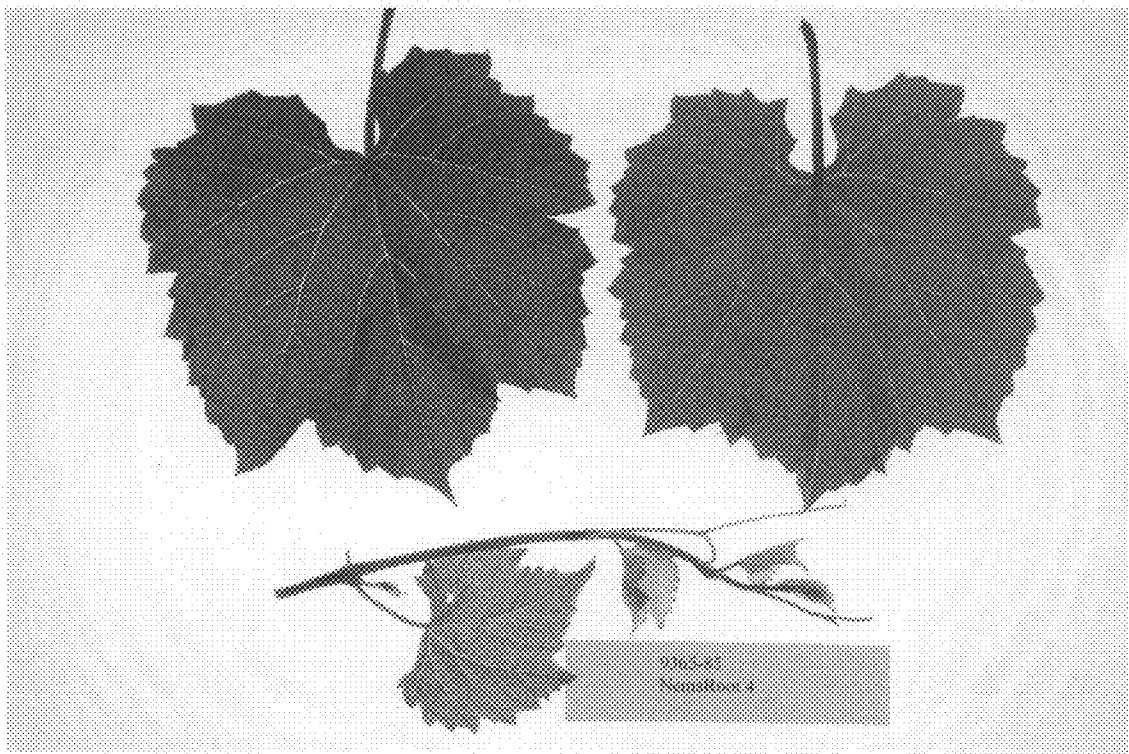


Figure 2