Inheritance & Genetic Mapping of *Xiphinema index* Resistance Derived from *Vitis arizonica*

by

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Declaration

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Summary

Grapevines are one of the most important and diverse crops in the world, but tend to be susceptible for numerous pests and diseases. The dagger nematode, *Xiphinema index* (*X. index*) is a well-known soil-borne pest of grapevine and vector of grapevine fanleaf virus. Several *Vitis* species showed resistance to this pest. Breeding efforts have been underway for several decades to create resistant rootstocks. However, conventional breeding efforts are time consuming due to grapevines being a perennial crop, its heterozygosity, as well as its long growth cycle. Breeding new grapevine varieties are also expensive and work intensive. The development of marker-assisted selection introduced a way to overcome some of the above-mentioned problems.

The aim of this study was to broaden the genetic evaluation and breeding efforts for improved *X. index* resistance in grapevine rootstocks. In 2007 several crosses were made in the University of California, Davis vineyards. The background for all these crosses consisted of *V. arizonica*. These *V. arizonica* plants are part of a collection obtained by H.P. Olmo during the 1960's. In recent studies it was established that *X. index* resistance is controlled by a single dominant gene. The 0701 (R8916-07 (Wichita Refuge x b40-14) x R8916-32), 0704 (161-49C x b40-14) and 0705 (161-49C x R8916-22) populations were created to confirm the homozygous nature of b40-14, a *V. arizonica* accession. In addition, several *V. arizonica* species were screened to confirm their resistance or susceptibility towards *X. index* feeding. The 0705 population was also used to create a genetic map for *X. index* resistance.

In this study a new and improved screening method was developed to inoculate vines under greenhouse conditions. This screening method proved to be quicker and less damaging on the nematodes than traditional systems. Control varieties were used and O39-16, a commercial rootstock showed no damage, even with high nematode pressure, whereas *V. rupestris* Saint George had severe root damage and decline after eight weeks of exposure.

A range of *V. arizonica* accessions was tested for their resistance to *X. index* feeding. Of the 18 genotypes tested, half showed resistance and the rest were susceptible. It is possible that these genotypes are not pure *V. arizonica* genotypes. Genotypes with *V. arizonica* in the background were also tested. Wichita Refuge was used as a susceptible female parent and the progeny were expected to be heterozygous resistant. Some of the progeny allowed low levels of feeding damage, which may have been the result of the more effective inoculation method described above.

The 0701 population confirmed the hypothesized model of 3:1 (Resistance (R):Susceptible (S)) segregation although 13 of the genotypes showed significantly higher gall numbers than the susceptible female parent. The possibility of transgressive segregation exists, but needs to be confirmed. All progeny from the 0704 population should be heterozygous resistant, but a 1:1 (R:S) segregation pattern was observed. The 0705 population was created as a mapping population to study *X. index* resistance. This population was also tested in the greenhouse for its *X. index* resistance and was expected to segregate 1:1 (R:S). The X^2 analysis did not fully support this model.

A genetic map covering all 19 linkage groups, and positioning 175 polymorphic SSR markers was created for the 164 progeny in the 0705 population. MapQTL analysis revealed a major QTL on linkage group 9 and two minor QTL's on groups 13 and 19. The major QTL placed between markers VMC1c10 and CTG1032918 with a LOD score of 33.4 explaining 70.5% of the phenotypic variance for *X. index*. This QTL is the second major QTL discovered for *X. index* resistance.

With the discovery of a second major QTL, the two types of resistance can be pyramided. Work is underway to saturate the area around the major QTL on linkage group 9 and to move towards physical mapping of *X. index* resistance. The b40-14 *V. arizonica* accession is also known for its resistance to Pierce's disease and the possibility of simultaneous expression of two types of resistance is created. The 0705 population can also be used to evaluate phenotypical characteristics in the field to determine if useful rootstocks can be selected. Taken together, the results obtained in this study provide improved methods and highly characterized plant populations to support the efforts in obtaining improved *X. index* resistance in grapevine rootstocks.

Opsomming

Wingerde is van die belangrikste en mees diverse gewasse op aarde, maar hulle neig om vir ‘n verskeidenheid plae en siektes vatbaar te wees. Die dolk-aalwurm, *Xiphinema index* (*X. index*), is ‘n bekende grondgedraagde plaag van wingerd en ‘n vektor vir wingerd- netelblaarvirus. Verskeie *Vitis*-spesies toon weerstand teen hierdie plaag. Daar word reeds vir dekades pogings aangewend om weerstandbiedende onderstokke te kweek. Konvensionele kweekpogings is egter tydrowend omdat wingerd ‘n meerjarige gewas is, op grond van die heterosigositeit van die gewas, sowel as die lang groeisiklus. Dit is ook duur en arbeidsintensief om nuwe wingerdvariëteite te kweek. Die ontwikkeling van merker-ondersteunde seleksie het dus ‘n metode verskaf om sommige van bogenoemde probleme te oorkom.

Die doelwit van hierdie studie was om die genetiese evaluerings- en kweekpogings vir verbeterde *X. index*-weerstand in wingerd-onderstokke te verbreed. In 2007 is verskeie kruisings in die wingerde by die Universiteit van Kalifornië, Davis gemaak. Die agtergrond vir al hierdie kruisings het bestaan uit *V. arizonica*. Hierdie *V. arizonica*-plante vorm deel van ‘n versameling wat in die 1960’s deur H.P. Olmo verkry is. In onlangse studies is daar bepaal dat *X. index*-weerstand deur ‘n enkele dominante geen beheer word. Die 0701 (R8916-07 (Wichita Refuge x b40-14) x R8916-32), 0704 (161-49C x b40-14) en 0705 (161-49C x R8916-22) bevolkings is geskep om die homosigotiese geaardheid van b40-14, ’n *V. arizonica*-afstammeling, te bevestig. Daarbenewens is verskeie *V. arizonica*-spesies gesif om hulle weerstand teen of vatbaarheid vir *X. index* voeding te bevestig. Die 0705 bevolking is ook gebruik om ‘n genetiese kaart vir *X. index*weerstand te skep.

In hierdie studie is ‘n nuwe en verbeterde siftingsmetode ontwikkel om wingerdstokke onder glashuistoestande te inokuleer. Daar is gewys dat hierdie siftingsmetode vinniger en minder skadelik vir die aalwurms as tradisionele metodes is. Beheervariëteite is gebruik en O39-16, ‘n kommersiële onderstok, het geen skade getoon nie, selfs met hoë aalwurmdruk, terwyl *V. rupestris* Saint George ernstige wortelskade en agteruitgang na agt weke se blootstelling getoon het.

‘n Verskeidenheid *V. arizonica*-afstammelinge is vir hulle weerstand teen *X. index*-voeding getoets. Van die 18 genotipes wat getoets is, het die helfte weerstand getoon en die res was vatbaar. Dit is moontlik dat hierdie genotipes nie suiwer *V. arizonica*-genotipes was nie. Genotipes met *V. arizonica* in hulle agtergrond is ook getoets. Wichita Refuge is as ‘n vatbare vroulike ouer gebruik en die verwagting was dat die nageslag heterosigoties weerstandbiedend sou wees. Sommige van die nageslag het lae vlakke van voedingskade toegelaat, wat moontlik die gevolg was van die meer doeltreffende inokulasiemetode wat hierbo beskryf word.

Die 0701 bevolking het die veronderstelde model van 3:1 (Weerstandbiedend (W):Vatbaar (V)) segregasie bevestig, hoewel 13 van die genotipe noemenswaardig hoër galgetalle as die vatbare vroulike ouer getoon het. Die moontlikheid van transgressiewe segregasie bestaan, maar dit moet nog bevestig word. Alle nageslag van die 0704 bevolking behoort heterosigoties weerstandbiedend te wees, maar ‘n 1:1 (W:V) segregasiepatroon is waargeneem. Die 0705 bevolking is as ‘n karteringsbevolking geskep om *X. index*-weerstand te bestudeer. Hierdie bevolking is ook in die glashuis vir sy *X. index*-weerstand getoets en daar is verwag dat dit 1:1 (W:V) sou segregeer. Die X^2 analise het nie hierdie model ten volle ondersteun nie.

‘n Genetiese padkaart wat al 19 skakelingsgroepe en die posisies van 175 polimorfiese SSR merkers toon, is vir die 164 afstammelinge in die 0705 bevolking geskep. MapQTL analise het ‘n groot kwantitatiewe eienskap lokus (QTL) op skakelingsgroep 9 en twee kleiner QTL’e op

groepe 13 en 19 onthul. Die groot QTL is tussen merkers VMC1c10 en CTG1032918 met ‘n LOD telling van 33.4 geplaas en het 70.5% van die fenotipiese variansie van *X. index* verklaar. Hierdie QTL is die tweede groot QTL wat vir *X. index*-weerstand ontdek is.

Met die ontdekking van ‘n tweede groot QTL, kan die twee soorte weerstand gepiramideer word. Werk word reeds onderneem om die area rondom die groot QTL op skakelingsgroep 9 te versadig en om na die fisiese kartering van *X. index*-weerstand te beweeg. Die b40-14 *V. arizonica*-afstammeling is ook bekend vir sy weerstand teen Pierce se siekte en die moontlikheid word geskep vir die gelyktydige uitdrukking van twee soorte weerstand. Die 0705 bevolking kan ook gebruik word om die fenotipiese kenmerke in die veld te evalueer om te bepaal of bruikbare onderstokke geselekteer kan word. In kombinasie behoort die resultate wat in hierdie studie verkry is, verbeterde metodes en hoogs gekarakteriseerde plantbevolkings te lewer wat die pogings sal ondersteun om verbeterde *X. index*-weerstand in wingerd-onderstokke te verkry.

Dedication

This dissertation is dedicated to

Danie van Zyl

Biographical sketch

Sonet van Zyl was born in Gauteng, South Africa on 23 November 1977. She matriculated at Brandwag High School, Benoni in 1995. In 1996 she enrolled at Stellenbosch University and obtained a BSc(Agric)-degree in Viticulture and Genetics (Plant breeding) in 2000. The following year, she enrolled for a MscAgric-degree in Viticulture at the same University. She completed this degree in 2003 with a thesis entitled “The influence of an open air hydroponic system on the production of table grapes: A case study”. From November 2002 until February 2007 Sonet was employed by the Agricultural Research Council (Infruitec-Nietvoorbij) where after she enrolled for her PhD(Agric)-degree at Stellenbosch University. All the research conducted for her PhD studies were completed at the Department of Viticulture and Enology, University of California, Davis.

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Preface

This dissertation is presented as a compilation of 7 chapters. Each chapter is introduced separately and is written according to the style of the *South African Journal for Enology & Viticulture.* Contributions towards the data analyses for Chapters 3, 4 and 5 were made by Dr. Andrew Walker and for Chapter 6 by Dr. Summaira Riaz.

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Chapter 1

General introduction and project aims

Chapter 1. General introduction and project aims

1.1 Introduction

Grapevine is one of the most important crops worldwide. Grapes are grown for the purpose of wine making, distilling, fresh fruit, raisins and juice production and their cultivation dates back more than 8,000 years to ancient Mesopotamia (Fisher *et al.,* 2004). Strong evidence was found that all *V. vinifera* populations originated from the Near East (Myles *et al.,* 2010). With the domestication of grapevines, their associated pests and diseases were spread to most of the grape growing countries in the world (Esmenjaud & Bouquet, 2009). Wild grape species such as *Muscadinia rotundifolia* were shown to be resistant to several of these pests and diseases, but their fruit quality is usually unacceptable for wine making and the fresh fruit market. Although several *Vitis* species are cultivated throughout the world, most commercial grape cultivars are *V. vinifera* species and although they exhibit excellent fruit characteristics, they are susceptible to most important pests and diseases (Riaz *et al.,* 2004).

Soil-borne pests are of particular importance. The dagger nematode, *Xiphinema index* (*X. index*), is one of the most damaging root pests associated with grapevine due to its ability to vector grapevine fanleaf virus (GFLV), which causes fanleaf degeneration (Hewitt *et al.,* 1958). This disease is becoming increasing damaging in the world's vineyards due to the lack of crop rotation and fallow periods, and restrictions of the use of environmentally damaging nematicides and fumigants. The quest for *X. index* resistant rootstocks has been underway for decades (Kunde *et al.,* 1968; Harris, 1983; Meredith *et al.,* 1982; Coiro *et al.,* 1985) and several studies were conducted to find resistance in grapevines against GFLV (Staudt & Weischer, 1992; Walker *et al.,* 1985). Resistance in one plant source against both *X. index* and GFLV has yet to be found. It was shown that current grape cultivars from different areas are still very similar since its domestication and due to vegetative propagation. However, the grape gene pool is still highly heterozygous that provides a benefit for future breeding efforts (Myles *et al.,* 2010). Therefore, the search should continue as grape species are extremely diverse and have valuable sources of genes for resistance to diseases, insects and abiotic stresses (Mullins *et al.,* 1992).

Grapevine is a perennial woody plant species with a long growth cycle and a high level of heterozygosity (Salmaso *et al.,* 2004), which makes conventional breeding efforts time consuming. Grapevine breeding is also work-intensive and costly. The introduction of marker assisted selection (MAS) created the opportunity to overcome some of the mentioned disadvantages. MAS can screen progeny in the early seedling stage and allow rapid selection of progeny before they are planted in the vineyard, thus saving time, space and money. Simple sequence repeat (SSR) or microsatellite markers are very useful for MAS due to their hypervariability, abundance, reproducibility and codominant nature (Scott *et al.,* 2000). MAS has been used to expedite breeding in a number of grape breeding programs for traits such as resistance to *X. index* (Xu *et al.*, 2008), phylloxera (Zhang *et al.,* 2009), powdery mildew (Donald *et al.,* 2002; Barker *et al.,* 2005), downy mildew (Bellin *et al.,* 2009) and Pierce's disease (Krivanek *et al.,* 2006); and for berry characteristics such as seedlessness (Bouquet & Danglot, 1996; Striem *et al.,* 1996; Doligez *et al.,* 2002).

The grapevine genome has been sequenced (Velasco *et al.,* 2007; Jaillon *et al.,* 2007) and genetic and physical maps are becoming more available. Genetic information is increasingly used to guide breeding efforts in grapevine (Myles *et al.,* 2010). Technologies to enhance classical breeding and selection of grapevine can benefit significantly from the availability of the grapevine genome sequence. Genome mapping can identify the genetic location of mutants,

recombinants, and qualitative and quantitative loci. Mapping studies resolve the relationships between the marker loci and the targeted trait and require segregating populations, marker data sets and high quality phenotypical data. Related technologies such as functional genomics where genetic loci are assigned functions will further benefit these initiatives. Considering the rapid development of these new technologies, no limitations are foreseen for the acquisition of information on the molecular level (Martínez-Zapater *et al.,* 2010).

1.2 Project aims

This dissertation aims to broaden the breeding and genetic evaluation of grapevine rootstock genotypes for resistance to *X. index.* Previous research at the University of California, Davis determined that at least one accession of *V. arizonica* has resistance to *X. index*, and that its resistance is controlled by a single dominant gene. The work undertaken in this study will characterize this resistance in populations created from other accessions of *V. arizonica* by creating genetic maps and identifying genetic markers associated with resistance. The specific aims include:

1. The selection of parents and performing crosses to establish mapping populations as well as the phenotypical characterization of these populations.
2. The development and the evaluation of optimized *X. index* inoculation methods under greenhouse conditions by using highly resistant and highly susceptible commercial rootstock cultivars.
3. The verification of previous results of *V. arizonica* types and their progeny, and the verification that the pure *V. arizonica* type, b40-14, is homozygous resistant to *X. index* feeding.
4. To determine the inheritance of *X. index* resistance in *V. arizonica* by screening progeny from crosses made with the commercial rootstock, 161-49C.
5. To create a genetic framework map for *X. index* resistance derived from a population with a pure *V. arizonica* background.

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Chapter 2

Literature review

Xiphinema index and its relationship to grapevines: A review

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Chapter 2. Literature review: *Xiphinema index* and its relationship to grapevines: A review

2.1 Introduction

Grapevines are cultivated in temperate and Mediterranean climates around the world. Grapevines have been moved between countries and continents, following human migration and settlement as well as imported and cultivated in numerous countries; all these factors have increased the incidence and spread of injurious pests and diseases (Esmenjaud & Bouquet, 2009). Three major pests attack the root system of grapevines: grape phylloxera (*Daktulosphaira vitifoliae* Fitch); ground pearls (*Margarodes* spp.); and a wide range of root-feeding nematodes. These pests damage roots leading to their decay, prevent new root development, and can result in vine decline and eventual death. The initial impact of these pests may be less severe, but the impact over years becomes more intensified and causes significant losses (De Klerk & Loubser, 1988). Nematodes associated with vine damage are root-knot nematodes (*Meloidogyne* spp.), citrus nematodes (*Tylenchulus semipenetrans*), root-lesion nematodes (*Pratylenchus vulnus*) and dagger nematodes (*Xiphinema* spp.) (Nicholas *et al.,* 2007).

All of the economically important nematodes of grapevines are present in South Africa (Smith, 1977). Because the dagger nematodes are often associated with woody plants and are generally associated with specific viruses, which they carry from plant to plant through feeding, they are considered to be major pests. More than 170 species of *Xiphinema* have been identified on a wide range of hosts worldwide. Approximately 69 *Xiphinema* species have been reported in South Africa, although only four were implicated in plant virus transmission: *X. americanum* Cobb, *X. diversicaudatum* Thorne, *X. index* Thorne and Allen, and *X. italiae* Meyl (Loubser & Meyer, 1987a); the first three of which are common in South African vineyards (Malan, 1995). They are found in a variety of soils and are migratory ectoparasites (Shurtleff & Averre III, 2000). This review will focus on X. index specifically, its interaction with grapevines and its role as vector for (GFLV).

2.2 The classification, description and identification of *Xiphinema index*

Xiphinema index is from the order *Dorylaimida,* suborder *Dorylaimina,* and superfamily *Dorylaimoidea*, family *Longidoridae*, subfamily *Xiphineminae* and genus *Xiphinema* (Taylor & Brown, 1997). The genus *Xiphinema* was first described by Thorne (1939) and *X. index* was first identified and described by Thorne & Allen (1950).

The body of an adult female *X. index* is about 3 mm long. The lip region is hemispherical and almost continuous with the body. The odontostyle is approximately 126 μm long, the odontophore 70 μm and has large flanges. There is a guide ring at approximately 108 μm from the anterior end (Decraemer & Geraert, 2006). The female body is elongate-cylindrical, forming an open spiral with a greater curvature in the posterior half. The cuticle is thick with fine, superficial striations. Eight or nine lateral body pores are present in the oesophageal region, 13 or 14 between the oesophagus and vulva, and 21 or 22 between the vulva and anus (Siddiqi, 1974). The female has one or two ovaries, which are usually paired and reflexed, one reduced and extending anteriorly, the other posteriorly (Shurtleff & Averre III, 2000). Reproduction is parthenogenetic and males are extremely rare. Their body shape is the same as for the females (Siddiqi, 1974). Males have two opposed, outstretched testes, and the

spicules are strong with lateral guiding pieces (Shurtleff & Averre III, 2000). Both males and females have short, dorsally rounded tails. The tail has a terminal peg situated ventrally and is 8-12 μm long. This peg is a distinct characteristic of the species (Fig. 2.1) (Luc & Cohn, 1982).

Descriptions of this nematode have varied for example; the listed length of females ranges from 2.8 to 3.4 mm, and the odontostyle length vary from 120 to 144 mm (Barsi, 1989; Coiro, *et al*., 1992; Lamberti *et al*., 1985; Thorne & Allen, 1950). However, the soil environment might play a role in this variation since this factor is often ignored during collection (Prins, 1997). In 1977, Garau & Prota described the four juvenile stages of *X. index* using three different measurements: body length, functional odontostyle length and replacement odontostyle length. However, the data showed considerable variability within each of these measurements, particularly across juvenile stages. Separation of the first and second stage juveniles was particularly difficult, but with any single measurement used, the third and fourth stages were readily identified with a high degree of assurance (Garau & Prota, 1977).

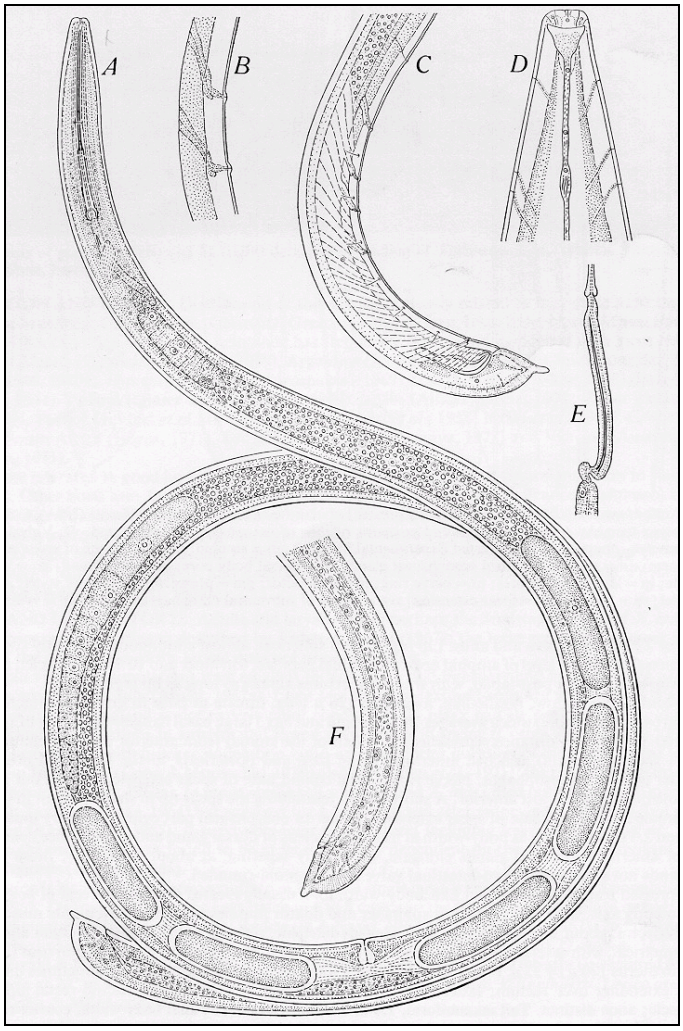

Figure 2.1 *X. index* as described by Thorne & Allen, 1950. A: Female. B: Detail of supplements. C: Male posterior. D: Head end showing amphid. E. Replacement spear in anterior portion of oesophagus of larvae. F: Female posterior (Siddiqi, 1974).

It is important to be able to identify different species of *Xiphinema* from each other. *Xiphinema index, X. diversicaudatum, X. vuittenezi and X. italiae* are closely related taxonomically, and therefore difficult to distinguish with morphological and morphometrical characters. This has led to molecular efforts, using PCR (Polymerase Chain Reaction) techniques and species-specific primers (Esmenjaud & Bouquet, 2009) to distinguish the

species. Specific regions were sequenced in one population of each species and species-specific primers were developed from the sequencing data to detect individuals, even when the numbers were low (Wang *et al.,* 2002). Similarly, Hübschen *et al.,* (2004b) developed species-specific ribosomal primers for seven *Longidorid* species to facilitate taxonomic identification for non-specialists. These primers were tested for sensitivity and selectivity on closely related *Longidorid* species and proven to be highly specific in detecting all developmental stages within one species, and also in detecting a single target nematode from a community (Hübschen *et al.,* 2004b). The same group also developed and validated specific primers for *X. index, X. diversicaudatum* and *X. vuittenezi* detection (Hübschen *et al.,* 2004a).

2.3 Range, habitat, biology and culturing of *Xiphinema index*

Dagger nematodes are found in all soil types. In South Africa 16 species of *Xiphinema* were found in soils analyzed from five viticultural regions in the Cape Province, and *X. index* was present in three of these regions (Malan & Meyer, 1994). The population of *X. index* decreases with soil depth. More that 92% of all nematodes are found in the 0-300 mm zone where most vine roots occur (De Klerk & Loubser, 1988). Earlier research done in California, showed that *X. index* could be found as deep as 360 cm (Raski *et al.,* 1965a), and are likely to be found wherever roots are. In a different study, the highest number of individuals occurred at 40-110 cm depth, corresponding to the two layers where the highest densities of fine roots were observed (Villate *et al.,* 2008). Light to medium textured soils seem to be preferred with a pH between 6.5 and 7.5 (Siddiqi, 1974). Based on a study done in a Barossa Valley vineyard in Australia, the best time to determine *X. index* densities was in the late spring (Quader *et al.,* 2003).

Temperature is an important modulating factor on the reproduction and life cycle of *X. index*, which is typically associated with grapevines in warm climates. The *X. index* population increased more rapidly as the soil temperature increased from 16-28°C. In Italy, it was found that *X. index* numbers are lower in the winter (Coiro *et al.,* 1987; Coiro *et al.,* 1991), but a study in California found that the populations peaked in the winter (Feil *et al.,* 1997) perhaps because sampling was more accurate in moist soils. A study done in England under experimental conditions showed that *X. index* egg-laying peaked during summer months, with maximum populations in autumn, and lowest populations in spring (Siddiqi, 1974).

Xiphinema index has been shown to survive in a wide range of soil temperatures ranging from -11°C to 35°C, but constant temperatures for 10 days of 45°C or -22°C killed the nematodes. Fluctuations in diurnal temperatures also lowered *X. index* survival rates (Cotten *et al.,* 1971). Females typically produce an egg every 24-26 days when the temperature is above a minimum daily threshold of 10°C. Eggs are laid singly in the soil close to the feeding site (Weischer & Wyss, 1976) and the life cycle takes 3-5 months to complete at 28°C, but slows down to 7-9 months at lower temperatures (Nicholas *et al.,* 2007). Reproduction rate has been shown to be highest at 29.4°C (Siddiqi, 1974). As mentioned, reproduction is by parthenogenesis (Dalmasso, 1975) and a single larva is capable of generating a population. Eggs hatch in 6-8 days, and the first molt takes place outside the egg 24-48 hours after hatching. Dagger nematodes have four juvenile stages; the 2nd, 3rd and 4th molts occur at six-day intervals (Siddiqi, 1974). The opportunity for increasing genetic diversity through sexual recombination in *X. index* is low because reproduction is almost entirely parthenogenetic (Dalmasso, 1975). Sexual reproduction has not been reported and males constitute only about 2.7% of the population. A small percentage of the females have spermatozoa present in the uterus (Luc & Cohn, 1982). Initial studies found that the *X. index*

genome consisted of 20 chromosomes, and suggested that it might be a tetraploid (Dalmasso & Younes, 1969), but it was later reported that the genome consisted of 10 chromosomes (Dalmasso, 1975).

Earlier studies showed substantial variations in reproduction rates and life cycle stages under greenhouse conditions (Cohn & Mordechai, 1970; Coiro *et al.*, 1990); *X. index* reproduced faster in non-clay soils under these conditions (Coiro *et al.,* 1987). Moreover, fine sand and sandy loam soils with a soil moisture content of 10-15% induced higher reproduction results than coarse sand (Sultan & Ferris, 1991). In a Californian greenhouse study the cycle from egg to female has been reported to be 22-27 days (Pearson & Goheen, 1988), whereas others report on a 60-day life cycle (McKenry, 2000). Individuals can live for many years (Nicholas *et al.,* 2007) as confirmed by a French report that claimed survival in dry soil for four years (Demangeat, *et al.,* 2005). In a study done by Brown & Coiro (1985), it was shown that the longevity of *X. index* on *Ficus carica* was 60-64 weeks, with a total reproductive capacity of 140-160 progeny. Longevities and reproductive capacities for female *X. index* from Italy and the U.S.A. were similar when raised on *F. carica* (Brown & Coiro, 1985).

2.3.1 Effect of *Xiphinema index* feeding on grapevines

Xiphinema index feeding initially causes a swollen club-like gall on root tips, which varies in size based on the size and vigor of the root. The feeding wound then becomes reddish brown to black, and forms slightly sunken lesions on the roots (Shurtleff & Averre III, 2000). Infested root systems are stunted and have a witch's broom appearance after successive rounds of new roots branching and being damaged from behind the original damaged root tip (Pearson & Goheen, 1988). Extensive root damage eventually results in reduced shoot growth and yield. Common symptoms of *X. index* feeding are plant stunting, chlorosis, root swellings or galls and root necrosis (Fig. 2.2) (Shurtleff & Averre III, 2000). The number of galls formed has been correlated with the size of a nematode population and with size of the root system in potted plants (Xu *et al.,* 2008). The clubbed galls suggest that the nematodes discharge some substance into the roots to induce swelling (O'Bannon & Inserra, 1990) and this galling has been shown to occur as early as 24 hours after feeding (Fisher & Raski, 1967). Nematode feeding damage induces water and nutrient stress, which in turn reduces vine vigor and yield. Penetration of roots by nematodes also makes them more susceptible to root-rotting fungi (Nicholas *et al*., 2007), which contributes to vine death.

The foliage symptoms caused by root damage from *X. index* feeding are similar to those caused by root rots, drought and other root-feeding pests. Soil conditions can also restrict root growth and consequently damage done by *X. index* can be made worse. These conditions include drought, compact soils, shallow water tables, saline soils and highly acidic or alkaline soils. In addition, it is common to have more than one type of nematode attacking the roots, which often intensifies the damage (Nicholas *et al*., 2007).

The combined effect of *X. index* feeding and its association with GFLV may kill grapevines (Nicholas *et al*., 2007). Cultural practices, which put grapevines under stress, such as girdling, can further intensify the deleterious effects of nematode feeding (Raski, 1955). If soil and cultural conditions are favorable, infested grapevines are able to better tolerate the presence of nematodes (Anwar *et al.,* 2003).

Figure 2.2 Feeding damage (galling) caused by *X. index* on roots of St. George, a highly susceptible variety.

All stages, including adult females, move through the soil to find and feed on roots (Nicholas *et al.,* 2007). *X. index* prefer to feed near the root tips (Weischer & Wyss, 1976) by inserting their mouth parts (stylets) into the root tissue (Fig. 2.3) (De Klerk & Loubser, 1988). This nematode perforates 5-7 cells deep with a twisting action of the odontostyle, followed by rhythmical contractions of the oesophageal bulb and feeding actions (Taylor & Brown, 1997; Weischer & Wyss, 1976). The time period *X. index* stay at one feeding site can vary from several minutes to several days. Root areas already fed on attract more nematodes and can result in crowding (Weischer & Wyss, 1976).

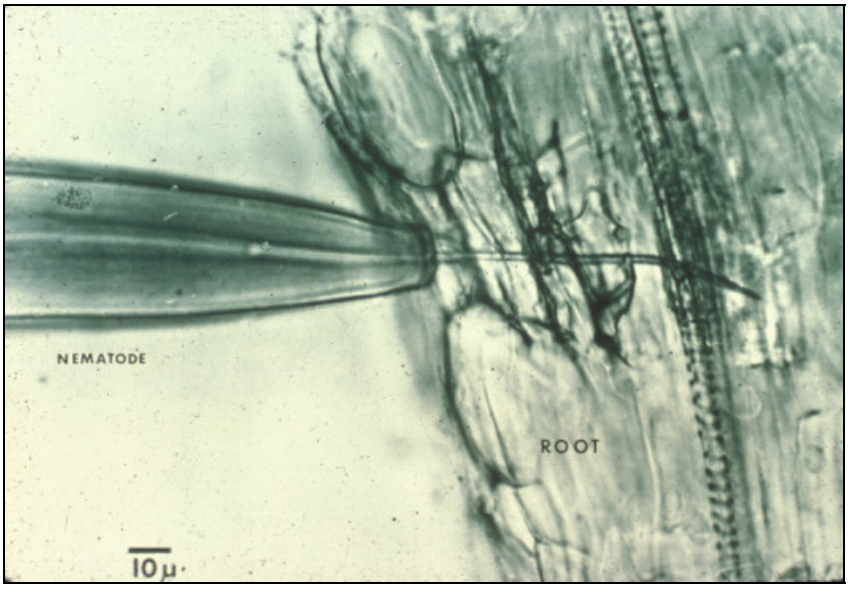

Figure 2.3 Nematode feeding on grapevine roots (Wylie *et al.,* 2004).

2.3.2 Non-grape hosts

Xiphinema index has been reported to attack figs, roses and citrus (Nicholas *et al.,* 2007). In Italy, *X. index* was also found on the roots of mulberry trees (Siddiqi, 1974). *Xiphinema* species in general are associated with root damage on ornamental shrubs, corn, lawn grasses, oats, roses, pines, peanuts (Garrett *et al.,* 1966), as well as pistachio (Weiner & Raski, 1966). Coiro & Serino (1991) reported that *X. index* reproduction could occur on petunia and tomato, which render them hosts. A lesser extent of reproduction was found on *Chenopodium amaranticolor* and tobacco plants, showing that some herbaceous plants may be suitable as bait plants, but differences in host status are likely between different *X. index* populations (Coiro & Serino, 1991). Brown & Coiro (1985) reported that *F. carica* can be a more suitable host for *X. index* than *V. vinifera* under controlled greenhouse conditions. They found that *Olea europaea, Citrus aurantium* and four tomato cultivars were poor hosts (Brown & Coiro, 1985).

2.3.3 *In vitro* culture

A quick method to screen grapevines for *X. index* resistance does not exist. *In vitro* dual culture on grape roots might overcome this problem. For *in vitro* culture to be successful, nematodes have to be surface sterilized. In 1978, Wyss successfully surface sterilized *X. index* using a 0.03% NaN_3 solution. The nematodes were transferred to a 0.6% agar media where they were left to feed on fig roots. In 1983, Bleve-Zacheo & Zacheo did a similar study, but they used a 2% agar media. In both these studies, *X. index* were alive and feeding on fig roots within a few days. They observed reproduction and growth of juveniles *in vitro.* However, a study done by Bavaresco & Walker (1994) on different sterilization methods showed that no nematodes survived the NaN_3 treatment. The only surface sterilization treatment *X. index* survived was a Sigma A-7292 antibiotic antimycotic compound. After this treatment root tip swelling and egg production were observed after 50 days, whereas, first stage larvae were observed after 60 days (Bavaresco & Walker, 1994).

2.3.4 Extraction methods

Nematodes can be extracted from plants and soil in several ways. Soil samples are usually taken near the vine up to a depth of 600 mm (Quader *et al.,* 2003), and the method of extraction is usually dependent on the nematode species (Brown & Boag, 1988) and soil type (Viglierchio & Schmitt, 1983). Brown and Boag (1988) showed that care should be taken when handling soil samples containing virus vector nematodes. It was shown that *X. index* were more susceptible to rough handling than some *Longidorus* species, and that dropping soil samples can kill nematodes (Brown & Boag, 1988). Four different methods for nematode extraction are summarized in table 2.1.

Table 2.1 Nematode extraction methods.

Method	Advantage	Disadvantage	References
Cobb's sieving and gravity method	- Rapid method - Larger soil samples used	- Samples not always clean - Egg and juveniles not - retained	- Shurtleff & Averre III, 2000 - Viglierchio & Schmitt, 1983
Baermann funnel method	- Active adult and juveniles extracted - Used in combination - with first method - Limits soil and root - debris	- Time consuming: - hours to days - depending on sample - size, temperature - species - Anaerobic conditions in funnels	- Evans *et al*., 1993 - Brown & Boag, 1988 - Shurtleff & Averre III, 2000 - Viglierchio & Schmitt,1983

Table 2.1 *(cont.)*

Method	Advantage	Disadvantage	References
Mist extraction method	- No anaerobic conditions	- Most time consuming method	- Shurtleff & Averre III, 2000 - Viglierchio & Schmitt, 1983
Centrifugal flotation method	- Active and sedentary nematodes recovered - Good for large samples	- High mortality rate for *X. index*	- Shurtleff & Averre III. 2000 - Viglierchio & Schmitt. 1983

2.4 *Xiphinema index* as a vector for grapevine fanleaf virus

Grapevine fanleaf virus is a member of the nepovirus (nematode vectored polyhedral particle shape) group (Pearson & Goheen, 1988). This group contains 37 viral species that have isometric particles of about 28 nm in diameter. One-third of the viruses in this group are known to be transmitted by nematodes (Taylor & Brown, 1997).

2.4.1 Genetics of the grapevine fanleaf virus

Nepoviruses are positive-sense single-stranded RNA viruses, and have two genomic RNA's. The larger one is referred to as RNA1 and the smaller as RNA2. The large RNA1 molecule carries the genetic determinants for host-range, seed transmissibility and some types of symptom expression, while the small RNA2 molecule contains genes for the coat protein, nematode transmissibility and some symptom expression (Taylor & Brown, 1997). Full-length cDNA clones of GFLV RNA1 and RNA2 have been constructed for the synthesis of infectious transcripts (Viry *et al.,* 1993).

The determinants responsible for the specific spread of GFLV by *X. index* are located within the 513 C-terminal residues of the polyprotein encoded by RNA2. Findings suggest that the coat protein provides the basic determinants for the specificity of GFLV transmission by *X. index* (Belin *et al.,* 2001). In 2004 it was confirmed that the viral coat protein was the key determinant for GFLV transmission of GFLV (Andret-Link *et al.,* 2004a). Genetic variability exists within the RNA2 molecule of GFLV (Pompe-Novak *et al.,* 2007). Multiple interspecies recombination events were identified within the RNA2 molecule of strains from GFLV and the arabis mosaic virus (Vigne *et al.,* 2008).

GFLV can be inoculated by grafting so that the impact of virus resistance can be studied without the impact of nematode feeding or transmission. Approach grafting techniques were used to study GFLV resistance in *V. vinifera* (Walker & Meredith, 1989) and Bouquet (1981) also used graft transmission to examine resistance in *Vitis* species. Valat *et al.* (2000) developed a biolistic method to inoculate *Vitis* species with GFLV to enable the examination of GLFV genetics and resistance on a molecular level. However, consistent detection of the virus in grapevine tissue after bombardment was not successful. The transmission and infectivity of GFLV might also vary based on variation among virus strains (Valat *et al.,* 2003). Fattouch *et al.* (2005) detected and characterized two different strains of GFLV in Tunisia. Different grapevine samples were subjected to ELISA (enzyme-linked immunosorbent assay) techniques and then amplified by using RT-PCR (Reverse Transcription Polymerase Chain Reaction). The PCR products were used for RFLP (Restriction Fragment Length Polymorphism) analysis and data showed a clear distinction between two GFLV strains. This study was the first report to show molecular variability of GFLV (Fattouch *et al.,* 2005).

2.4.2 Symptoms caused by the grapevine fanleaf virus

Grapevine fanleaf virus is one of the oldest viruses of *V. vinifera* (Pearson & Goheen, 1988), and is still one of the most economically important pathogens (Vigne *et al.,* 2005). Records of this disease date back 200 years, and it is believed that GFLV may have existed in the Mediterranean Basin and the Near East since the earliest cultivation of grapes (Pearson & Goheen, 1988).

Vines infected with GFLV are generally seen in patches within a vineyard (Andret-Link *et al.,* 2004b; Nicholas *et al*., 2007), and are normally smaller than healthy vines (Golino *et al.,* 1992). In 1954 Hewitt documented the symptoms and the use of indicator plants for GFLV. The impact of GFLV varies with the tolerance of the cultivar, and more tolerant cultivars can continue to produce good crops (Pearson & Goheen, 1988).

The disease is characterized by four distinct symptoms.

1. Infected leaves exhibit widely open petiolar sinuses and abnormally gathered primary veins causing a fan-like shape (Fig. 2.4a). This leaf deformity gave origin to the name of the virus (Pearson & Goheen, 1988). Leaf and shoot deformities develop early in the season but fade later (Hewitt, 1954). Vine shoots can also be malformed, showing abnormal branching, double nodes, short internodes and zig-zag growth (Raski *et al.,* 1983).
2. Yellow mosaic develops on leaves of affected vines in early spring. Specks vary from a few scattered spots to total yellowing. In summer the vegetation resumes its normal color (Pearson & Goheen, 1988).
3. Bunches are fewer and smaller than usual with shot berries and irregular ripening (Fig. 2.4b) (Pearson & Goheen, 1988). The GFLV can cause up to 80% reduction in fruit set. Symptoms can be confused with herbicide damage and mite injury (Nicholas *et al*., 2007).
4. Affected vines show yellow vein banding along the main veins of mature leaves. These symptoms are seen in mid to late summer (Fig. 2.4c). Discolored leaves show little malformation (Pearson & Goheen, 1988). This symptom has been shown to be the result of cross infection with yellow speckle viroid (Szychowski *et al.,* 1995).

Figure 2.4 A. Grapevine leaves showing the fan-like symptoms of the GFLV. **B.** Vines infected with GFLV show smaller, fewer bunches per vine with a high number of shot berries. **C.** Late-summer yellow vein-banding symptoms of vines infected with GFLV.

2.4.3 Diagnosis and detection of grapevine fanleaf virus

Grapevine fanleaf virus is one of a number of viruses for which woody indexing is used to verify virus-free status. The rootstock variety St. George is the standard indicator for the presence of GFLV, but symptoms are common on most *V. vinifera* varieties. Woody indexing involves grafting a candidate plant bud onto the highly reactive indicator variety. This index requires at least 18 months for reliable assays with grapevine viruses (Alley, 1955).

To accelerate the time required for detection of GFLV infection, serological techniques such as ELISA were developed (Rowhani, 1992). However, immunoassays are much less sensitive than techniques based on nucleic acid hybridizations (Fuchs *et al*., 1991) and PCR. Both RT-PCR (Fattouch *et al.,* 2001) and immunocapture (IC)-RT-PCR (Acheche *et al*., 1999) have been shown to be successful as very sensitive GFLV detection methods.

In 2001, Fattouch *et al.* developed a RNA oligoprobe capture technique to detect GFLV in grapevine tissue. This procedure was compared to an IC technique using commercial antibodies. Grapevine fanleaf virus isolates from vineyards in northern Tunisia showed negative results with IC-RT-PCR, but were detected by the RNA oligoprobe capture technique (Fattouch *et al.,* 2001). A method to detect GFLV from a single nematode from field or greenhouse soils was developed by Demangeat *et al.* (2004). The method is based on the use of a bead mill to disrupt the nematodes, and then amplifying a 555 bp fragment of the coat protein by using RT-PCR. *Styl* RFLP analysis on the coat protein amplicon is used in addition to RT-PCR to enable the GFLV isolate carried by a single nematode to be characterized (Demangeat *et al.,* 2004).

Significant progress has been made on the elucidation of the functions of most GFLV proteins, specifically those involved in the virus multiplication cycle, RNA replication, cell-to-cell movement and transmission by *X. index.* New insights into the genomic variability among isolates from naturally infected vineyards have also been made (Andret-Link *et al.,* 2004b).

2.4.4 Grapevine fanleaf virus acquisition and transmission

In 1958, Hewitt *et al.* showed that *X. index* is the natural vector of the GFLV, and that GFLV is soil-borne and not air-borne. This study was also the first to prove that nematodes can vector soil-borne viruses, and that spread was typically slow and in a concentric pattern (Hewitt *et al*., 1958).

Laboratory methods for assessing the transmission of nepoviruses were established by Trudgill *et al*. (1983). Nematode vectors that feed on plant roots can transmit viruses in all development stages, but GFLV is lost with each molt and needs to be reacquired (Taylor & Raski, 1964). However, GFLV is not passed through nematode eggs (Taylor & Raski, 1964; McFarlane *et al.,* 2002). *Xiphinema index* has the ability to ingest GFLV particles from an infected grapevine, retain the virions at specific retention sites within its feeding apparatus and subsequently infect a recipient vine when feeding (Andret-Link *et al.,* 2004b). The virus also occurs in grapevine pollen (Cory & Hewitt, 1968), but not in seeds (Shurtleff & Averre III, 2000).

The virus is acquired by *X. index*, feeding first on the roots of an infected vine and then transferring the virus by feeding on healthy vines (Leavitt, 2000). A single brief feeding on an infected vine root can make nematodes viruliferous. The nematode can retain the virus for up to eight months in the absence of host plants or up to three months when feeding on resistant host plants. The minimum GFLV acquisition threshold for transmission from *X. index* to the grapevine was established by Alfaro & Goheen (1974), and proved to be five minutes. The

virus has no measurable effect on the rate of reproduction of its vector, but improved its survival rate during starvation (Das & Raski, 1969).

In laboratory and greenhouse studies, temperature, soil moisture, the host plant, the population and developmental stages of the nematode and even the size of the pot affected the rate of virus transmission. In general, increasing the acquisition and transmission access periods from hours to several weeks increased the frequency of transmission (Shurtleff & Averre III, 2000). The virus is acquired and transmitted with an access time of 5-15 minutes in a soil temperature of 13-24°C (Siddiqi, 1974). Even when *X. index* does not carry the virus, roots are still damaged (McKenry, 1992). The nematodes retain the ability to transmit the virus for 4-8 weeks when feeding on non-viruliferous plants (Taylor & Raski, 1964) and for up to nine months under starvation conditions (Raski & Hewitt, 1960). Successful virus transmission requires that infective virus particles be inoculated into plant cells that are healthy and undamaged (O'Bannon & Inserra, 1990).

2.4.5 Vector method and grapevine fanleaf virus spread and specificity

According to Pearson & Goheen (1988), GFLV's natural host range is limited to *Vitis* species. Recent studies showed that Bermuda grass in Iran is infected with GFLV. The virus was detected by RT-PCR using two different pairs of GFLV specific primers and ELISA. However, the Bermuda grass expressed few or no symptoms of GFLV infection (Izadpanah *et al*., 2003). In addition to *X. index*, *X. italiae* has been reported to spread GFLV (Cohn *et al.,* 1970), but these results were not corroborated (Esmenjaud & Bouquet, 2009). Long-range spread of the GFLV is limited to the spread of infected plant material. Short-range spread depends on nematodes (Pearson & Goheen, 1988).

The transmission process is characterized by a high degree of specificity between GFLV and *X. index.* Viruses are attached to the cuticular lining and the lumen of the odontophore and the pharynx (Decraemer & Geraert, 2006). They are shed with the cuticle when the nematode molts (Shurtleff & Averre III, 2000). During feeding, virus particles dissociate from the cuticular lining at the retention site and are carried by the saliva of the nematode to the grapevine plant cells. Dissociation of the virus particles occurs when saliva passes through the lumen of the oesophagus and absorbs the virus at the retention site. Virus particles are released into the grapevine cells during the initial feeding phases (O'Bannon & Inserra, 1990). Limited information is available on the mechanisms of the transmission process of GFLV (Belin *et al.,* 2001).

2.5 Management strategies for *Xiphinema index* and grapevine fanleaf virus

Each disease and pest requires a different control strategy. For example, foliar diseases of grapes need specific weather patterns, some diseases and pests spread quickly, others slowly, and viruses live within the vine. Nematodes are primarily spread by the movement of contaminated soil or infested plant material sources (Nicolas *et al*., 2007). Preventative measures for controlling *X. index* and GFLV are usually the best (Hewitt, 1954), but not always practical given limited availability of desired varieties and clones. It is helpful to plant only certified planting stock (Golino, 1993), but studies have shown that healthy grapevines can become infected with GFLV within three years after planting (Hewitt *et al.,* 1962).

2.5.1 Grapevine rootstocks

The use of resistant rootstocks on which fruiting cultivars are grafted is often the best way to overcome nematode problems in perennial crops. Rootstocks for use against the *X. index* / GFLV disease complex must resist both the nematode and virus. However, resistance to both does not exist within commercial rootstocks (Harris, 1983; Meredith *et al.,* 1982). The 110R rootstock, which is often used in South Africa for its phylloxera resistance and good vigor, is susceptible to *X. index* feeding. However, Harmony, Freedom, 3309C and Schwarzmann had some degree of resistance (Harris, 1983; Malan & Meyer, 1993). More rootstock examples are named and described in table 2.2 for its resistance or susceptibility towards *X. index* feeding.

Table 2.2 Description of rootstock characteristics in terms of *X. index* resistance with S = susceptible, R = resistant and MR = moderately resistant.

Rootstock	Genetic origin	Resistance	Reference
110R	*V. berlandieri* x *V. rupestris*	S	Malan & Meyer, 1993
Harmony	(*V. longii* x Othello) x Dog Ridge	R	Harris, 1983
Freedom	(*V. longii* x Othello) x Dog Ridge	R	Harris, 1983
3309C	*V. rupestris* x *V. riparia*	S	McKenry *et al.,* 2004
Schwarzmann	*V. riparia*	MR	Harris, 1983
O39-16	*V. vinifera* x *M. rotundifolia*	R	McKenry *et al.,* 2004
Ramsey	*V. champini*	S	Ambrosi *et al.,* 1966
Dog Ridge	*V. rupestris* x *V. candicans*	S	Ambrosi *et al.,* 1966
Fairy	Not known	MR	Ambrosi *et al.,* 1966
Jacquez	*V. aestivalis* x *V. cinerea* x *V. vinifera*	S	Ambrosi *et al.,* 1966
775 Paulsen	*V. berlandieri* x *V. rupestris*	S	Ambrosi *et al.,* 1966

2.5.2 Hot water treatment, heat therapy and somatic embryogenesis

A common means of spreading *X. index* is by the distribution of infested dormant rootings or bench grafts from nurseries or from vineyards where rootstocks are planted between rows in infested areas and then later moved to other areas. A hot water (52°C) agitated soak for five minutes is recommended for treatment of infested materials (Nicholas *et al.,* 2007). However, to avoid damaging roots or buds, accurate temperature control is essential, and low numbers of nematodes may survive (Raski *et al.,* 1965b).

Grape viruses are widely spread and controlling the distribution of infected plant materials was the genesis of clean stock/certification programs in the world's grape growing regions. Infected plants can be freed of viruses by heat therapy and/or meristem culture (Torres-Viñals *et al.,* 2004). Meristem culture is effective in eliminating phloem-limited viruses, while heat therapy is normally required for viruses that readily invade plant meristems such as nepoviruses (Gambino *et al.,* 2009). Buds from a candidate vine of unknown virus status can be grafted onto a nurse plant and heat-treated in a growth chamber at 37°C for two to three months. After this treatment the buds are forced to grow and the resulting shoots are checked for the presence of virus by indexing or PCR-based testing. Heat therapy works because RNA based viruses degrade at high temperature and are eliminated before plant cells can be damaged. The process is not highly efficient, but was widely used in the past (Gifford & Hewitt, 1961). Alternatively, a small segment, less than one mm, of the shoot tip can be excised and grown in sterile culture. In many cases this small piece of tissue has escaped virus infection and can be grown into a new plant (Barlass & Skene 1978) whose virus infection can be verified free of virus by indexing and PCR testing. In some cases these two techniques can be combined but in most cases meristem culture is effective (Gambino *et al.,* 2009).

Somatic embryogenesis has also been used to efficiently eliminate several phloem-limited viruses from grapevine material (Goussard *et al.,* 1991). By using this technique, GFLV was eliminated from grapevine tissue in combination with heat therapy of the explants (Goussard & Wiid, 1992). In a study done by Gambino *et al.* (2009), it was possible to eliminate GFLV from plantlets by using somatic embryogenesis without using heat therapy with a success rate close to 100%. The virus was however detected in all tested anthers and ovaries by using RT-PCR techniques, but not in the regenerated plantlets two years after transfer to greenhouse conditions (Gambino *et al.,* 2009).

2.5.3 Crop rotation and fallow periods

Before vineyards are replanted with grapevines, the land can be cropped with cereals or grains to suppress grapevine-attacking nematodes. Some crops can increase nematode populations, as is the case with growing pumpkins or tomatoes before replanting grapevines (Nicholas *et al.,* 2007). An early study done by Raski (1955) suggested that three years is an adequate period for crop rotation. However, more recent studies suggest that *X. index* infested sites should be left fallow or rotated to crops other than grapes or figs for at least 10 years (McKenry, 2000). In moist sterile soil without food, *X. index* died after 9-10 months, but survived for 4-5 years in soil where grapevines were removed, but roots remained (Raski *et al.,* 1965a). Since vine roots decay very slowly and act as a reservoir for *X. index*, it is beneficial (but not necessarily economically viable) to wait at least six to ten years before replanting (Golino *et al.,* 1992). It must also be kept in mind that GFLV can be detected in nematodes kept in dry soil without roots for four years (Demangeat *et al.,* 2005).

2.5.4 Nematicides

Before planting, the soil may be fumigated although such treatments rarely penetrate to depths greater than one meter, and thus do not eradicate nematodes on deep perennial root systems (Lear *et al.,* 1981). This is especially true for California where the soils are often deep and fine-structured (Raski *et al.,* 1983). Broad-spectrum fumigants are expensive, but they also kill soil insects, fungi and weeds as well as beneficial organisms. Before nematicides and fumigants can be applied, the soil must be ripped and cleared of as many old roots as possible and dried to as great a depth as possible (Nicholas *et al.,* 2007).

Non-fumigant nematicides can be applied to established vineyards by using soil drenches or applied through the drip irrigation system. These nematicides must be applied with care, as they are toxic to humans and may leave residues in or on fruit (Nicholas *et al.,* 2007). Due to the high toxicity levels of nematicides and because they are unsafe for the environment and human health, their use is becoming highly restricted in the world's vineyards (Bouquet *et al.,* 2000).

2.5.5 Breeding *Xiphinema index* and grapevine fanleaf virus resistant vines

Breeding fanleaf degeneration resistant grape rootstocks would be an obvious step in the process of controlling this disease, however as with all perennial crops the process can be slow and difficult (Esmenjaud & Bouquet, 2009). Resistance to GFLV has been identified in *Muscadinia rotundifolia* (Bouquet *et al.* 2000; Walker & Jin, 2000) and in some Middle Eastern *V. vinifera* cultivars (Walker *et al*., 1985), although these latter sources have not been further studied.

Resistance to *X. index* has been found in a number of *Vitis* species, notably *V. arizonica*, *V. candicans*, *V. rufotomentosa* and *V. solonis* (Kunde *et al.,* 1968), and *M. rotundifolia* (Bouquet *et al.,* 2000). A breeding program at the University of California, Davis found that two *V. vinifera* x *M. rotundifolia* (VR) hybrids, O39-16 and O43-43, were highly resistant to *X. index* and prevented fanleaf degeneration. These two rootstocks were patented and released (Walker *et al.,* 1991), although the recommendation for O43-43 was subsequently withdrawn due to insufficient phylloxera resistance (Walker *et al.,* 1994). Once these rootstocks were used in field situations it became clear that although they had strong resistance to *X. index* feeding, they did not prevent the vectoring of GFLV as *X. index* probed for feeding sites. Feeding sites are usually swollen and distorted at the root tip (Catalano *et al.,* 1991). However, although scions grafted on these rootstocks became infected with GFLV, disease was not expressed (Walker *et al.,* 1994, Walker & Wolpert, 1994). Unfortunately these hybrids cannot be used as parents in future crosses due to sterility from the incomplete pairing of chromosomes resulting from *Vitis* (2n=38) x *Muscadinia* (2n=40) crosses (Walker *et al.,* 1994).

The need for a broader range of rootstocks with strong resistance to fanleaf degeneration continues, and efforts to discover strong sources of *X. index* resistance have built on the work of Kunde *et al*. (1968) mentioned above. Coiro *et al*. (1985) found that *V. riparia* and hybrids containing *V. riparia* also had degrees of resistance. *Muscadinia* species have also been studied to determine their *X. index* resistance and consequently their resistance to the vectoring of GFLV. *Xiphinema index* was found to attack *Muscadinia* roots very reluctantly and the few feeding sites that developed rapidly became necrotic indicating a high hypersensitivity. It was thought that this reaction prevented viruses from being transmitted, suggesting that these species were resistant to *X. index* feeding and the transmission of GFLV (Staudt & Weischer, 1992). The basis for GFLV resistance in grapevines is not yet fully understood and need further investigation.

2.6 Inheritance and mapping of DNA markers for *Xiphinema index*

The highly heterozygous nature of grapevine made it a difficult crop to explore its natural genetic diversity. But grapes are also further developed in terms of its breeding history and domestication compared to other perennial crops (Myles *et al.,* 2010). The publication of the first grapevine genome sequence (Jaillon, *et al*., 2007; Velasco *et al.,* 2007) provided a new generation of molecular tools for grapevine breeding efforts. Doors were opened to identify genes responsible for agronomic traits and disease resistance as well as the assignment of biological functions to annotated sequences (Martínez-Zapater *et al.,* 2009). Technologies such as QTL- and linkage disequilibrium-based mapping are implemented to better understand the genetic structure of grapevines. Only a small portion of the genetic diversity of grapes has been explored. The grape genome sequence in addition with rapidly developing technologies will provide easier ways to improve existing grape cultivars while incorporating specific traits and disease resistance (Myles *et al.,* 2010; Martínez-Zapater *et al.,* 2009).

Since the 1970s, the University of California, Davis has been developing rootstocks to resist fanleaf degeneration. As part of this effort, *V. rupestris* x *M. rotundifolia* hybrids were produced, and 60 of 200 seedlings tested highly resistant to *X. index* feeding, several of which, including R8913-02 and R8913-21, were also resistant to the root-knot nematode and phylloxera. Genetic mapping efforts found that the RAPD (Random Amplified Polymorphic DNA) marker OPA-12 (Operon) was tightly linked to *X. index* resistance (Walker & Jin, 1998). These *V. rupestris* x *M. rotundifolia* seedling populations were later found to be largely mistaken outcrosses of *V. rupestris* by forms of *V. arizonica* (Riaz *et al.,* 2007). Two half siblings, R8909-

15 x R8909-17 were used to create a mapping population, 9621, in which resistance to *X. index* segregated as a single dominant resistance gene. Initial mapping efforts used AFLP (Amplified Fragment Length Polymorphism) technology to identify over 500 segregating markers on 19 linkage groups (Walker & Jin, 2000). This map was later used to position resistance to the bacterial causal agent of Pierce's disease, *Xylella fastidiosa* (Doucleff *et al*., 2004).
Previous work found that *V. arizonica* was resistant to *X. index* (Kunde *et al*., 1968) and suggested that resistance was inherited as a single heterozygous gene (Meredith *et al.,* 1982). More recently, the 9621 population has been mapped with highly informative and co-dominant simple sequence repeat (SSR) markers further positioning resistance to *X. fastidiosa*, and placing a major quantitative trait locus (QTL) for *X. index* resistance (*XiR1*) on chromosome 19 (Xu *et al*., 2008).

These studies as well as the agronomical importance of nematodes and the viruses they vector prompt interest in determining the extent of *X. index* resistance in *V. arizonica* and whether other accessions had the same degree and genetics of resistance to this nematode pest.

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Chapter 3

Research results

Optimizing a pot culture screen for evaluating grapevine resistance to *Xiphinema index*

This manuscript will be submitted for publication in
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Chapter 3. Optimizing a pot culture screen for evaluating grapevine resistance to *Xiphinema index*

3.1 Introduction

The dagger nematode, *Xiphinema index* (*X. index*) is a destructive pest that feeds on grapevine roots. This nematode is even more important because of its ability to vector grapevine fanleaf virus (GFLV) (Hewitt *et al.,* 1958), and because it is present in most grape-growing countries in the world (Aballay *et al.,* 2009; Feil *et al.,* 1997; Harris, 1983a; Malan & Meyer, 1993). *Xiphinema index* has the ability to survive in vineyard soil and retain the virus without host plants for several years (Raski *et al.,* 1965; Demangeat *et al.,* 2005). Since soil fumigation is costly and only provides a temporary solution (Lear *et al.,* 1981), breeding *X. index* resistant rootstocks would provide an environmentally sensitive and long-term solution to this nematode pest.

Resistance to *X. index* has been identified in several *Vitis* species (Kunde *et al.,* 1968; Coiro & Brown, 1984), and a number of screening techniques for use under greenhouse conditions have been described (Meredith *et al.,* 1982; Harris, 1983b; McKenry *et al.,* 2001). However, most of these techniques require long periods of time to fully assess resistance or are limited by the need for large numbers of nematodes, which must be laboriously extracted from infested soils prior to inoculating trials (Malan & Meyer, 1993; Xu *et al.,* 2008). Efforts to simplify the technique and to reduce the time needed to accurately detect feeding damage have been successful (Jin, 1997; Xu *et al*., 2008), but the nematode inoculum must still be acquired from infested soil.

Extraction of nematodes from soils is performed to define and quantify the species present, or to obtain nematodes for experimental purposes (Viglierchio & Schmitt, 1983). This second objective presents problems given the typically low and unevenly distributed populations in the soil. The extraction method used depends on the nematode species and the goal of the research project. For example, the density flotation technique where nematodes (already extracted from the soil) are immersed in a centrifuge tube filled with a layered gradient of increasing sucrose concentrations can damage some nematode species. *Xiphinema index* showed increased injury, specifically disruption of membrane function, with increased hyper tonicity of the solution (Viglierchio & Yamashita, 1983). Thus, the density flotation technique is not recommended for extracting live *X. index* from soil samples. The recommended methods for successfully extracting *X. index* from soil samples are a combination of decanting and sieving with a Baermann funnel (Viglierchio & Schmitt, 1983). These methods are effective for the larger sized nematodes such as *X. index,* but eggs and juveniles are normally lost. *Xiphinema index* is also susceptible to rough handling and nematodes in bags of soil can be damaged by rough handling or over-heating (Brown & Boag, 1988).

In an effort to reduce the mechanical damage and mortality rate of *X. index* during extraction and to bolster screening efficiency by including juveniles and eggs, a new inoculation method was tested in this study. We tested the effectiveness of planting grapevines directly into infested soil, thereby ensuring that the nematodes were minimally disturbed and that juvenile stages and eggs were present. It was hoped that this method might also reduce the exposure time required to see galls, and result in higher gall numbers in a shorter period of time. Thus, a better distinction between resistant and susceptible genotypes might be made in a shorter period of time, especially for genotypes that might be moderately resistant and allow minimal numbers of galls to form.

3.2 Materials and Methods

3.2.1 Plant material

The commercial rootstock St. George (*Vitis rupestris*) was used in this study as the susceptible genotype (Pongrácz, 1983), and O39-16 (*V. vinifera x Muscadinia rotundifolia*) was used as the resistant genotype (McKenry *et al.,* 2004). Two node green cuttings with the bottom bud removed were used to create sets of five single vine replicates for each treatment. All the cuttings were treated with 1,000 ppm solution of Wood's Rooting Compound (Earth Science Production Corp., Wilsonville, OR) for 5 sec and inserted into cellulose sponges and placed on a mist propagation bench with intermittent mist controlled by a moisture sensing switch and 30°C bottom heat. After about two weeks these cuttings developed sufficient roots to be transplanted into 1,300 cm^3 plastic pots. The plants were watered on a daily basis and trimmed when needed to ensure uniform size.

3.2.2 Treatments and experimental design

Two different methods of nematode inoculation were tested on the above-mentioned commercial rootstocks. Plants of both cultivars were kept uninoculated as controls. The plants in both methods were exposed to nematode feeding for either four or eight weeks to determine the optimal time for sufficient gall formation as well as the effect of the different inoculation pressures on the cultivars.

The first inoculation method was based on the traditional method where nematodes were extracted from the soil and inoculated into pots taking care to place the nematodes close to the root mass of the plants. A combination of the Cobb's sieving and Baermann funnel techniques (Agrios, 1997) was used to extract the nematodes from soil originally collected from a highly infested site in Oakville, California. This soil had been previously used for *X. index* and grapevine fanleaf virus research and was kept in large bins. The Baermann funnels used for nematode extraction were 11 cm in diameter and made of glass. Natural rubber tubing with metal clamps was attached to the spouts. Metal wire screens were placed on top of the funnels, covered with a single tissue paper (Kimwipe, Kimberly-Clark, Neenah, WI). Soil samples were mixed with water and placed on the counter for 15-20 seconds for heavier particles to settle, with the nematodes still in suspension. The suspension containing the nematodes was poured through a 100-mesh sieve to trap adult nematodes. The sieve was rinsed from the back to collect nematodes into a 100 mL glass beaker. This process was repeated twice with the same soil solution. The runoff containing the nematodes was poured into the Baermann funnel, and the nematodes were left to migrate through the tissue paper and collect in the base of the funnel. After 48 hours adult nematodes and juveniles that failed to migrate through the funnel, were collected from the base of the funnel and counted using a light microscope. A dissecting microscope was used to positively identify *X. index* after they were extracted. The nematodes were kept in a water suspension and gently mixed on a magnetic stir plate. A counting dish was used to count the nematodes in a 2.5 mL aliquot to determine the number of *X. index* / mL. After an average of *X. index* / mL was determined, five plants from each genotype were inoculated with approximately 200 nematodes by equally pipetting them into four pencil holes near the roots. This method is referred to as the pipette inoculation method. After the study concluded, nematodes were extracted from the soil in the pots to determine nematode densities (Table 3.1).

The second inoculation method was divided into two treatments where the replicates for each cultivar were directly planted into *X. index*-infested soil (Table 3.1). The first treatment consisted of an equal part mixture of soil collected from highly infested bins and a soil mix consisting of three parts coarse sand and one part crushed lava rock. The second treatment consisted of 25% soil collected from the same highly infested bins mixed with 75% of the coarse sand/crushed lava rock mix. The fill sand and crushed lava rock mix was steam-sterilized before use. These soil combinations were mixed thoroughly and gently to ensure even nematode distribution. Soil samples were taken randomly after mixing and nematodes were extracted to determine the concentration and distribution of *X. index*. Rooted plants were potted into either of these two mixtures of infested soil. Soil samples were taken again after the testing period from individual pots and the nematodes were extracted to determine nematode densities by using the combination method of Cobb's sieving and Baermann funnel technique (Agrios, 1997).

Table 3.1 The experimental layout of the two different nematode inoculation methods and their exposure times to feeding (n=5).

Four week exposure		Eight week exposure	
Treatment	**Cultivar**	**Treatment**	**Cultivar**
Pipette inoculated	St. George	Pipette inoculated	St. George
25% soil mix	St. George	25% soil mix	St. George
50% soil mix	St. George	50% soil mix	St. George
No inoculation	St. George	No inoculation	St. George
Pipette inoculated	O39-16	Pipette inoculated	O39-16
25% soil mix	O39-16	25% soil mix	O39-16
50% soil mix	O39-16	50% soil mix	O39-16
No inoculation	O39-16	No inoculation	O39-16

After nematode inoculation of all plants, the pots were placed in the greenhouse in a completely randomized design. Inoculated plants were kept on a separate bench within the greenhouse to minimize contamination. The controls for the greenhouse were set for a temperature range of 25-30°C. Plants were hand watered and kept moist on the surface to prevent drying out, but care was taken against over-watering to prevent anaerobic conditions that can damage roots and nematodes, and wash nematodes out of the pots.

3.2.3 Evaluation of *Xiphinema index* resistance

After four weeks of *X. index* exposure in the greenhouse, plants from each treatment, including control plants, were carefully taken out of their pots and the roots were rinsed from soil and debris. Root systems were inspected and galls were counted using a 10X-illuminated magnifying glass. If no galls were detected the plant was considered resistant, but if two or more galls were counted, the plant was considered susceptible. After counting the galls, the roots were carefully removed from the stem of the plant and placed into a brown paper bag. These bags were labelled and stacked in a heated drying room for two weeks. Root weights (g) were then determined by using a bench-top scale. The entire procedure was repeated for the second set of plants after eight weeks of exposure to *X. index* feeding.

JMPSAS (version 8.0) was used to do statistical analyses on all the data. The different inoculation techniques were compared separately for the four and eight-week exposure period and the two cultivars were compared separately. Analysis of variance was done on the number of galls, root weights and nematode numbers. To determine significant differences between inoculation methods, a mean comparison was done for all pairs by using Tukey-Kramer HSD.

3.3 Results

After four and eight weeks of exposure to *X. index* feeding, gall numbers on the roots of O39-16 and St. George were determined for each inoculation treatment. Root dry weights and the number of adult *X. index* extracted from the soil were also recorded. The mean values are summarized in Tables 3.2 and 3.3. No plants died during the study.

Table 3.2 Gall numbers, root weights and the numbers of *X. index* found on St. George and O39-16 after four weeks of exposure. Mean separation data is applicable across rows of data and not within columns.

Genotype	Treatment	Gall number			Root mass (g)		*X. index* number	
		Mean		STDEV	Mean	STDEV	Mean	STDEV
St. George	Pipette inoculation	16	A	7.9	2.3	1.5	0	0.0
St. George	25% infested soil mix	67	B	26.5	0.6	0.2	31	25.5
St. George	50% infested soil mix	126	C	39.1	1.0	0.9	81	31.6
St. George	No inoculation	0	A	0.0	1.7	0.9	0	0.0
O39-16	Pipette inoculation	0	A	0.0	2.0	0.4	0	0.6
O39-16	25% infested soil mix	0	A	0.0	1.2	0.5	14	8.9
O39-16	50% infested soil mix	0	A	0.0	0.9	0.2	31	19.8
O39-16	No inoculation	0	A	0.0	2.8	1.0	0	0.0

* Values followed by a different letter are significantly different (Tukey's Kramer HSD at p<0.05).

Table 3.3 Gall numbers, root weights and the number of *X. index* found on St. George and O39-16 after eight weeks of exposure. Mean separation data is applicable across rows of data and not within columns.

Genotype	Treatment	Gall number			Root mass (g)		*X. index* number	
		Mean		STDEV	Mean	STDEV	Mean	STDEV
St. George	Pipette inoculation	16	A	8.8	2.8	1.7	3	2.2
St. George	25% infested soil mix	188	B	32.5	1.2	0.5	112	11.1
St. George	50% infested soil mix	217	C	81.6	1.7	1.1	132	40.7
St. George	No inoculation	0	A	0.0	1.5	0.7	0	0.0
O39-16	Pipette inoculation	0	A	0.0	3.2	1.5	1	0.7
O39-16	25% infested soil mix	0	A	0.0	1.8	0.6	74	7.5
O39-16	50% infested soil mix	0	A	0.5	2.2	0.9	134	34.0
O39-16	No inoculation	0	A	0.0	2.5	0.9	0	0.0

* Values followed by a different letter are significantly different (Tukey's Kramer HSD at p<0.05).

3.3.1 Average gall numbers

No galls developed on the roots of O39-16 in any of the treatments after four weeks of exposure to *X. index.* One possible gall was counted on a single replicate of the 50% soil inoculum treatment after eight weeks, but was not significantly different (*p*-value = 0.3966) from the 25% soil inoculum or the pipette inoculum treatments.

After four and eight weeks of exposure, no galls were detected on roots of the St. George uninoculated control plants (Figure 3.1). The pipette inoculated St. George had the same number of galls after four and eight weeks of inoculation. Gall numbers were significantly different between pipette inoculation, 25% soil mix and 50% soil mix treatments after four weeks (*p*-value = 0.0002) and the same relationship was found after eight weeks (*p*-value < 0.0001) (Tables 3.2 and 3.3).

3.3.2 Average root mass

St. George did not show any significant differences in average root mass among treatments. This result was the same for four weeks and eight weeks of exposure. O39-16 showed no significant difference in root mass among treatments after eight weeks of exposure, but there was a significant difference after four weeks with a *p*-value of 0.0006 (Table 3.4 and Figure 3.1).

Table 3.4 Differences in average root weights (g) of O39-16 and St. George after four or eight weeks of exposure to *X. index* feeding using three inoculation techniques. Significant differences in root mass was denoted with letter symbols.

Genotype	Time	*p*-value	Root weights (g)			
			Control	Pipette inoculation	25% soil mix	50% soil mix
O39-16	4 Weeks	0.0006	2.8 A	2.0 AB	1.2 BC	0.9 C
O39-16	8 Weeks	0.2159	2.5	3.2	1.8	2.2
St. George	4 Weeks	0.0577	1.7	2.3	0.6	1.0
St. George	8 Weeks	0.1462	1.5	2.8	1.2	1.7

* Values followed by a different letter are significantly different (Tukey's Kramer HSD at p<0.05).

The average root weights were compared to the average number of galls for both rootstocks. After four weeks of exposure there was no correlation between the average gall numbers and average root mass for St. George (R^2 = 0.166213). There were no galls on the roots of O39-16 to make a comparison. After eight weeks there were also no correlation for St. George (R^2 = 0.012347) and O39-16 (R^2 = 0.015627).

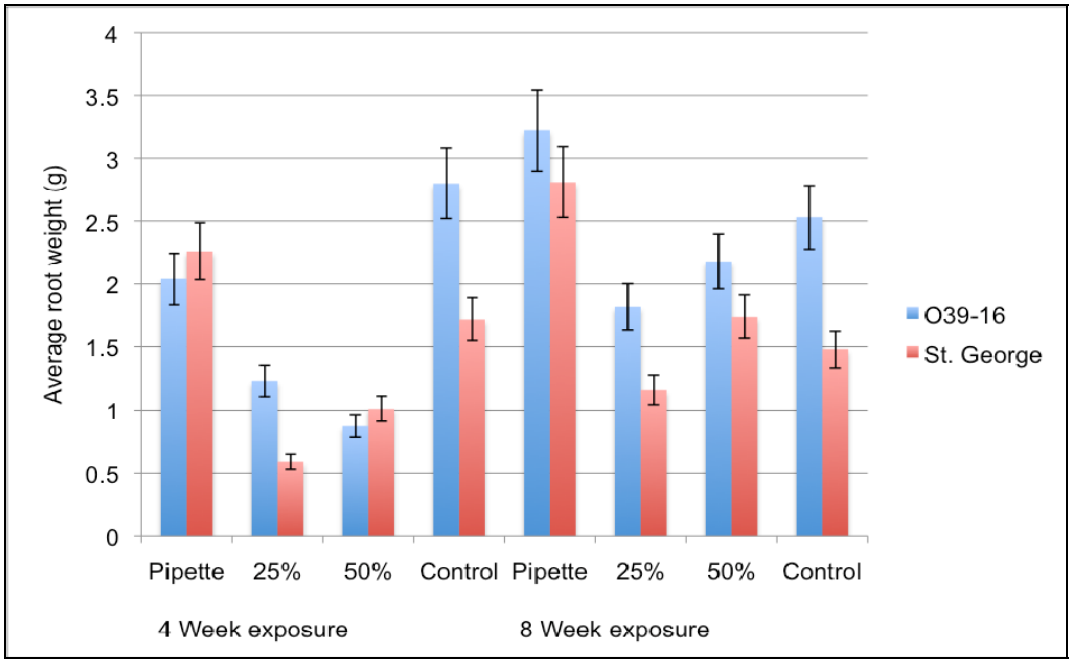

Figure 3.1 The average root weights of potted plants of St. George and O39-16 after four or eight weeks of exposure to *X. index* using three inoculation techniques.

3.3.3 Average nematode numbers extracted

Between 40 and 50 adult nematodes were extracted from the 50% soil mix and between 20 and 30 nematodes for the 25% soil mix before the study was conducted. After four and eight weeks of exposure, no nematodes were found in the pots of the control plants. The number of

nematodes on O39-16 was significantly lower for the pipette inoculation method compared to the 25% and the 50% soil mix after four and eight weeks of exposure (Table 3.5). The same was true for St. George after eight weeks of exposure, but not after four weeks. St. George in the 50% soil mix had significantly higher nematode numbers than the 25% soil mix and the pipette inoculation for both cultivars after four and eight weeks of *X. index* exposure (Figure 3.2).

Table 3.5 Differences in average number of *X. index* in potted plants of O39-16 and St. George after four or eight weeks of exposure to feeding under three inoculation techniques. Mean separation data is applicable across rows of data and not within columns.

Genotype	Time	*p*-value	*X. index* numbers			
			Control	Pipette inoculation	25% soil mix	50% soil mix
O39-16	4 Weeks	0.0010	0 B	0 B	14 AB	31 A
O39-16	8 Weeks	<0.0001	0 C	1 C	74 B	134 A
St. George	4 Weeks	<0.0001	0 B	0 B	31 B	81 A
St. George	8 Weeks	<0.0001	0 B	3 B	112 A	132 A

* Values followed by a different letter are significantly different (Tukey's Kramer HSD at p<0.05).

The average nematode numbers were compared to the average gall numbers between the different treatments. A significant correlation was found for St. George after four weeks of exposure. The higher the gall count, the higher the number of nematodes in the soil (R^2 = 0.790605). No correlation was found for O39-16 after four weeks. After eight weeks of exposure there was a significant correlation between the gall numbers and the number of nematodes for St. George (R^2 = 0.733494), these values were not correlated for O39-16 (R^2 = 0.287804).

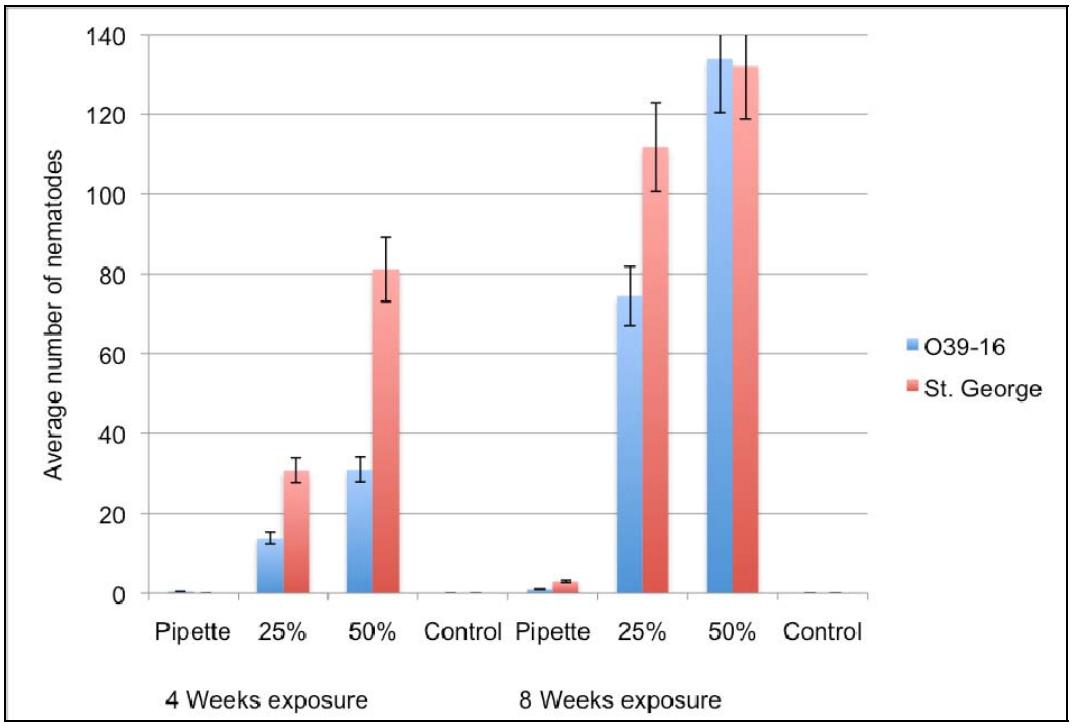

Figure 3.2 The average number of nematodes in the soil of potted plants of St. George and O39-16 after four or eight weeks of exposure to *X. index* using three inoculation techniques.

3.4 Discussion

Depending on the species of nematode, a series of extraction methods have been established and improved, and greenhouse studies on the introduction of nematodes to plants have been described. Jin (1997) examined *X. index* population increases and galling of grape roots under greenhouse conditions. Nematodes were extracted by using the Cobb's sieving method in combination with the Baermann funnel technique. Plants were observed for 12 months although high numbers of nematodes were found after only three to four months. Wheeler (2006) extracted *X. index* from heavily infested soil and used 100 nematodes to pipette inoculate a pot and after six weeks low gall numbers were detected. Roncoroni (2004) used the same extraction method as Jin (1997) and Wheeler (2006), but found more than 10 galls after only four weeks of *X. index* feeding. Roncoroni (2004) tested a plant population with *V. rupestris*, *V. arizonica* and *V. candicans* in the background where Wheeler (2006) tested the *Vitis* species collected from northern Mexico by Dr. Olmo. These northern Mexico species included *V. acerifolia / girdiana*, *V. arizonica*, *V. arizonica / candicans*, *V. treleasei* and others. Jin (1997) tested other *Vitis species* including*V. berlandieri x V. rufotomentosa* crosses and *V. rupestris* x *M. rotundifolia* hybrids.

In an effort to improve upon the traditional inoculation technique — extraction of nematodes from a soil sample, collection via sieving and Baermann funnel and then inoculating with a suspension of nematodes — an inoculation system using a known infested soil was tested. Using *X. index* infested soil saved time and labour, and was less damaging to the nematodes. The concentration of nematodes can be estimated by taking a few random soil samples before and after diluting with uninfested soil mix so that a consistent inoculum concentration can be used with multiple tests. In addition, this method should also retain eggs and juvenile stages of *X. index,* which can feed sooner and expand the population increasing the severity of the test. More galls were detected after four weeks on St. George with the 25% and the 50% inoculum than with the pipette inoculation technique. These levels were also higher when compared to previous studies in the lab that had used hand picked inoculation (Jin, 1997; Roncoroni, 2005; Wheeler, 2006). The disadvantages of using an infested soil might be the uneven distribution of *X. index* between pots, the occurrence of other nematode species that can have an impact on *X. index*'s survival and feeding, and the rapid production of high gall numbers, which can decay roots and prevent the observation of galls. Optimally a clean greenhouse culture of *X. index* is maintained for inoculation purposes. Susceptible grapevine genotypes such as St. George can be used for culturing *X. index*; *Ficus carica* can be used to isolate the impact of *X. index* from GFLV as *F. carica* hosts the nematode but not the virus (Winterhagen *et al.,* 2007).

3.4.1 Average gall numbers

There were no galls on the St. George and O39-16 control plants after four and eight weeks of *X. index* exposure. Even when the inoculated and control plants were in close proximity, no cross contamination occurred. O39-16 only showed one possible gall after eight weeks with the 50% soil mix treatment. This gall might have been the result of a root deformation and not because O39-16 resistance failed under high *X. index* pressure. O39-16 plants should be exposed to a soil-based inoculum with high *X. index* numbers for a prolonged period of time to determine whether O39-16's resistance can be compromised.

The average gall number was significantly higher for the 50% soil mix compared to the 25% soil mix, which was significantly higher than the pipette inoculation after four weeks. The resulting differences were greater after eight weeks. This indicates that the soil-based inoculum

is more effective than the pipette inoculation method. The pipette inoculation method does introduce a known number of adult *X. index* into the soil, but no eggs or juvenile stages are present. In addition, the extraction method damages the nematodes and only a few may be left to adjust to their new environment and to start feeding on the roots. Pipette inoculation might be more effective if the plants were exposed beyond eight weeks. The St. George plants that were planted in infested soil (25% and 50% mix) had higher gall numbers than the pipette inoculation method and more nematodes were extracted from the pots with higher gall numbers. The higher gall numbers were likely the result of the relatively undisturbed nematodes (i.e. no physical damage as caused by the pipette inoculation method) and the presence of eggs and juvenile stages, which should result in higher numbers of nematodes capable of feeding. However, after eight weeks the galls became difficult to count on the roots for both the 25% and 50% soil mixes because the roots were decaying. It is recommended that plants inoculated with the soil-based methods be monitored closely to determine the optimum level of galling versus root decay. The results presented here suggest four to six weeks is optimum.

3.4.2 Average root mass

St. George had lower root weights than O39-16 for all the treatments, except for the pipette inoculation method and the control after four weeks. This difference seems to be best explained by difference between the two rootstocks. No correlation was found between the average root mass and the average gall numbers for any of the treatments, which corresponds with the studies done by Roncoroni (2004) and Wheeler (2006). However, Xu *et al.* (2008) found an inverse correlation between gall numbers and root weights.

3.4.3 Average number of nematodes recovered

The average number of nematodes recovered was higher from the pots with the 50% soil mix compared to the 25% soil mix and the pipette inoculation for both cultivars. Nematode numbers would be expected to decline on O39-16 due to its strong resistance, but this study showed high numbers of nematodes even though no galling was detected, especially after eight weeks. The average number of nematodes recovered from the 50% soil mix (133.8) was significantly higher than the pipette inoculation method (1.0) as well as the 25% soil mix (74.4). These results indicate that the number of nematodes does not decline in the presence of highly resistant rootstocks. They also suggest that *X. index* may be feeding and reproducing on O39-16 roots, but not inducing gall formation. Alternatively, high numbers of *X. index* were in the 50% soil inoculum and they remained in the pots through the eight-week testing period.

This study found a correlation between gall numbers and the number of *X. index* recovered from the soil. The higher the gall number on the susceptible genotype, the higher the number of nematodes recovered, which was also noted by Jin (1997) and Xu *et al*. (2008). The results presented here support the use of a soil-based inoculum when screening for *X. index* resistance. This method seems to produce more severe feeding, higher resulting nematode numbers, more distinct separation of resistant and susceptible genotypes based on the number of galls produced and takes less time and effort.

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Chapter 4

Research results

Evaluating *Vitis arizonica* for *Xiphinema index* resistance

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Chapter 4. Evaluating *Vitis arizonica* for *Xiphinema index* resistance

4.1 Introduction

Vitis vinifera is widely grown in many environments around the world. It tolerates low fertility soils, lime and drought, and its cultivars include wine, table and raisin grapes. However, it is susceptible to a broad array of diseases and pests. Vineyard production can be significantly impacted by viral, bacterial and fungal disease, as well as soil-borne pests such as phylloxera and nematodes.

The dagger nematode, *Xiphinema index*, is a particularly important soil-borne pest. Its feeding causes club shaped galls on young root tips, which induces secondary branching of roots that are then also fed upon, leading to masses of short galled roots that function poorly and soon decay. The damage to the root system is severe, but while feeding on the roots *X. index* also vectors grapevine fanleaf virus (GFLV) (Hewitt *et al*., 1958). Grapevine fanleaf virus causes fanleaf degeneration, a severe disease that greatly impacts berry set and reduces crop yields by 80% or more (Pearson and Goheen 1988). Nematicides and fumigants have been used to control *X. index* and reduce the spread and severity of fanleaf degeneration. However, these pesticides have not been effective at eliminating *X. index* from soils due to the deeply rooted nature of grape roots and and *X. index*'s ability to survive on these deep roots (Raski *et al*., 1983; Raski & Goheen, 1988); crop rotation or long-term fallow periods are not economical for vineyards.

Research has been underway for many decades to identify *X. index* resistant grape species. In 1968, Kunde *et al.* evaluated different *Vitis* species for resistance to *X. index*, and found that *V. arizonica, V. candicans, V. rufotomentosa, V. smalliana* and *V. solonis* exhibited high resistance. The resistance ratings were based on visible root galls and the change in the nematode numbers over a period of eight months. This study stimulated efforts that tested hybrids among these species, rootstocks and other species like *Muscadinia rotundifolia* for *X. index* resistance (Harris, 1983; Meredith *et al*., 1982; Coiro *et al*., 1990; Malan & Meyer, 1993). *Vitis vinifera* and *M. rotundifolia* hybrids were also developed by Olmo to utilize the strong and broad pest and disease resistance of *M. rotundifolia* (Olmo, 1986). Lider and Goheen later used these so-called VR hybrids as rootstocks in a trial to test rootstock selections for resistance to fanleaf degeneration. The VR rootstock O39-16 was commercially released from these trials (Walker *et al.* 1991). Field trials have confirmed its resistance to *X. index* feeding (McKenry *et al*. 2001) and its ability to induce tolerance to fanleaf degeneration (Walker *et al.* 1994). Although O39-16 has strong *X. index* resistance, it lacks resistance to root-knot nematodes and cannot be hybridized with other species to improve it because it is a hybrid of a 2n = 38 (*Vitis*) species and 2n = 40 (*Muscadinia*) species.

Recently, *V. arizonica* has been re-examined for use in breeding due to its strong resistance to *X. index* feeding (Roncoroni & Walker, 2004) as well as *Xylella fastidiosa*, the bacterial agent for Pierce's disease (Krivanek *et al.,* 2005). *Vitis arizonica,* also known as canyon grape and Arizona grape, is indigenous to Arizona, Utah, New Mexico, Nevada, Texas and northern Mexico (Gucker, 2006). In 1961 H.P. Olmo collected seeds from a wide range of wild *Vitis* species, including various forms of *V. arizonica* grapes in northern Mexico (Figure 4.1). He established a male and female sibling vine from each of these sites, which are now kept at the United States Department of Agriculture National Clonal Germplasm Repository in Davis, California (www.ars.usda.gov/Main/docs.htm?docid=12254). *Xiphinema index* is not known to

be present in Mexico (Siddiqi, 1974), although some *Vitis arizonica* genotypes from this country exhibit resistance to *X. index* as shown in previous studies (Wheeler, 2006). However, other *Xiphinema* species, for example *X. americanum*, do exist in Mexico (Siddiqi, 1973). *Xiphinema americanum* is known to be a vector of peach rosette mosaic virus (PRMV) (Ramsdell *et al*., 1983; Ramsdell *et al*., 1996) and grape yellow vein virus (Pearson & Goheen, 1988), but not GFLV. It is possible that Mexican grape species have an evolved resistance to *X. americanum* and that this mechanism is capable of resisting the closely related *X. index*.

In 2006, Wheeler determined the extent of *X. index* resistance of the *V. arizonica* collection from Olmo by placing them into groups based on plant morphology and subsequently testing them for *X. index* resistance. The study revealed new sources of *X. index* resistance that can be applied in future studies.

In this study, accessions of *V. arizonica* and related hybrid forms collected by Olmo were also examined and compared to the Wheeler (2006) study to establish the level of their resistance to *X. index* feeding damage using a novel *X. index* inoculation method (method as described in Chapter 3 of this dissertation). The progeny from crosses with these parents were also tested. The results from this study will be used to select optimal parents for breeding efforts and to develop additional populations for the genetic mapping of *X. index* resistance.

Figure 4.1 *Vitis* collection sites in Mexico where H.P. Olmo collected seeds in 1961.

4.2 Materials and Methods

4.2.1 Plant material

Herbaceous cuttings of the Olmo Mexican *Vitis* collection (the b-series) were collected from the UC Davis or the USDA Germplasm collection sites. These cuttings were used to create subsets of four replicates for each of the 18 genotypes that appeared according to M.A. Walker to have at least some *V. arizonica* parentage (Table 4.1). These cuttings were dipped into a 1,000 ppm solution of Wood's Rooting Compound (IBA/NAA mix – Earth Science Production Corp., Wilsonville, OR) for a few seconds and then inserted into small cellulose sponges. The cuttings were then placed in trays on a propagation bed with intermittent mist, controlled by a moisture sensor and 30°C bottom heat. After two weeks these cuttings developed sufficient roots to be transplanted into 1,300 cm^3 pots.

Table 4.1 Details of *Vitis arizonica* types studied. The UC Davis and USDA collection columns refer to the block number, row number and vine position. The DVIT codes are plant identification codes used at the USDA National Clonal Germplasm Repository, Davis, California.

Olmo code	Olmo collection site	DVIT code	UC Davis collection	USDA Germplasm collection	Species
b40-13	Chihuahua	1863	M25: 1-2	S33: 9-10	*arizonica*
b40-14	Chihuahua	1864	M25: 59-60	S33: 11-12	*arizonica*
b40-29	Chihuahua	1865	M25: 61-62	S33: 13-14	*arizonica*
b40-34	Chihuahua	1866	M25: 3-4	S33: 15-16	*arizonica*
b40-50	Chihuahua	1867	M25: 63-64	S33: 17-18	*cinerea*
b40-51	Chihuahua	1868	M25: 5-6	R18: 28	*cinerea*
b40-61	Hildago Parral	1870	M26: 1-2	S29: 27-28	*arizonica*
b41-13	Ciudad Mante	1872	M26: 9-10	S42: 12-13	*arizonica*
b41-23	Ciudad Mante	1873	M26: 5-6	S34: 24-25	*cinerea*
b41-47	Linares	1875	M26: 7-8	R18: 30-31	*cinerea*
b42-11	Linares	1876	M25: 15-16	R19: 1-2	*cinerea*
b42-24	Loreto	1877	M25: 17-18	Dead	*arizonica*
b42-26	Loreto	1874	M26: 11-12	Dead	*arizonica/girdiana*
b42-33	Linares	1878	M25: 19-20	S33: 19-20	*cinerea/candicans*
b42-34	Linares	1879	M26: 13-14	Not available	*cinerea*
b43-15	Guadalupe	1884	M25: 25-26	S38: 27-28	*arizonica/candicans*
b44-22	Nuevo Laredo	1893	M26: 27-28	S35: 1-2	*cinerea var. helleri*
b45-26	Monterrey	1900	M26: 35-36	R13: 30-31	*cinerea*

The progeny from three populations of crosses among three of the *V. arizonica* types, b40-14, b40-29 and b42-26 crossed to two *V. rupestris* selections A. de Serres and Wichita Refuge were also tested in this study. These populations and their progeny were designated as the D, Q and R series (see Table 4.2) (Riaz *et al.,* 2007). DNA was collected from all the individuals and four SSR markers were used to verify their identity after being moved to a new field location. The primers used were VMC2a3, VMC2g2, VMC5a10 and VMC6e10. These SSR markers are described in the NCBI databases (UniSTS) and can be found at http://www.ncbi.nlm.nih.gov/. *Vitis rupestris* cv. St. George was used as a *X. index* susceptible control and O39-16 (*V. vinifera x Muscadinia. rotundifolia*) was used as the resistant control in

this study. The plant material and control plants were prepared in the same way as for the *V. arizonica* types.

Table 4.2. Details of the *V. arizonica* –based populations studied.

Female Parent	Male Parent	Number of Progeny
V. rupestris A. de Serres	*V. arizonica*/*girdiana* b42-26	11 (D-series)
V. rupestris Wichita Refuge	*V. arizonica* b40-29	1 (Q-series)
V. rupestris Wichita Refuge	*V. arizonica* b40-14	46 (R-series)

4.2.2 Soil inoculum

All plants were transplanted into *X. index*-infested soil mix. The soil mix consisted of 50% soil collected from a known highly infested site near Oakville, Napa County, California. The remaining 50% was made up from three parts coarse sand and one part crushed lava rock. The sand and lava rock mix was steam-sterilized before mixing. These soil combinations were mixed thoroughly to ensure even nematode distribution. Five soil samples were taken randomly after mixing and nematodes were extracted to determine the distribution and concentration of *X. index*. Rooted plants were potted with the nematode infested soil mix. After these studies were concluded, soil samples were taken again from the combined pot soil and the nematodes were extracted to determine nematode numbers.

Nematodes were extracted by using a combination of Cobb's sieving and Baermann funnel techniques (Agrios, 1997). The Baermann funnels used for nematode extraction were 11 cm in diameter and made of glass. Natural rubber tubing with metal clamps was attached to the spouts. Metal wire screens were placed on top of the funnels, covered with a single paper tissue (Kimwipe, Kimberly-Clark, Neenah, WI). Soil samples were mixed with water and placed on the counter for 15-20 seconds to let heavier particles settle, with the nematodes still in suspension. The suspension containing the nematodes was poured through a 100-mesh sieve to trap adult nematodes. The sieve was rinsed from the back to collect nematodes in a 500 mL glass beaker. This process was repeated twice with the same soil. The runoff containing the nematodes was poured into the Baermann funnel, and the nematodes were left to migrate through the tissue and collect in the base of the funnel. After 48 hours the nematodes were collected from the base of the funnel and counted using a light microscope. This method extracts the adult *X. index* nematodes that are capable of moving through the tissue, but not the juvenile stages or eggs.

4.2.3 Experimental design

All three populations were potted into *X. index*-infested soil and placed in the greenhouse in a completely randomized design. Inoculated plants were kept on separate benches within the greenhouse to minimize contamination. The room controllers were set to 25 to 30˚C. Plants were kept moist on the surface to prevent them from drying out, but care was taken not to over-water, which might wash nematodes out from the soil and damage roots. An automated drip irrigation system was used where each pot had one emitter (1 L / hour). The system was set to irrigate once every 24 hours for 30 seconds.

4.2.4 Evaluation of *Xiphinema index* resistance

Extra St. George plants were also potted so that *X. index* feeding damage could be assessed on their roots to determine how many weeks after potting the test plants should be evaluated. After six weeks, roots on these susceptible test plants were well galled and the decision was made to evaluate all of the plants. They were taken out of their pots and the soil was washed from the roots. Root systems were inspected and galls were counted using a 10X-illuminated magnifying glass. If one or fewer galls were detected, the plant was considered resistant, but if two or more galls were counted, the plant was considered susceptible. The control plants, O39-16 and St. George were also examined for galls.

4.3 Results

4.3.1 *Vitis arizonica* accessions (b-series)

Eighteen genotypes were tested and nine were susceptible to *X. index* feeding, based on the number of their root galls. Nine of the genotypes had fewer than 2 galls and were judged to be resistant. Of these, only the data from b42-24 was compromised since three of the replicates did not take after transplant and the data for this accession is represented by a single replicate (Figure 4.2 and Addendum 1). All the other genotypes had complete sets of four replicates each. Analysis of variance showed a significant difference among the mean values for the 18 genotypes with $p<0.0001$, and Tukey-Kramer HSD analysis confirmed these differences (Table 4.3). These nine genotypes had been tested earlier (Wheeler, 2006), but in that study the b41-23, b41-47 and b42-11 tested as susceptible, with 2.75, 5.00 and 6.25 average galls, respectively. Of the susceptible genotypes, b40-13 had galls on all four replicates, but had the lowest average (10.75) gall number of the susceptible genotypes. Three of the genotypes, b40-50, b45-26 and b40-13 tested resistant in the Wheeler (2006) study, but had 86.75, 70.00 and 10.75 galls on average in this test (Table 4.3). All the replicates of O39-16 were resistant as expected, and St. George had consistently high numbers of galls on all replicates with an average gall number of 99.25. Only b44-22 had a higher number of galls than the St. George susceptible control (162 galls).

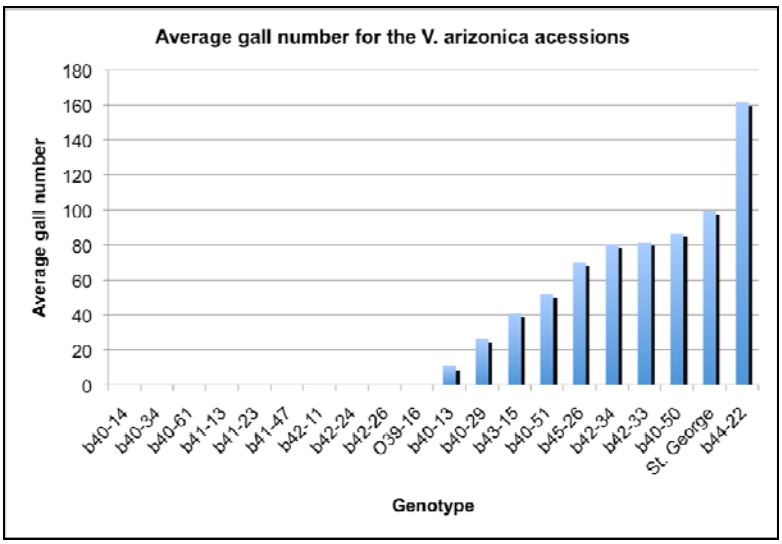

Figure 4.2 Distribution of mean gall values of the *V. arizonica* "b-series" genotypes and controls.

Table 4.3 Mean galls recorded in this study as compared to the status of a previous study done on different *Vitis* species. The letters R and S refer to resistant and susceptible genotypes respectively. Additional data is presented in Addendum 1.

Genotype	Mean galls	Mean separation	This study	Wheeler, 2006
b40-13	11	DE	S	R
b40-14	0	E	R	R
b40-29	26	CDE	S	S
b40-34	0	E	R	R
b40-50	87	B	S	R
b40-51	52	BCD	S	S
b40-61	0	E	R	R
b41-13	0	E	R	R
b41-23	0	E	R	S
b41-47	0	E	R	S
b42-11	0	E	R	S
b42-24	0	CDE	R	-
b42-26	0	E	R	R
b42-33	82	B	S	S
b42-34	80	B	S	-
b43-15	41	BCDE	S	S
b44-22	162	A	S	S
b45-26	70	BC	S	R
O39-16	0	E	R	R
St. George	99	B	S	S

* Mean values followed by a different letter are significantly different (Tukey's Kramer HSD at $p<0.05$).

4.3.2 *Vitis arizonica* progeny (D-, Q- and R-series)

A total of 58 genotypes from the D-, Q- and R-series (Riaz *et al.,* 2007) were tested for resistance. Six genotypes from the R-series allowed limited *X. index* feeding galls on one or more replicates after six weeks of exposure (Figure 4.3), but no galls were found on any of the roots of the D- and Q-series. A total of 50 genotypes for the three populations did not allow *X. index* feeding damage (Addendum 2). A previous study (Jin, 1997) found D8911-04 and Q8918-04 to be susceptible to *X. index* feeding and all genotypes in the R-series to be resistant. All four replicates of R8916-02 and R8919-01 died during this study, whereas D8909-03, D8913-03 and R8916-05 had three surviving replicates.

The resistant control rootstock, O39-16 showed no evidence of *X. index* feeding damage, and all four replicates of St. George had high numbers of galls after four weeks with an average gall number of 124.

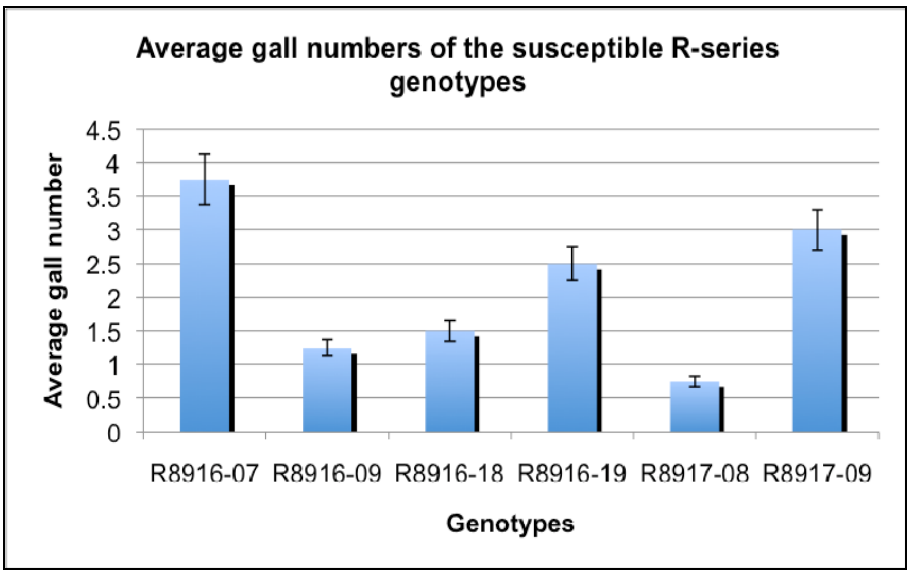

Figure 4.3 Distribution of mean gall values of the six susceptible R-series genotypes.

Analysis of variance between the means of all the genotypes showed a significant difference between the resistant and susceptible genotypes (Table 4.4), but a Tukey-Kramer HSD comparison did not distinguish the means of the susceptible genotypes.

Table 4.4 Analysis of variance on the means of progeny tested for *X. index* resistance in the D-, Q- and R-series.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	55	122.98077	2.23601	1.9191	<0.0009
Error	165	192.25000	1.16515		
C. Total	220	315.23077			

4.3.3 *Xiphinema index* recovery

Before inoculation of the b-series and the D-, Q-, and R-series, five random soil samples were collected from the infested soil mix and the adult *X. index* were recovered to establish the concentration of nematodes and how evenly they were distributed (Table 4.5). The same infested soil mix was used for the b-series and the D-, Q- and R-series. After the study concluded, five soil samples were randomly taken from the collected soil to determine the average number of nematodes present.

Table 4.5. *Xiphinema index* numbers before and after the six-week screening of the b-series and the D-, Q-, and R-series.

Soil Sample	1	2	3	4	5	Average
b-series						
Samples before inoculation	22	19	30	24	27	24
Samples after study	40	31	23	27	34	31
D-, Q- and R-series						
Samples before inoculation	15	22	18	26	29	22
Samples after study	30	17	20	19	22	22

Analysis of variance was done on the data and no significant difference between the mean values for nematode numbers of b-series before and after the study was found (Table 4.6). The same result for the D-, Q- and R-series was found (Table 4.7).

Table 4.6 Analysis of variance on the means of the nematode numbers before and after the study was done on the b-series.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	1	108.90000	108.900	3.5822	0.0950
Error	8	243.20000	30.400		
C. Total	9	352.10000			

Table 4.7 Analysis of variance between the means of the nematode numbers before and after the study was done on the D-, Q- and R-series.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	1	0.40000	0.4000	0.0138	0.9092
Error	8	231.20000	28.90000		
C. Total	9	231.60000			

4.4 Discussion

H.P. Olmo collected *V. arizonica* plants from different areas in Northern Mexico (commonly known as the Olmo b-series accessions). In this study we found nine of these to be resistant to *X. index* feeding and nine others to be susceptible. Six genotypes out of the eighteen tested had a different response toward *X. index* feeding in a previous study (Table 4.2) done by Wheeler (2006). Six of the resistant genotypes appeared to be *V. arizonica* or *V. arizonica* hybrids with related species (Table 4.2). Given the past work of Kunde *et al.* (1968), which determined that *V. arizonica* was resistant to *X. index*, and the discovery and mapping of *XiR1,* a resistance gene from *V. arizonica* (Xu *et al.* 2008), it was expected that all or most of the *V. arizonica* genotypes tested here would be resistant, whereas only four of the six tested were resistant. Most of the *V. arizonica* types were from near Chihuahua in north/central Mexico, but only two of the four *V. arizonica* types collected from this area tested resistant. Two *V. cinerea/V. arizonica* hybrids from the same area tested susceptible to *X. index* feeding. This might suggest that some of the genotypes thought to be pure *V. arizonica* were in fact hybridized with another species, or that there is more variability than expected in *V. arizonica*'s resistance. The two *V. arizonica* types, one from Hildago Parral and the other from Ciudad Mante tested resistant in both this study and previous work done by Wheeler, 2006. Pure *V. cinerea* collected in the Linares area tested resistant in this study, but was classified as an off-type in Wheeler's study. However, Kunde *et al.* (1968) classified *V. cinerea* as susceptible. It is possible that these genotypes were incorrectly labelled or collected in the above-mentioned studies. These plants should be retested in terms of species as well as nematode resistance to determine their true origin and status.

It is also puzzling why Mexican *Vitis* species would be resistant to *X. index*, a nematode thought to have originated in the Middle East (Hewitt *et al*. 1958) where it probably co-evolved with GFLV (Hewitt, 1985). European literature refers back 200 years to GFLV and the consensus is that GFLV may have existed in the Near East and Mediterranean Basin since the start of grape cultivation (Pearson & Goheen, 1988). References from Lebanon suggest that GFLV entered the country with imported grafts, but *X. index* was associated with fig trees in

fields where no grapevines were found. This indicates that *X. index* and GFLV have been present in the Middle East for a long time (Hanna *et al.*, 2008). Research done in Iran indicated high numbers of *X. index* present on cultivated soils as well as natural woodlands where wild grapevines are common, especially in the Caspian forests. However, the occurrence of GFLV was isolated in Iran (Mojtahedi *et al.,* 1980).

An alternative scenario is that these Mexican species have evolved resistance to a different *Xiphinema* or Longidorid species and this resistance mechanism provides protection against *X. index*. *Xiphinema americanum* is widely found in Mexico where grapes are grown (Siddiqi, 1973). Other *Xiphinema* species found in Mexico are *X. basiri* (Norton *et al*., 1984), *X. californicum* (Lamberti & Golden, 1986) and *X. diversicaudatum* (Norton *et al.,* 1984). No reference to the geographical locations within Mexico of these nematodes could be found.

In a previous study done by Jin (1997), the genotypes D8911-04 and Q8918-04 were susceptible to *X. index* feeding, although they were resistant here. It is possible that D8911-04 and Q8918-04 might be off-types in this or Jin's study, since the plant material for these studies were not collected from the same sites as this collection had to be relocated. Although DNA testing was done to confirm that the transfer was done properly from the old block to the new one, retesting of these two accessions would help clarify the contradictory test results. Jin (1997) also tested the R-series for resistance to *X. index* and he found all the individuals to be resistant. The parentage of this population is *V. rupestris* Wichita Refuge (susceptible to *X. index*) x *V. arizonica* b40-14, the latter tested resistant to *X. index* feeding in this study. Previous work found that *X. index* resistance in b40-14 is controlled by a single homozygous locus (Walker & Jin, 2000), thus all progeny from crosses between Wichita Refuge and b40-14 should be resistant to *X. index.* However, six genotypes had galls after six weeks of exposure to *X. index* feeding; although the gall numbers were very low, with the highest average less than four. The study presented here used an infested soil inoculation system (see Chapter 3), which may have retained more active nematodes that fed more aggressively on the R-series progeny; especially since eggs, active juveniles and non-disturbed adults are present in the soil-based inoculation but not in the traditional inoculation system based on the extraction and inoculation of adult nematodes. The higher inoculum pressure might have caused the true level of possible moderate resistance of these six genotypes to appear. The consistently low numbers of galls found on these individuals indicates that the level of their susceptibility was very low if compared to the susceptible control. The susceptible St. George plants back this theory up since they showed extensive feeding damage on all replicates (Addendum 2). However, the susceptibility of these accessions is further documented by earlier testing of R8916-07 (Chapter 5) where it also had low numbers of galls. It is recommended that these six R-series genotypes be retested with both hand inoculation of extracted adult nematodes and the infested soil inoculation system to verify these results of moderate resistance. It would also be useful to test these inoculation systems and genotypes over short (4 to 6 weeks) and long (8 weeks and longer) to acquire a more accurate indication of their status.

The results from this study were used to develop a study of the inheritance of resistance to *X. index* within *V. arizonica* (Chapter 5) and the development of framework genetic map of this resistance (Chapter 6).

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Addendum 1. Screening data for the *Vitis arizonica* species collected by H.P. Olmo in Mexico.

Genotype	Gall numbers				Average gall number	STD DEV	SE	Resistance ranking
	Rep 1	Rep 2	Rep 3	Rep 4				
b40-13	16	7	8	12	11	4.11	0.63	S
b40-14	0	0	0	0	0	0.00	-	R
b40-29	28	0	41	36	26	18.30	1.79	S
b40-34	0	0	0	0	0	0.00	-	R
b40-50	49	110	65	123	89	35.37	1.90	S
b40-51	51	78	46	32	52	19.26	1.34	S
b40-61	0	0	0	0	0	0.00	-	R
b41-13	0	0	0	0	0	0.00	-	R
b41-23	0	0	0	0	0	0.00	-	R
b41-47	0	0	0	0	0	0.00	-	R
b42-11	0	0	0	0	0	0.00	-	R
b42-24	Dead	Dead	0	Dead	0	0.00	-	R
b42-26	0	0	0	0	0	0.00	-	R
b42-33	126	67	100	34	82	39.95	2.21	S
b42-34	64	55	113	89	80	26.15	1.46	S
b43-15	32	35	41	56	41	10.68	0.83	S
b44-22	186	152	139	170	162	20.56	0.81	S
b45-26	27	102	56	95	70	35.09	2.10	S
O39-16	0	0	0	0	0	0.00	-	R
St. George	56	75	142	124	99	40.41	2.03	S

Addendum 2. Screening data for the D-, Q- and R-series.

Genotype	Gall numbers				Average gall number	STD DEV	SE	Resistance ranking
	Rep 1	Rep 2	Rep 3	Rep 4				
D8908-02	0	0	0	0	0	0.00	-	R
D8909-03	Dead	0	0	0	0	0.00	-	R
D8909-04	0	0	0	0	0	0.00	-	R
D8909-15	0	0	0	0	0	0.00	-	R
D8911-04	0	0	0	0	0	0.00	-	R
D8911-06	0	0	0	0	0	0.00	-	R
D8913-02	0	0	0	0	0	0.00	-	R
D8913-03	0	0	Dead	0	0	0.00	-	R
D8913-21	0	0	0	0	0	0.00	-	R
D8913-22	0	0	0	0	0	0.00	-	R
D8913-38	0	0	0	0	0	0.00	-	R
Q8918-04	0	0	0	0	0	0.00	-	R
R8914-01	0	0	0	0	0	0.00	-	R
R8914-02	0	0	0	0	0	0.00	-	R
R8914-04	0	0	0	0	0	0.00	-	R
R8915-01	0	0	0	0	0	0.00	-	R
R8915-05	0	0	0	0	0	0.00	-	R
R8915-06	0	0	0	0	0	0.00	-	R
R8916-02	Dead	Dead	Dead	Dead	-	-	-	-
R8916-04	0	0	0	0	0	0.00	-	R
R8916-05	Dead	0	0	0	0	0.00	-	R
R8916-07	7	1	3	4	4	2.50	0.65	S
R8916-08	0	0	0	0	0	0.00	-	R
R8916-09	0	0	5	0	1	2.50	1.12	S
R8916-11	0	0	0	0	0	0.00	-	R
R8916-12	0	0	0	0	0	0.00	-	R
R8916-14	0	0	0	0	0	0.00	-	R
R8916-15	0	0	0	0	0	0.00	-	R
R8916-16	0	0	0	0	0	0.00	-	R
R8916-17	0	0	0	0	0	0.00	-	R
R8916-18	4	2	0	0	2	1.92	0.78	S
R8916-19	0	7	3	0	3	3.32	1.05	S
R8916-20	0	0	0	0	0	0.00	-	R
R8916-21	0	0	0	0	0	0.00	-	R
R8916-22	0	0	0	0	0	0.00	-	R
R8916-23	0	0	0	0	0	0.00	-	R
R8916-24	0	0	0	0	0	0.00	-	R
R8916-25	0	0	0	0	0	0.00	-	R
R8916-26	0	0	0	0	0	0.00	-	R
R8916-27	0	0	0	0	0	0.00	-	R

Addendum 2. *(cont.)*

Genotype	Gall numbers				Average gall number	STD DEV	SE	Resistance ranking
	Rep 1	Rep 2	Rep 3	Rep 4				
R8916-29	0	0	0	0	0	0.00	-	R
R8916-30	0	0	0	0	0	0.00	-	R
R8916-31	0	0	0	0	0	0.00	-	R
R8916-32	0	0	0	0	0	0.00	-	R
R8917-02	0	0	0	0	0	0.00	-	R
R8917-04	0	0	0	0	0	0.00	-	R
R8917-06	0	0	0	0	0	0.00	-	R
R8917-07	0	0	0	0	0	0.00	-	R
R8917-08	1	0	0	2	1	0.96	0.55	S
R8917-09	12	0	0	0	3	6.00	1.73	S
R8918-05	0	0	0	0	0	0.00	-	R
R8918-09	0	0	0	0	0	0.00	-	R
R8918-10	0	0	0	0	0	0.00	-	R
R8918-11	0	0	0	0	0	0.00	-	R
R8918-13	0	0	0	0	0	0.00	-	R
R8919-01	Dead	Dead	Dead	Dead	-	-	-	-
R8919-02	0	0	0	0	0	0.00	-	R
R8919-03	0	0	0	0	0	0.00	-	R
O39-16	0	0	0	0	0	0.00	-	R
St. George	73	69	165	188	124	61.65	2.77	S

Chapter 5

Research results

Evaluating the inheritance of *Xiphinema index* resistance derived from three grapevine populations with *Vitis arizonica* backgrounds

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Chapter 5. Evaluating the inheritance of *Xiphinema index* resistance derived from three grapevine populations with *Vitis arizonica* backgrounds

5.1 Introduction

Vitis vinifera L. is one of the most important horticultural crops in the world and is considered the predominant grapevine species used in commercial grape production. This species is used in the table, wine and raisin grape industries because of its excellent fruit quality and its ability to adapt to a wide range of climatic conditions (Alleweldt & Possingham, 1988). However, *V. vinifera* is susceptible to a wide range of pests and diseases. This is particularly true for their roots, which are susceptible to attack by plant-parasitic nematodes such as the root-knot nematode (*Meloidogyne* spp.), lesion nematode (*Pratylenchus vulnus*), citrus nematode (*Tylenchulus semipenetrans*), and the vector of grapevine fanleaf virus – the dagger nematode (*Xiphinema index*) (Nicholas *et al.,* 2007).

In most grape growing regions of the world, grape cultivars are grafted onto rootstocks to provide resistance to grape phylloxera (*Daktulosphaira vitifoliae*), nematodes and other soil related factors. Research has shown that *Vitis* species vary in tolerance to the parasitic nematode, *X. index* (Malan & Meyer, 1993). The rootstock Ramsey, often incorrectly known as Salt Creek, is widely used in the South African table grape industry, and is moderately resistant to *X. index*, but it induces excessive vigour in scions, which can lead to fruit quality issues related to insufficient light exposure (Pongrácz, 1983).

Strong resistance to this nematode was found in *Muscadinia rotundifolia* Small (Walker & Jin, 2000) as well as *V. arizonica* Englm. (Kunde *et al.,* 1968). *Xiphinema index* also vectors grapevine fanleaf virus (GFLV) (Hewitt *et al.,* 1958), which is a very serious virus disease worldwide. Olmo created the rootstock, O39-16 (*V. vinifera x M. rotundifolia*), which was released by Lider and Goheen (Walker *et al*., 1991), and was tested extensively in field trials where fanleaf degeneration was prevalent (Walker *et al.,* 1994a; Walker *et al.,* 1994b). O39-16 is the only rootstock recommended in the United States for GFLV-infected sites (Christensen *et al.,* 2003).

In this study, *X. index* resistance derived from *V. arizonica* was tested by performing three crosses and studying the inheritance of *X. index* resistance in these populations under greenhouse conditions. The crosses used the R8916-series originating from b40-14, a pure form of *V. arizonica* collected near Chihuahua, Mexico by H.P. Olmo in 1961, as a source of resistance against *X. index.* The b40-14 genotype displays staminate flower phenotypes, and also has resistance to Pierce's disease (Krivanek *et al.,* 2005) in addition to *X. index* resistance (Jin, 1997). Previous work found that *X. index* resistance in b40-14 is controlled by a single homozygous locus (Walker & Jin, 2000), suggesting that all progeny from crosses with b40-14 should be resistant to *X. index.* Apart from the R8916-series used as parent in the crosses, 161-49C, a commercial rootstock (*V. berlandieri* Planch. x *V. riparia* Michx.) with moderate susceptible to *X. index* feeding (Pongrácz, 1983) was also used. The controls used in this study were *V. rupestris* Scheele cv. St. George, also known as Rupestris du Lot and 039-16. The former is resistant to phylloxera (Galet, 1998), but is highly sensitive to *X. index* feeding (Pongrácz, 1983) and therefore a good choice to use as a control plant for *X. index* susceptibility, whereas 039-16 with its exceptional resistance to *X. index* feeding (McKenry *et al.,* 2001; McKenry *et al.,* 2004) was used as a control plant to indicate resistance. Three crosses and their progeny (0701, 0704 and 0705) will be discussed in terms of their

resistance or susceptibility against *X. index*, as well as characteristics of the inheritance of the traits. In this study possible transgressive segregation was observed for all three populations.

5.2 Materials and Methods

5.2.1 Plant material and crosses

A series of crosses were made in May 2007 to establish populations to study the inheritance of *X. index* resistance originating from *V. arizonica*. These populations and their backgrounds are summarized in Table 5.1.

Table 5.1 Crosses made during the 2007 growing season (R = resistant and S = susceptible) and the controls used in the study.

Genotype	Female Parent	Male Parent	Objective	References
0701	R8916-07 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14)	R8916-32 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14)	F1 siblings crossed to confirm homozygous resistant b40-14 with 3:1 (R:S) segregation expected	Jin, 1997
0704	161-49C (*V. berlandieri* x *V. riparia*)	b40-14 (*V. arizonica*)	Cross with commercial rootstock to further confirm homozygous resistant b40-14 with no segregation expected	Pongrácz, 1983; Wheeler, 2006
0705	161-49C (*V. berlandieri* x *V. riparia*)	R8916-22 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14)	Establish mapping population with 1:1 (R:S) segregation	Pongrácz, 1983; Jin, 1997
O39-16	*V. vinifera* Almeria	*M. rotundifolia*	Resistant control	Christensen *et al.*, 2003
St. George	*V. rupestris*	*V. rupestris*	Susceptible control	Christensen *et al.*, 2003

No emasculation was needed for the crosses since the female parents are pistillate and male parents are staminate. The crosses were harvested in the fall of 2007 and the berries were removed from the rachis and pedicels. The berries were crushed in plastic bags and then poured onto different sized mesh screens to separate the seeds from the pulp and skins. The floating seeds were separated from the rest by placing them in water, after which the viable (sinking) seeds were air-dried and then placed in cold storage (4°C) for three months. After cold stratification in peat moss, the seeds were germinated and grown under greenhouse conditions. In total, 296 plants from the three populations were transferred to a field location at University of California, Davis in 2008. After two growing seasons all plants were visually scored to determine their flower types.

Herbaceous cuttings were collected from the 0701 and 0704 populations to create sets of four replicates for each genotype. These cuttings were treated with Wood's Rooting Compound (Earth Science Production Corp., Wilsonville, OR), which contains Indole-3-butyric acid (IBA) and Naphthalene Acetic acid (NAA). All the cuttings were inserted into small cellulose sponges prior to propagation. The cuttings were then placed on a mist propagation bed with intermittent mist and 30°C bottom heat. After two weeks these cuttings developed sufficient roots to be transplanted into 1,300 cm^3 plastic pots. The susceptible St. George and resistant O39-16 control plants and the population's parents were included in this evaluation of *X. index* feeding resistance.

For the 0705 population both hardwood and herbaceous cuttings were collected. Hardwood cuttings were collected during the winter for all the progeny and stored in a cold room

(4°C) for four weeks. Plant material was cut into two-node segments with the bottom bud removed. The plant material was treated with the same rooting hormone and placed in callusing boxes (30 x 30 x 50 cm). The cuttings were covered with a moist perlite/vermiculite mix (ratio = 3:1) and kept in a 27°C room with a constant relative humidity of 98% for three weeks to promote root growth. The cuttings were then potted into small cardboard sleeves and transferred to a mist bed with bottom heat in the greenhouse for two weeks, and the surviving cuttings were planted in plastic pots to establish roots and shoots over a period of two to four weeks. The genotypes that did not survive this process were propagated from herbaceous cuttings as described previously. The parents as well as b40-14, St. George and O39-16 were included as controls in both groups.

5.2.2 DNA extraction and genotype verification

Young leaf and shoot tissue from populations 0701, 0704 and 0705 were used for DNA extraction. A modified CTAB (cetyltrimethylammonium bromide) was used to extract the DNA from plant tissue as described by Lodhi *et al.,* (1994). Approximately 0.5 g of leaf and shoot tissue were placed in Bioreba grinding bags and frozen until needed. Five mL extraction buffer containing ß-mercaptoethanol (0.5% v/v) was pipetted into each bag, and samples were ground with a Homes 6 mechanical homogenizer (Bioreba, Longmont, CO). Two mL of each homogenized sample was pipetted into a 2 mL Eppendorff tube and centrifuged at 8500 rpm for five minutes. The supernatant was discarded without disturbing the pellet. The pellet was resuspended in 0.8 mL high salt lysis buffer and 0.2 mL 5% sarcocyl solution were added after the samples were incubated at 65°C for 45 minutes. Extraneous material was denatured by adding 0.8 mL chloroform:isoamyl alcohol (21:1). The DNA was then precipitated with 90 µL 3 M sodium acetate and 900 µL isopropanol where after the DNA pellet was washed with 70% ethanol and resuspended in 100 µL TE buffer.

Eight simple sequence repeat (SSR) markers were used to screen the progeny for the elimination of off-types that did not represent the parental genetic profile (Tables 5.2 and 5.3). The VMC and VrZag markers are described in the NCBI databases (UniSTS) and can be found at http://www.ncbi.nlm.nih.gov/. The CTG markers are described in the UC Davis *Vitis*-EST database (http://cgf.ucdavis.edu/).

Table 5.2 Marker sources and references used to screen the populations for off-types.

Marker	Source	Reference
VMC2c3 VMC3b7.2 VMC2f10 VMC2h10	Vitis Microsatellite Consortium	Agrogene SA (Moissy Cramayel, France), now Eurofins (http://eurofins.com/)
VrZag47 VrZag64	University of Agriculture, Vienna, Austria	Sefc *et al.* (1999)
CTG1010193 CTG1009382	University of California Davis, USA	http://www.cgf.ucdavis.edu/

Table 5.3 Primer sequences and amplicon sizes used to screen the populations for off-types.

Marker	Amplicon size (bp)	Forward sequence	Reverse sequence
VMC2c3	193	TGCAATCCCATTATTATCTCTT	AATATTTGTAGAATGGTGCTTTT
VMC3b7.2	105	TGTCTTTGATACCCAATCGAACT	ATTCCTCCTGGTTTTGAAACTCT
VMC2f10	101	AGATTCTTCTGATGGTGTTGGG	ATCAGAGCTCCTCTTTCCTTCC
VMC2h10	132	TTCACTTTCCTCAGTTTCTCGG	TGCCACCTACACTGTGAGATTC
VrZag47	185	GGTCTGAATACATCCGTAAGTATAT	ACGGTGTGCTCTCATTGTCATTGAC
VrZag64	176	TATGAAAGAAACCCAACGCGGCACG	TGCAATGTGGTCAGCCTTTGATGGG
CTG1010193	286	GCAAAAAGCCACAAGCAAAT	TCCTTTGGCCAGACCTACAC
CTG1009382	168	GCCATTGCATTTCAGTTGAG	GCAAATGAGCAATGCAAGAA

5.2.3 Preparation of *Xiphinema index*-infested soil inoculum

The progeny from the crosses and the relavent controls were inoculated by transplanting them into *X. index*-infested soil for resistance/susceptibility screening purposes. This soil was collected from near Oakville, Napa County, California, where previous sampling had found 300 to 500 adult nematodes per liter of soil. Soil was collected at and near these sampling sites and returned to the University of California, Davis in large coolers. Upon return a soil mix was constructed by mixing one part of the infested clay:loam soil with two parts of a coarse sand/crushed lava rock (3:1 mix). The soil mix for the 0705 population consisted of one part soil collected from the same highly infested site, and one part of the same combination of coarse sand and crushed lava rock. The fill sand and lava rock mixes were steam-sterilized before use. These soil combinations were gently and thoroughly mixed to ensure even nematode distribution. Soil samples were taken randomly after mixing and nematodes were extracted to evaluate the uniformity of the mixture.

After the screening trials were concluded, soil samples were taken from the control and parent plants in the 0705 population. The soil from the 0701 study was mixed and five random soil samples were taken for nematode extraction. The same was done for the 0704 study. The soil volume used for each sample in the three populations was 1,300 cm^2. Nematodes were extracted by using a combination of Cobb's sieving and Baermann funnel techniques (Agrios, 1997; Shurtleff & Averre III, 2000). The Baermann funnels used for nematode extraction were 11 cm in diameter and made of glass. Natural rubber tubes with metal clamps were attached to the spouts. Metal wire screens were placed on top of the funnels, covered with a single Kimwipe tissue (Kimberly-Clark, Neenah, WI). Soil samples were mixed with water and placed on the counter for 15-20 seconds for heavier particles to settle, with the adult nematodes still in suspension. The suspension containing the nematodes was poured through a 100-mesh sieve to trap adult nematodes. The sieve was gently rinsed from the back to collect nematodes in a 500 mL glass beaker. This process was repeated twice with the same soil. The runoff containing the nematodes was then poured into the Baermann funnel, and the nematodes were left to migrate through the Kimwipe and collect in the base of the funnel. After 48 hours the clamps were released and the nematodes were collected and counted using a light microscope.

5.2.4 Experimental design for infections

All three populations were potted into *X. index*-infested soil and placed on benches in the greenhouse in a completely randomized design. Colour coded labels were used to identify genotypes, parents and controls. Greenhouse controls were set for a temperature range of between 24 and 28°C. During the summer months the greenhouse was white washed to moderate high temperatures, and lights were added during the winter to extend daylight hours.

The 0701 and 0704 populations were watered using an automated drip irrigation system, whereas the 0705 population was hand watered as needed. Plants were kept moist on the surface to prevent drying out, but care was taken to avoid over-watering, which damages grape roots and can wash nematodes out of the pots.

After four weeks of exposure to *X. index* feeding, some of the susceptible St. George plants were unpotted and inspected for feeding damage. Based on the monitoring of the degree of feeding damage observed, the total exposure for the 0701 and 0704 populations, parents and remaining controls were four weeks, whereas the 0705 population and its controls were kept in *X. index*-infested soil for six weeks based on responses of the St. George controls in this test block.

5.2.5 Evaluation of *Xiphinema index* resistance and susceptibility

After four to six weeks of growth in the *X. index* infested soil, all plants from the various populations were taken out of their pots and soil and debris were rinsed from the roots. Root systems were inspected and galls were counted; when needed a 10X illuminated magnifying glass was used. If one or no galls were detected the plant was considered resistant. If two or more galls were counted on any one of the replicates, the plant was considered susceptible. If the number of galls counted on the 0701 and 0704 plants exceeded 50, counting stopped. Galls were counted up to 200 per root system for the 0705 population. Chi-square tests were performed to test goodness of fit to the expected segregation ratio for each population.

After the galls on the 0705 population were counted, the roots were cut from the stem and placed into a brown paper bag. These bags were labelled and stacked in a heated drying room for two weeks. Root weights (g) were then determined by using a bench-top scale for only the 0705 population.

5.3 Results

5.3.1 Genotype verification

Before any greenhouse testing was initiated on the 0701, 0704 and 0705 populations, the DNA of all genotypes was screened to eliminate off-types. One hundred and seventy five genotypes from the 0701 population were tested, but only 56 were true-to-type (Table 5.4). Fifty genotypes were tested from the 0704 population, and only five were off-types. Three additional genotypes in this population died during the initial seedling stage, which left 42 genotypes for *X. index* screening trials. Of the 200 seedlings tested in the 0705 population, 164 were true-to-type and five genotypes died during the seedling phase. Only the true-to-type and viable genotypes were transferred to the field at the University of California, Davis. Four replicates of each genotype in each progeny were generated either by hardwood or herbaceous cuttings to perform subsequent tests.

Table 5.4 Results of SSR-marker testing for true-to-type verification in the 0701, 0704 and 0705 populations.

Population	Genotypes tested	True-to-type genotypes	Off-types	Non-viable genotypes
0701	175	56	108	11
0704	50	42	5	3
0705	200	164	31	5

5.3.2 Distribution of root galls in the progenies, parents and controls

0701

A total of 56 genotypes were tested in the 0701 population and 37 had one or no galls on the roots after four weeks of exposure to *X. index* feeding. Nineteen genotypes had more than two galls with a range of between 2 and 47 (Figure 5.1). Forty-eight of the genotypes had relatively even gall numbers across their four replicates. During the *X. index* screening period one replicate of the 0701-015 genotype died, two of the replicates did not have galls, but the remaining replicate had 20 galls and the genotype was considered to be susceptible. Six susceptible genotypes had galls on only some of the replicates: 0701-008, 0701-014, 0701-027, 0701-066, 0701-116 and 0701-161 (Addendum 1). The pistillate parent of the population, R8916-07, had galls on three of the four replicates with an average of 7.25 galls, and 13 of the progeny had higher gall numbers than this parent. The staminate parent, R8916-32, and the resistant control, O39-16, did not allow any galling on any of the replicates. The susceptible control, St. George, had consistent and high numbers of galls across all four replicates with an average of 50. Analysis of variance found a significant difference between the means of the 0701 population with $p<0.0001$ (Table 5.5).

Table 5.5 Analysis of variance for the 0701 population.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	55	19482.958	354.236	5.6594	<0.0001
Error	167	10452.917	62.592		
C. Total	222	29935.874			

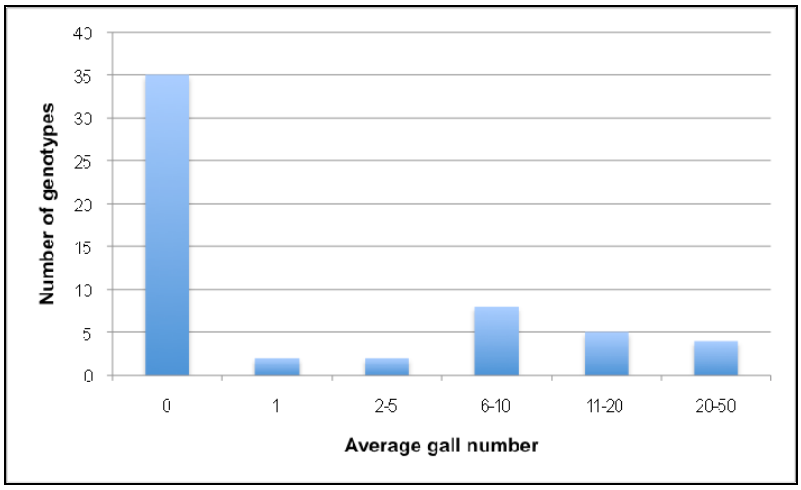

Figure 5.1 The distribution of *X. index* feeding galls on the roots of genotypes in the 0701 population.

0704

Of the 42 genotypes tested in the 0704 population, 16 had galls after four weeks (Figure 5.2). The gall numbers were on average lower than for the 0701 population, except for St. George, which had an average of 37.75 galls across four replicates. Thirteen of the genotypes judged to be susceptible had one or more replicates with no galls. Three genotypes had one or more replicates that died during the study period. 0704-029 had only one surviving plant with a gall count of three (Addendum 2), and it was judged to be susceptible. The susceptible pistillate parent, 161-49C, had galls on all four replicates, although the numbers were fairly low with an

average of 6.0. Three susceptible genotypes had higher gall numbers than 161-49C, but these levels were not significantly different from 161-49C (p-value = 0.57). The resistant staminate parent, b40-14 had no galls on any of the four replicates. Both control plants behaved as expected with high gall numbers on all plants for St. George and no galls on plants of O39-16. Analysis of variance on the mean values for 0704 was highly significant (Table 5.6).

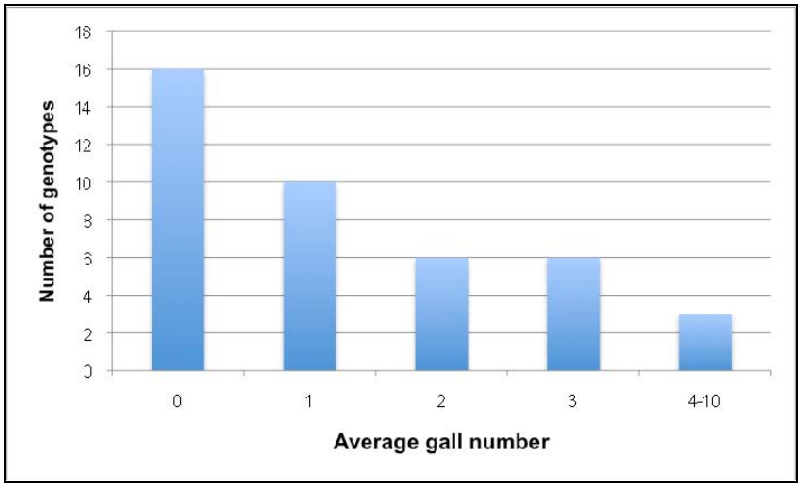

Figure 5.2 The distribution of *X. index* feeding galls on the roots of genotypes in the 0704 population.

Table 5.6 Analysis of variance for the 0704 population.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	41	796.0808	19.4166	6.4931	<0.0001
Error	121	361.8333	2.9904		
C. Total	162	1157.9141			

The 0701 and 0704 populations were screened at the same time and prior to screening, five random soil samples, 1,300 cm^2 each, from the nematode-infested inoculum were used for nematode extraction. The average adult nematode count for these samples was 47 (Table 5.7). After the four-week screening period, five random soil samples were taken again to establish an increase/decrease in nematode numbers. The average nematode count was 54. Analysis of variance found no significant difference between the nematode numbers before and after screening (p-value = 0.0572).

Table 5.7 The number of adult nematodes present in the soil inoculum before and after the four-week screening of the 0701 and 0704 populations.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average
Before screening	52	35	60	48	41	47
After screening	75	27	66	49	52	54

0705

Testing of the 0705 population was done in two parts due to the difficulty in propagating some of the progeny from woody cuttings. A second set of genotypes was propagated from herbaceous cuttings, and the data from both sets were combined. The results for the woody and herbaceous cuttings of the resistant and susceptible parents and controls were consistent after

six weeks of *X. index* exposure, except for *V. rupestris* cv. Wichita Refuge, which did not have plants surviving from woody cuttings (Addendum 4 and 5).

After six weeks of *X. index* exposure, 94 genotypes were judged to be susceptible with two or more galls and 70 genotypes were judged to be resistant with no or one gall on average (Figure 5.3). Three of the susceptible genotypes had galls on two or three out of the four replicates. Only one replicate of the 0705-178 genotype survived, but it had 25 galls and was susceptible. Fourteen of the susceptible genotypes had one replicate that died during the study and eight of the genotypes had two dead replicates (Addendum 3). The replicates of the controls and the parents from both sets of the study were evaluated for galls (Addendum 4 and 5) and their numbers were relatively consistent. The root damage was severe on the highly susceptible genotypes, which made it difficult to accurately count galls. O39-16, R8916-22 and b40-14 had no galls after six weeks, which is consistent with results obtained with another separate infection (reported on in Chapter 3). The susceptible St. George had high numbers of galls on all ten of its replicates. 161-49C was difficult to propagate and had only seven replicates, all of which had low gall numbers with an average of 6.83. This result was similar to the values for 161-49C found in the screening of the 0704 population (Addendum 2). A total of 88 susceptible genotypes had a higher average gall numbers than 161-49C. The susceptible grandparent, *V. rupestris* cv. Wichita Refuge had five replicates in the second part of the study, and although the replicate plants grew poorly, high gall numbers were seen on all roots.

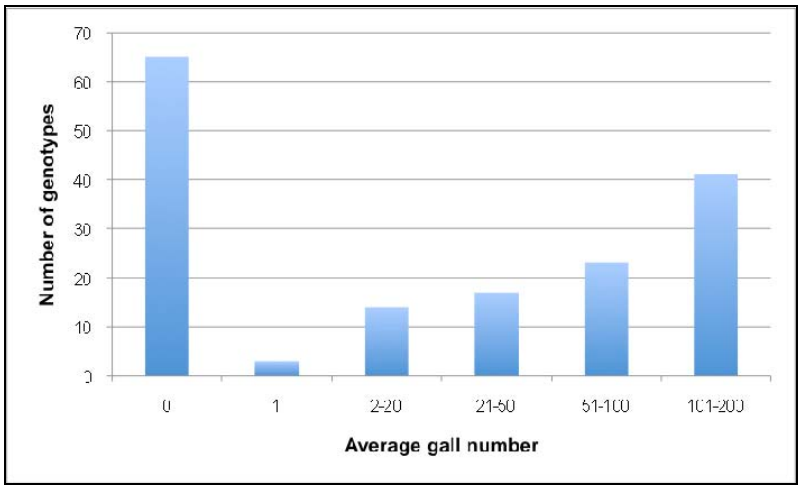

Figure 5.3 The distribution of *X. index* feeding galls on the roots of genotypes in the 0705 population.

Statistical analysis was also performed on the 0705 population to create resistant, susceptible and intermediate groupings of genotypes. Analysis of variance found significant differences between the mean values for the 0705 population (Table 5.8). Tukey-Kramer analysis using JMPSas (Version 8.0) was used on the entire population to separate the data into classes, but relatively high standard deviations prevented effective categorization of the data. JMPSas was also used to perform a multivariate cluster analysis (Addendum 6). The average gall numbers for each genotype clustered into 13 groups (Figure 5.4), ranging from highly resistant (0-1 galls) to highly susceptible (196-200 galls).

Table 5.8 Analysis of variance for the 0705 population.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	163	3652099.1	22405.5	76.1122	<0.0001
Error	459	135118.1	294.4		
C. Total	622	3787217.1			

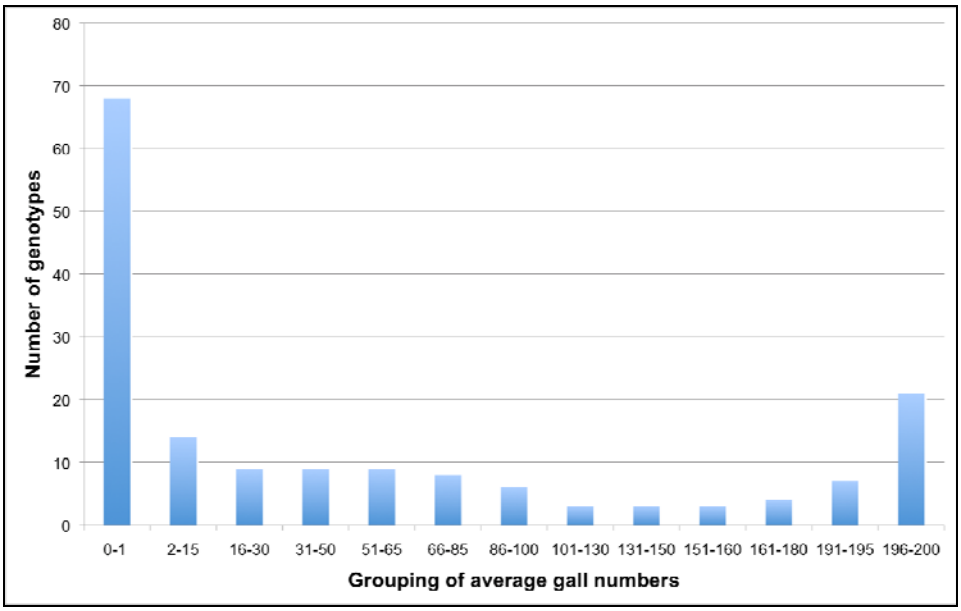

Figure 5.4 The grouping of average *X. index* feeding gall numbers for the 0705 population into 13 groups.

5.3.3 Segregation analysis

Chi-square tests were calculated to test the hypothesized inheritance models for each of the three populations. Table 5.9 shows a summary of the Chi-square results obtained. The 0701 population fit a 3:1 (R:S) segregation model suggesting a cross between two heterozygous resistant parents (Rr X Rr), with a p-value >0.10. The 0704 population fit a 1:1 (R:S) segregation model suggesting a cross between a heterozygous resistant parent by a homozygous susceptible parent (Rr X rr) with a p-value of >0.20, but did not fit the expected model of a RR x rr cross where all progeny should have been resistant. The 0705 population was expected to segregate 1:1 (R:S) based on a proposed model of a rr X Rr cross, which the data fit loosely, but the X^2-value of 3.52 was borderline significant at the 5% probability level. Nine genotypes had an average gall number below 10. When these genotypes were excluded from the analysis, the X^2-value changed to 1.86 with a p-value of >0.10. Marker assisted screening data determined a clear 1:1 segregation ratio with a X^2 value of 1.56 and a p-value of >0.20 (refer to Chapter 6 of this dissertation for data).

Table 5.9 Chi-square test results for the 0701, 0704 and 0705 populations.

Population	Observed		Total	Expected		X^2(df)*	*p*
	Resistant	Susceptible		Resistant	Susceptible		
0701	37	19	56	42	14	2.38 (1)	>0.10
0704	26	16	42	21	21	1.52 (1)	>0.20
0705	70	94	164	82	82	3.52 (1)	>0.05

* Degrees of freedom

5.3.4 Root quality and nematode distribution for 0705

Figure 5.5 shows the difference in the quality of roots and the degree of galling between resistant and susceptible genotypes in the 0705 population. These pictures were taken after the population was exposed to *X. index* feeding for six weeks, and illustrate the degree of galling across the four replicates of a highly susceptible and a resistant genotype. There was no correlation between the average root weights and average gall numbers (R^2 = 0.05866) across all genotypes (Figure 5.6). The susceptible genotypes were then considered to determine if a correlation between the average root weights and gall numbers existed, but none was found (R^2 = 0.193491).

Figure 5.5 Genotypes in the 0705 population without *X. index* feeding damage (left) and with *X. index* feeding damage (right) after 6 weeks of exposure.

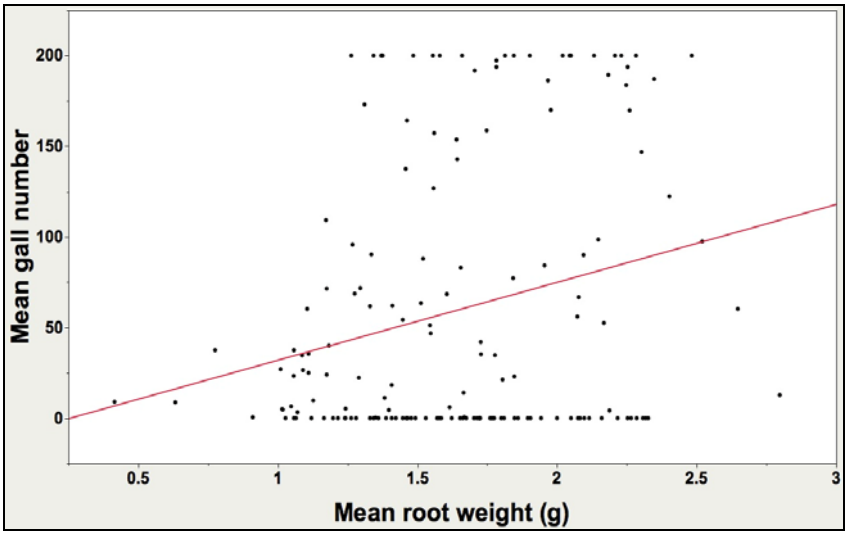

Figure 5.6 Regression analysis to determine if a correlation existed between root weight and *X. index* feeding gall number in the 0705 population after exposure to *X. index* feeding (R^2 = 0.05866). The mean gall numbers and root weights were used for all resistant and susceptible genotypes.

Before the 0705 population was inoculated with *X. index*-infested soil, nematodes were extracted from 10 random 1,300 cm^3 soil samples to quantify their distribution. After the study was completed, 10 soil samples were taken from the pots of the parents and controls. The number of nematodes present in the soil before and after the screening was consistent for both the first and second part of the 0705 study, except for O39-16 where the average number of nematodes was low in the first round. Table 5.10 presents the nematode numbers before and after the six-week screening for both rounds. Analysis of variance found a significant difference

between nematode counts before and after screening for R8916-22 (p-value = 0.0237) in the first round and for b40-14 (p-value = 0.0015) in the second round.

Table 5.10 Nematode numbers before and after the six-week screening of the 0705 population.

Sample	1	2	3	4	5	6	7	8	9	10	Average
Nematode numbers before inoculation in the first part of the study											
Soil	41	35	45	52	49	37	60	52	43	50	46
Nematode numbers after inoculation in the first part of the study											
161-49C	29	28	25	50	48	49	34	-	-	-	38
b40-14	30	23	25	75	32	31	64	24	24	39	37
R8916-22	36	66	47	48	108	43	82	85	60	76	65
O39-16	70	53	50	42	25	40	63	68	62	50	52
St. George	48	82	39	40	42	65	48	45	49	116	57
Nematode numbers before inoculation in the second part of the study											
Soil	32	41	55	57	51	68	45	48	43	60	50
Nematode numbers after inoculation in the second part of the study											
161-49C	44	43	51	25	60	29	35	38	-	-	41
b40-14	30	24	41	40	26	37	53	20	24	33	33
R8916-22	54	49	47	21	50	35	68	27	39	21	41
O39-16	70	53	50	42	25	40	63	68	62	50	52
St. George	48	82	39	40	12	65	48	45	49	116	54

5.4 Discussion

Previous work done on other *V. arizonica* accessions found this species to be an excellent source for resistance against *X. index* feeding (Kunde *et al.,* 1968). Roncoroni (2004) found genetic markers linked to the *X. index* resistance in *V. arizonica* (9621 population) and concluded that the resistance locus segregated as a single dominant gene. Xu *et al.* (2008) performed a quantitative trait loci (QTL) analysis on the same population and placed a major QTL near the SSR marker, VMC5a10 on chromosome 19. Xu *et al*. (2008) used a method for *X. index* inoculation based on extraction of nematodes from infested soils and then dispensing 100 nematodes in four evenly placed holes in the soil for each pot. Gall numbers averaged 20.4 ± 16.4 galls per replicate, which was lower than the average gall number (101.2) obtained in the current study of the 0705 population. A different inoculation method used in the 0701, 0704 and 0705 populations was chosen to save time and promote a more even *X. index* distribution in the soil. This method also retains the juveniles and eggs, which are lost during traditional extraction methods. This method proved to be better to separate degrees of resistance and susceptibility between genotypes and demonstrated that inoculum pressure was consistent throughout the studies. Care was taken to ensure that the environmental conditions were consistent, and the plants were genetically tested to ensure that the correct genotypes were studied. In all three populations, the controls, O39-16 and St. George behaved as expected and were always completely resistant and highly susceptible, respectively.

In this study the 0701 and 0704 populations were created to confirm the homozygous resistant trait of b40-14 and to aid in the data interpretation of the 0705 population, whereas the 0705 population was mainly created as a mapping population for a pure *V. arizonica* line.

Segregating populations of plants can exhibit phenotypes that range more widely than the parents (DeVicente & Tanksley, 1993). This is called transgressive segregation and is a phenomenon specific to segregating hybrid generations where a fraction of individuals exceed parental phenotypic values. Transgressive segregation facilitates the successful establishment

of hybrid lines, which is usually indirect (Rieseberg *et al.,* 1999), and appeared to be associated with inbreeding and is more common in plants than animals (Bell & Travis, 2005).

0701 population

The 0701 population was created by crossing two F_1 siblings from the R8916 population, created by crossing the susceptible *V. rupestris* Wichita Refuge x the resistant *V. arizonica* b40-14. b40-14 was thought to be homozygous resistant based on the testing results from progeny in the R8916 population (Jin, 1997). Thus, this population was created to confirm that b40-14 is homozygous resistant and that the R8916 progeny are heterozygous (Rr) resistant. Before any greenhouse studies were performed, the entire population was screened with SSR markers to eliminate off-types. Evaluation of this data found that the 0701 population had many more off-types (32%) than either the 0704 (84%) or 0705 (82%) populations. These off-types were likely produced by the windy conditions that existed during the pollination of the pistillate parent and the close proximity of numerous other staminate genotypes.

The greenhouse study confirmed the hypothesized model for 3:1 (R:S) segregation for two heterozygous (Rr) parents. However, both parents, R8916-07 and R8916-32 were expected to be highly resistant, yet the pistillate parent R8916-07 allowed limited *X. index* feeding damage on three of the four replicates (Addendum 1). In a previous study (Jin, 1997), all the genotypes from the R8916-series, including R8916-07 were tested and all were judged resistant to *X. index* feeding. Due to this inconsistency, further investigation was warranted (see Chapter 3 of this dissertation). One of the possible contributing factors to the observed inconsistency in resistance scoring of the parents is the specific *X. index* inoculation method used. Jin (1997) used the same method of *X. index* inoculation as Xu *et al.* (2008), which was different from the method used here. The *X. index* inoculation procedure used in the study presented here likely resulted in more intense feeding pressure due to the presence of nematode eggs and juvenile stages retained in the soil mix. This more severe screening may have resulted in the susceptibility observed in R8916-07. The same soil-based inoculum was used with all of the studied populations and with the R8916-series and the control cultivars. In addition, the grandparents, Wichita Refuge and b40-14, were highly susceptible and completely resistant, confirming the accuracy of the testing. Care was also taken to ensure that R8916-07 was indeed the correct genotype by double-checking the location where herbaceous cuttings were collected, and by testing and comparing DNA collected from these greenhouse studies.

Thirteen of the susceptible genotypes had a higher average gall number than the female parent, but they were in all cases lower or the same as the susceptible grandparent, Wichita Refuge. These differences however, were not statistically significant. If the female parent, R8916-07 was considered susceptible (rr instead of Rr), then the possibility of transgressive segregation exists, but the data does not support this (Figure 5.1 and Addendum 1).

Several studies done on grapevine have noted transgressive segregation. In a study done on cane characteristics, a proportion of the hybrids showed transgressive segregation with respect to cane length, thickness, number of nodes, number of branches and internodal length (Uppal & Sharma, 1977). In a study done on the inheritance of gall formation relative to phylloxera resistance, transgressive segregation was also observed (Roush *et al.,* 2007). Transgressive segregation was found in a study done on root-knot nematode resistance in cotton (Wang *et al.,* 2008). However, no specific reference could be found for transgressive segregation for nematode resistance within grapevine cultivars. Several explanations have been offered for transgressive segregation, but no concrete evidence on how this phenomenon is controlled has been published.

The susceptibility of R8916-07 cannot be explained by environmental factors, since the consistency of the greenhouse conditions were supported by the consistent results of the parents and control plants under the completely randomized design during the study. The impact of a higher *X. index* inoculum pressure might have caused the higher gall numbers on the 13 susceptible genotypes, but cannot explain the consistently lower gall numbers for the female parent. Transgressive segregation therefore remains a possible explanation and should be confirmed in the future by testing the 0701 population and the parents under higher inoculum pressure with longer exposure to *X. index* feeding. Crosses to explore this phenomenon can also be considered in future, i.e. if R8916-07 is crossed with b40-14, all progeny should be resistant whether R8916-07 is heterozygous resistant (Rr) or susceptible (rr). Unfortunately no phenotypic distinction would be possible for this cross. R8916-07 could however be crossed with R8916-22, the male parent in the 0705 population that is heterozygous resistant to *X. index* feeding. The suggested outcome for this cross should fit a 3:1 segregation model and if confirmed, susceptibility could be ruled out.

0704 population

To further confirm the homozygous resistant nature of b40-14, this genotype was crossed with a susceptible commercial rootstock. 161-49C, a *V. berlandieri* x *V. riparia* hybrid, was chosen because it is pistillate and assumed to be susceptible (Pongrácz, 1983). The progeny of this cross should not segregate, i.e. all should be heterozygous resistant to *X. index*, but instead a 1:1 (R:S) segregation ratio was observed. However, gall numbers were fairly low on the susceptible genotypes and in most cases only some roots of the replicates within these genotypes were galled. It is possible that the soil inoculum was not well enough mixed to ensure even distribution of the nematodes, resulting in uneven exposure of the replicates to *X. index* feeding. However, after the study was completed, nematodes were extracted from randomly chosen soil inoculum and consistent levels of nematode distribution were found (Table 5.5). 161-49C might have contributed to the 1:1 (R:S) segregation by having a gene or allele for resistance that is interacting with the resistance from b40-14. The segregation pattern and the lower than expected gall numbers, compared to the levels of galling seen on St. George and Wichita Refuge, therefore favors the hypothesis of two genes interacting. To establish if this is true, crosses need to be made within the 0704 population and some of the 0704 genotypes can be backcrossed with the parents. The following 0704 females can be considered for these crosses: 0704-028 and 0704-039 (susceptible); and 0704-030 and 0704-044, (resistant). Males to be considered are 0704-013 and 0704-046 for the resistant genotypes, with 0704-020 and 0704-033 as susceptible (Addendum 2). All these genotypes are established in the UC Davis vineyards.

The 161-49C pistillate parent was described as susceptible to *X. index* feeding (Pongrácz, 1983), but the low gall numbers observed in this study indicate that this rootstock may have some resistance. This conclusion was confirmed in the 0705 population, where the 161-49C female parent was also used and low gall numbers were also observed.

Of the susceptible genotypes, only three showed slightly higher, but not statistically significant, average gall counts than the susceptible parent. The higher counts can be explained by possible gall counting errors, or possible higher nematode numbers in the pots of these genotypes. As for the 0701 population, it is recommended to test the entire population under high *X. index* pressure for an extended time period to successfully separate the resistant and susceptible genotypes.

0705 population

The 0705 population was created to establish a mapping population where the *X. index* resistance of *V. arizonica* b40-14 could be studied. The cross was made between the susceptible commercial rootstock, 161-49C and the resistant *V. rupestris* Wichita Refuge x *V. arizonica* b40-14 selection, R8916-22. The 0705 plant replicates, controls and parents were subjected to *X. index* feeding for six weeks instead of the four-week testing duration used for the 0701 and 0704 populations. This extended testing resulted in higher gall numbers for the susceptible 0705 genotypes and the susceptible *V. rupestris* controls, but the resistant controls and the 161-49C parent had similar values as found with testing of the 0701 and 0704 populations (Addendum 3, 4 and 5). The decision to extend the test period to six weeks resulted from observations of galling on St. George roots after four weeks of exposure. After six weeks of exposure the St. George plants had an average gall number of 164.5 and 176.2 for the first and second part of the 0705 study respectively. The study done on *X. index* exposure time also indicated that six weeks of testing helped separate moderately resistant from moderately susceptible genotypes. These tests also found that eight weeks of exposure may leave many of the root systems too badly galled, leading to decay and inability to distinguish galls (see Chapter 3 for these results).

The 0705 population was expected to segregate 1:1 (R:S) but X^2 analysis did not fully support this model (Table 5.5). The X^2 value of 3.52 was borderline significant at the 5% level (*p*-value <0.10 but >0.05). It is possible that a small percentage of the genotypes might be off-types due to errors made during the collections of the green and hardwood cuttings. If the nine genotypes with an average gall number below 10 are excluded from the segregation analysis, the expected 1:1 segregation ratio is observed, but since the female parent, 161-49C, had low gall numbers in general, these nine genotypes were not excluded from the analysis. These genotypes should be tested again to determine whether they were useful recombinants for positioning potential resistance loci, or mistakes.

Accurate determination of gall numbers on the highly susceptible genotypes was difficult due to severe root damage, possibly as a result of the longer exposure to *X. index* feeding. The average number of galls in the susceptible genotypes was reproducible with low standard deviations: only five genotypes had mean galling values with relatively high standard deviations (Addendum 3). This data supports the even distribution of *X. index* in the soil mix, a condition that was further supported by the evaluation of *X. index* numbers before and after the test period. The uniform and effective nature of the screen was also evidenced by consistent results of the control and parent plants. The resistant parents, b40-14 and R8916-22 showed no galls, and the female grandparent, Wichita Refuge showed consistently high gall numbers. Galling on the female parent, 161-49C, was consistent with the results obtained in analysis of the 0704 population (Addendum 2, 4 and 5). Adult nematode counts in the soil before and after the study were fairly consistent, except for the first round tests with O39-16. In this test soil from the O39-16 pots had very few adult *X. index*, but after the second round of tests the nematode numbers were consistent with the other parents and controls. Since juvenile stages and egg masses are lost during the extraction process, the effectiveness of the *X. index* pressure in the soil cannot be fully determined. Uneven and higher numbers of *X. index* in the soil might have contributed to average gall numbers with high standard deviations, particularly with the susceptible genotypes. The additional two weeks of feeding exposure with the 0705 compared to 0701 and 0704 populations may also have played a role in the increased susceptibility of the population. The genotypes with average galling values with high standard deviations, and those with limited numbers of replicates should be repeated.

Eighty-eight of the 94 susceptible genotypes had higher average gall numbers than 161-49C, and in many cases significantly higher, suggesting that transgressive segregation occurred (Table 5.5). Although the 0705 study was conducted in two parts, the environmental and watering conditions were kept the same and resulted in consistent results for the parents and controls in the two-part study. Thus, greenhouse conditions should not have had an effect on the higher gall numbers for the susceptible genotypes. 161-49C was used as the female parent in both this population and the 0704 inheritance study. It seems possible that 161-49C is contributing to the high gall numbers in the susceptible genotypes perhaps with a second gene for susceptibility interacting with the *X. index* resistance gene. The 0705 population shows more concrete evidence that two major genes might be interacting where a susceptible gene is interfering with the alleles from the resistance gene from b40-14. It is possible that similar effects would have been seen in the 0704 population study if the feeding time had been similarly extended. A seedling population from a selfed 161-49C would be useful to study this effect but it cannot be made because 161-49C has pistillate flowers. The results obtained with the 0704 and 0705 crosses have provided evidence that 161-49C is somewhat resistant to *X. index* feeding, and it may accentuate the level of susceptibility when it is used as a parent. Crosses between some of the 0705 genotypes in different combinations of resistant and susceptible genotypes, and also backcrosses to the parents and grandparents might help to find the origin of the proposed susceptibility gene in 161-49C (Table 5.11).

Table 5.11 Proposed genotypes from the 0705 population to be used in backcrosses to the parents and grandparents.

Genotype	Origin	Flower Sex	Resistant or Susceptible
0705-020	This study	Male	Susceptible
0705-108	This study	Male	Resistant
0705-101	This study	Female	Susceptible
0705-005	This study	Female	Resistant
161-49C	*V. berlandieri x V. riparia*	Female	Some resistance
R8916-22	*V. rupestris* Wichita Refuge x *V. arizonica* b40-14	Male	Resistant
b40-14	*V. arizonica*	Male	Resistant
Wichita Refuge	*V. rupestris*	Female	Susceptible
O39-16	*V. vinifera x M. rotundifolia*	Sterile	Resistant (control)
St. George	*V. rupestris*	Male	Highly susceptible (control)

Crosses in the Table 5.8 can be combined in all possible combinations within the 0705 genotypes, as well as with the parents and grandparents. For instance, if 161-49C is considered rr (somewhat resistant), then a cross with 0705-020 (susceptible = rr) will leave all progeny susceptible. If 161-49C is crossed with 0705-108 (resistant = Rr), then half of the progeny will be resistant and the other half will be susceptible to *X. index* feeding. Also, if R8916-22 is crossed with 0705-005, then only a quarter of the progeny will be susceptible (Table 5.12). O39-16 and St. George proved to be good resistant and susceptible control plants respectively, but unfortunately O39-16 cannot be used as a parent since it is sterile due to the *Vitis x Muscadinia* (2n=39) parentage.

In conclusion: This study confirmed that a single dominant gene might control the inheritance of *X. index* resistance derived from *V. arizonica.* This is true for the *X. index* inheritance study conducted with the 0701 population. However, the inheritance of resistance might be more complex if a proposed second gene from a different source (161-49C) contributes to the resistance.

Table 5.12 Suggested segregation ratio's for the proposed crosses between some 0705 progeny and the parents and grandparents.

Female parent	Male parent	Segregation ratio
0705-101 (rr)	0705-020 (rr)	All susceptible
	0705-148 (Rr)	1:1 R:S
	R8916-22 (Rr)	3:1 R:S
	b40-14 (RR)	All resistant
0705-005 (Rr)	0705-020	1:1 R:S
	0705-148	3:1 R:S
	R8916-22	3:1 R:S
	b40-14	All resistant
161-49C (rr)	0705-020	All susceptible
	0705-148	1:1 R:S
	R8916-22 (0705 population)	1:1 R:S
	b40-14 (0704 population)	All resistant (not in this study)
Wichita Refuge (rr)	0705-020	All susceptible
	0705-148	1:1 R:S
	R8916-22	1:1 R:S
	b40-14 (R8916-series)	All resistant (Not in this study)

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Addendum 1 to Chapter 5. *Xiphinema index* galling numbers for genotypes in the 0701 (R8916-07 x R8916-32) population after a greenhouse resistance screen using an infested soil-based inoculation. The population parents and resistant and susceptible controls were included.

Genotype	Gall numbers				Average gall number	STD DEV	SE	Resistance ranking	Male or female
	Rep 1	Rep 2	Rep 3	Rep 4					
0701-007	0	0	0	0	0	0.000	-	R	M
0701-008	0	4	14	7	6	5.909	1.182	S	M
0701-010	9	14	20	20	16	5.315	0.670	S	F
0701-014	3	16	0	11	8	7.326	1.337	S	-
0701-015	20	0	Dead	0	7	11.547	2.582	S	M
0701-025	10	5	8	18	10	5.560	0.868	S	M
0701-027	0	9	0	0	2	4.500	1.500	S	-
0701-030	0	0	0	0	0	0.000	-	R	F
0701-035	15	14	3	4	9	6.377	1.063	S	F
0701-037	1	0	0	0	0	0.500	0.500	R	M
0701-042	20	20	50	100	48	37.749	2.739	S	M
0701-045	0	0	0	0	0	0.000	-	R	F
0701-046	0	0	0	0	0	0.000	-	R	M
0701-048	0	0	0	0	0	0.000	-	R	M
0701-050	2	20	3	3	7	8.679	1.640	S	F
0701-051	0	0	0	0	0	0.000	-	R	-
0701-059	0	0	0	0	0	0.000	-	R	M
0701-066	0	3	4	7	4	2.887	0.772	S	-
0701-078	0	0	0	0	0	0.000	-	R	F
0701-082	0	0	0	0	0	0.000	-	R	F
0701-084	0	0	0	0	0	0.000	-	R	-
0701-085	0	0	0	0	0	0.000	-	R	F
0701-086	0	0	0	0	0	0.000	-	R	F
0701-087	0	0	0	0	0	0.000	-	R	F
0701-093	0	0	0	0	0	0.000	-	R	F
0701-097	0	0	0	0	0	0.000	-	R	-
0701-099	0	0	0	0	0	0.000	-	R	M
0701-101	50	20	20	20	28	15.000	1.430	S	-
0701-105	0	0	0	0	0	0.000	-	R	M
0701-106	0	0	0	0	0	0.000	-	R	M
0701-109	0	0	0	0	0	0.000	-	R	-
0701-111	0	0	0	0	0	0.000	-	R	-
0701-116	26	0	4	8	10	11.475	1.861	S	F
0701-122	0	0	0	0	0	0.000	-	R	M
0701-123	0	0	0	0	0	0.000	-	R	M
0701-130	0	0	0	0	0	0.000	-	R	F
0701-131	0	0	0	0	0	0.000	-	R	-
0701-133	17	20	12	20	17	3.775	0.454	S	F
0701-135	0	1	0	1	1	0.577	-	R	-
0701-136	18	50	20	33	30	14.751	1.341	S	F

Addendum 1. *(cont.)*

Genotype	Gall numbers				Average gall number	STD DEV	SE	Resistance ranking	Male or female
	Rep 1	Rep 2	Rep 3	Rep 4					
0701-139	20	7	60	15	26	23.615	2.338	S	-
0701-140	0	0	0	0	0	0.000	-	R	-
0701-142	0	0	0	0	0	0.000	-	R	-
0701-145	0	0	0	0	0	0.000	-	R	F
0701-148	6	20	10	6	11	6.608	1.020	S	-
0701-149	0	0	0	0	0	0.000	-	R	M
0701-150	50	10	3	11	19	21.299	2.476	S	-
0701-152	0	0	0	0	0	0.000	-	R	F
0701-155	0	0	0	0	0	0.000	-	R	M
0701-161	0	8	4	0	3	3.830	1.106	S	M
0701-163	0	0	0	0	0	0.000	-	R	M
0701-165	0	0	0	0	0	0.000	-	R	M
0701-169	0	0	0	0	0	0.000	-	R	-
0701-171	4	11	7	14	9	4.397	0.733	S	M
0701-172	0	0	0	0	0	0.000	-	R	M
0701-175	0	0	0	0	0	0.000	-	R	F
O39-16	0	0	0	0	0	0.000	-	R	Sterile
St George	50	50	50	50	50	0.000	0.000	S	M
Wichita Refuge	20	20	50	100	48	37.749	2.739	S	F
b40-14	0	0	0	0	0	0.000	-	R	M
R8916-07	10	11	8	0	7	4.992	0.927	S	F
R8916-32	0	0	0	0	0	0.000	-	R	M

Addendum 2 to Chapter 5. *Xiphinema index* galling numbers for genotypes in the 0704 (161-49C x b40-14) population after a greenhouse resistance screen using an infested soil-based inoculation. The population parents and resistant and susceptible controls were included.

Genotype	Gall numbers				Average gall number	Std. Dev	SE	Resistance ranking	Male or female
	Rep 1	Rep 2	Rep 3	Rep 4					
0704-001	0	0	0	0	0	0.000	-	R	-
0704-003	0	0	0	0	0	0.000	-	R	-
0704-004	2	3	0	0	1	1.500	0.671	S	F
0704-005	2	0	3	3	2	1.414	0.500	S	F
0704-006	1	0	1	3	1	1.258	0.563	S	-
0704-009	0	0	0	0	0	0.000	-	R	M
0704-010	5	0	0	7	3	3.559	1.027	S	-
0704-011	0	0	0	0	0	0.000	-	R	-
0704-012	0	0	1	0	0	0.500	0.500	R	-
0704-013	0	0	0	0	0	0.000	-	R	M
0704-014	0	0	1	0	0	0.500	0.500	R	M
0704-015	0	1	1	0	1	1.000	0.707	R	M
0704-016	0	0	0	0	0	0.000	-	R	-
0704-018	5	0	0	0	1	2.500	1.118	S	F
0704-019	0	0	0	0	0	0.000	-	R	M
0704-020	10	6	15	3	9	5.196	0.891	S	M
0704-021	0	0	3	0	1	1.500	0.866	S	M
0704-023	0	0	0	0	0	0.000	-	R	-
0704-024	0	0	0	0	0	0.000	-	R	-
0704-025	1	1	Dead	0	1	0.577	0.408	R	-
0704-026	0	3	0	0	1	1.500	0.866	S	-
0704-027	0	0	0	0	0	0.000	-	R	M
0704-028	3	4	2	2	3	0.957	0.289	S	F
0704-029	Dead	Dead	3	Dead	3	-	-	S	F
0704-030	1	0	0	0	0	0.500	0.500	R	F
0704-031	1	0	4	3	2	1.826	0.645	S	M
0704-032	0	3	4	5	3	2.160	0.624	S	M
0704-033	2	4	6	0	3	2.582	0.745	S	M
0704-034	Dead	10	0	10	7	5.774	1.291	S	-
0704-035	4	0	0	0	1	2.000	1.000	S	M
0704-036	0	0	0	1	0	0.500	0.500	R	F
0704-037	0	0	0	0	0	0.000	-	R	-
0704-039	2	2	4	4	3	1.155	0.333	S	F
0704-040	0	0	1	1	1	1.000	0.707	R	M
0704-042	10	10	10	10	10	0.000	0.000	S	-
0704-043	0	8	0	0	2	4.000	1.414	S	F
0704-044	0	0	0	0	0	0.000	-	R	F
0704-045	0	0	0	0	0	0.000	-	R	F
0704-046	0	0	0	0	0	0.000	-	R	M
0704-048	0	0	0	0	0	0.000	-	R	M

Addendum 2. *(cont.)*

Genotype	Gall numbers				Average gall number	Std. Dev	SE	Resistance ranking	Male or female
	Rep 1	Rep 2	Rep 3	Rep 4					
0704-049	4	0	3	1	2	1.826	0.645	S	-
0704-050	0	0	0	0	0	0.000	-	R	-
O39-16	-	0	0	0	0	0.000	-	R	Sterile
St George	28	65	33	25	38	18.464	1.503	S	M
161-49C	10	1	4	9	6	4.243	0.866	S	F
b40-14	0	0	0	0	0	0.000	-	R	M

Addendum 3 to Chapter 5. *Xiphinema index* galling numbers for genotypes in the 0705 (161-49C x R8916-22) population after a greenhouse resistance screen using an infested soil-based inoculation. The population parents and resistant and susceptible controls were included.

Genotype	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Mean gall #	Std Dev	SE	Mean root #	Std Dev	SE	R/S	F/M
	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)								
0705-002	8	0.51	10	0.32	Dead	Dead	Dead	Dead	9	1.41	0.33	0.42	0.13	0.15	S	M
0705-003	10	0.66	5	0.74	10	0.45	10	0.68	9	2.50	0.42	0.63	0.13	0.08	S	F
0705-004	0	1.1	0	0.97	0	1.13	0	1.28	0	0.00	-	1.12	0.13	0.06	R	M
0705-005	0	1.30	0	1.25	0	1.09	0	1.32	0	0.00	-	1.24	0.10	0.05	R	F
0705-007	0	1.03	0	0.98	0	1.01	0	1.09	0	0.00	-	1.03	0.05	0.02	R	M
0705-008	0	2.11	0	1.99	Dead	Dead	Dead	Dead	0	0.00	-	2.05	0.08	0.04	R	M
0705-009	200	2.24	200	2.32	168	1.99	Dead	Dead	189	18.48	0.78	2.18	0.17	0.07	S	F
0705-010	200	1.06	167	1.57	200	2.08	200	2.11	192	16.50	0.60	1.71	0.50	0.19	S	F
0705-011	0	1.68	0	1.61	0	2.09	0	1.68	0	0.00	-	1.77	0.22	0.08	R	M
0705-012	200	1.33	167	1.20	188	1.34	137	1.37	173	27.60	1.05	1.31	0.08	0.03	S	F
0705-013	6	1.26	4	1.92	9	1.67	5	1.61	6	2.16	0.44	1.62	0.27	0.11	S	M
0705-015	0	1.45	0	2.28	0	1.39	0	1.69	0	0.00	-	1.70	0.41	0.16	R	M
0705-016	87	1.84	69	2.01	93	1.44	83	1.33	83	10.20	0.56	1.66	0.32	0.13	S	F
0705-017	89	1.24	34	1.17	57	1.12	61	0.89	60	22.56	1.45	1.11	0.15	0.07	S	F
0705-018	12	1.10	36	1.00	22	1.07	Dead	Dead	23	12.06	1.44	1.06	0.05	0.03	S	M
0705-020	200	1.31	200	1.50	200	1.37	200	1.30	200	0.00	0.00	1.37	0.09	0.04	S	M
0705-021	0	1.67	0	2.04	0	2.05	0	1.68	0	0.00	-	1.86	0.21	0.08	R	F
0705-022	200	2.15	200	2.39	200	2.15	Dead	Dead	200	0.00	0.00	2.23	0.14	0.05	S	F
0705-023	0	1.95	0	1.61	0	1.82	0	2.20	0	0.00	-	1.90	0.25	0.09	R	M
0705-024	0	2.11	0	1.78	0	1.55	0	1.60	0	0.00	-	1.76	0.25	0.10	R	M
0705-025	0	1.86	0	1.99	0	2.15	0	2.30	0	0.00	-	2.08	0.19	0.07	R	F
0705-026	178	2.20	167	1.75	200	1.95	135	2.01	170	27.07	1.04	1.98	0.19	0.07	S	M
0705-027	156	2.50	200	2.07	192	2.40	131	2.07	170	32.15	1.23	2.26	0.22	0.07	S	F
0705-028	0	2.05	0	2.16	0	2.44	Dead	Dead	0	0.00	-	2.22	0.20	0.08	R	F
0705-029	200	1.60	178	1.93	200	1.84	197	1.76	194	10.59	0.38	1.78	0.14	0.05	S	M
0705-030	17	1.94	26	2.18	11	1.73	31	1.37	21	8.96	0.97	1.81	0.34	0.13	S	F
0705-031	125	1.77	110	1.26	137	1.51	178	1.29	138	29.17	1.24	1.46	0.24	0.10	S	F
0705-033	159	2.10	200	2.24	200	1.78	186	1.75	186	19.33	0.71	1.97	0.24	0.09	S	M

Addendum 3. *(cont.)*

Genotype	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Mean gall #	Std Dev	SE	Mean root #	Std Dev	SE	R/S	F/M
	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)								
0705-034	60	0.94	93	1.26	71	1.29	62	1.21	72	15.11	0.89	1.18	0.16	0.07	S	M
0705-035	0	1.69	0	1.76	0	1.44	0	1.45	0	0.00	-	1.59	0.16	0.07	R	F
0705-036	200	1.63	200	2.22	200	2.14	200	1.39	200	0.00	0.00	1.85	0.40	0.15	S	F
0705-038	0	0.94	0	1.02	0	1.13	0	0.98	0	0.00	-	1.02	0.08	0.04	R	F
0705-039	200	1.80	200	1.82	200	1.82	Dead	Dead	200	0.00	0.00	1.81	0.01	0.00	S	M
0705-040	200	2.58	200	2.17	200	2.01	200	2.07	200	0.00	0.00	2.21	0.26	0.09	S	F
0705-041	45	2.01	72	1.96	57	2.14	93	2.20	67	20.69	1.27	2.08	0.11	0.04	S	F
0705-042	0	1.56	0	1.68	0	1.74	0	1.91	0	0.00	-	1.72	0.15	0.06	R	-
0705-043	32	0.92	Dead	Dead	43	0.63	Dead	Dead	38	7.78	0.90	0.78	0.21	0.16	S	-
0705-044	78	1.51	56	1.45	52	1.40	68	1.69	64	11.82	0.74	1.51	0.13	0.05	S	M
0705-045	0	1.86	0	1.65	0	1.74	0	1.83	0	0.00	-	1.77	0.09	0.04	R	F
0705-046	0	1.73	0	1.96	0	1.37	0	2.04	0	0.00	-	1.78	0.30	0.11	R	F
0705-047	0	1.27	0	1.3	0	1.51	Dead	Dead	0	0.00	-	1.36	0.13	0.06	R	F
0705-048	0	1.46	0	1.21	0	1.26	0	1.12	0	0.00	-	1.26	0.14	0.06	R	F
0705-049	0	1.36	0	1.34	0	1.65	0	1.44	0	0.00	-	1.45	0.14	0.06	R	-
0705-050	70	1.44	45	1.61	26	1.51	76	1.23	54	23.13	1.57	1.45	0.16	0.07	S	M
0705-051	200	2.11	200	2.07	200	2.02	200	2.33	200	0.00	0.00	2.13	0.14	0.05	S	M
0705-052	0	1.48	0	2.15	0	2.49	0	1.27	0	0.00	-	1.85	0.57	0.21	R	F
0705-053	0	2.11	0	1.94	0	2.50	0	2.20	0	0.00	-	2.19	0.23	0.08	R	F
0705-054	0	1.55	0	2.38	0	1.84	0	1.67	0	0.00	-	1.86	0.37	0.13	R	F
0705-055	0	1.74	0	1.63	0	1.34	0	1.41	0	0.00	-	1.53	0.19	0.08	R	F
0705-056	155	1.32	116	3.06	129	2.35	89	2.88	122	27.46	1.24	2.40	0.78	0.25	S	F
0705-057	0	1.25	57	1.80	200	1.52	17	1.85	69	90.86	5.49	1.61	0.28	0.11	S	M
0705-058	25	2.95	62	2.38	103	2.40	51	2.86	60	32.45	2.09	2.65	0.30	0.09	S	F
0705-060	42	1.54	37	1.49	56	1.45	52	1.71	47	8.77	0.64	1.55	0.11	0.05	S	F
0705-061	0	1.41	Dead	Dead	Dead	Dead	0	1.25	0	0.00	-	1.33	0.11	0.07	R	F
0705-062	147	1.59	117	1.77	126	1.44	181	1.77	143	28.43	1.19	1.64	0.16	0.06	S	M
0705-063	0	2.00	0	1.87	0	1.79	0	1.96	0	0.00	-	1.91	0.09	0.03	R	F
0705-064	200	1.43	200	1.30	200	2.23	200	1.36	200	0.00	0.00	1.58	0.44	0.17	S	-

Addendum 3. *(cont.)*

Genotype	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Mean gall #	Std Dev	SE	Mean root #	Std Dev	SE	R/S	F/M
	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)								
0705-065	0	2.01	0	2.44	0	2.50	Dead	Dead	0	0.00	-	2.32	0.27	0.10	R	M
0705-066	56	1.26	79	1.30	92	1.19	60	1.43	72	16.82	0.99	1.30	0.10	0.04	S	F
0705-067	0	2.49	0	1.97	0	2.13	0	2.42	0	0.00	-	2.25	0.24	0.08	R	M
0705-068	200	1.87	200	1.90	200	1.83	200	2.01	200	0.00	0.00	1.90	0.08	0.03	S	M
0705-069	0	1.31	0	1.26	0	1.55	0	1.57	0	0.00	-	1.42	0.16	0.07	R	M
0705-071	55	1.43	34	1.47	68	1.30	91	1.44	62	23.87	1.52	1.41	0.08	0.03	S	F
0705-072	27	1.01	Dead	Dead	44	1.21	Dead	Dead	36	12.02	1.43	1.11	0.14	0.09	S	M
0705-073	0	1.14	0	1.52	0	1.21	0	1.50	0	0.00	-	1.34	0.20	0.08	R	F
0705-074	0	1.00	1	0.82	Dead	Dead	Dead	Dead	1	0.71	0.71	0.91	0.13	0.09	R	F
0705-075	0	1.70	0	1.75	0	1.78	0	1.65	0	0.00	-	1.72	0.06	0.02	R	M
0705-077	0	1.64	0	1.35	0	1.26	0	2.60	0	0.00	-	1.71	0.61	0.23	R	F
0705-079	98	1.18	57	1.34	86	1.60	120	1.22	90	26.26	1.38	1.34	0.19	0.08	S	M
0705-080	200	1.77	200	1.54	200	1.60	200	1.73	200	0.00	0.00	1.66	0.11	0.04	S	M
0705-081	200	1.29	200	1.14	200	1.67	200	1.39	200	0.00	0.00	1.37	0.22	0.10	S	M
0705-082	7	1.05	10	1.10	12	1.23	Dead	Dead	10	2.52	0.47	1.13	0.09	0.05	S	F
0705-083	0	2.11	0	1.28	0	1.64	0	1.58	0	0.00	-	1.65	0.34	0.13	R	M
0705-085	0	1.29	0	1.48	0	1.27	0	1.51	0	0.00	-	1.39	0.13	0.05	R	F
0705-087	200	2.76	159	1.11	176	2.33	200	2.79	184	20.01	0.74	2.25	0.79	0.26	S	F
0705-088	17	1.30	23	1.07	25	1.13	31	1.20	24	5.77	0.59	1.18	0.10	0.05	S	M
0705-089	196	1.68	127	1.70	167	1.62	125	1.56	154	34.17	1.38	1.64	0.06	0.02	S	F
0705-090	200	1.25	200	2.07	200	1.52	200	1.38	200	0.00	0.00	1.56	0.36	0.14	S	F
0705-091	0	1.39	0	2.05	0	1.31	0	1.11	0	0.00	-	1.47	0.41	0.17	R	F
0705-092	200	2.97	200	2.34	200	2.42	200	2.20	200	0.00	0.00	2.48	0.34	0.11	S	F
0705-093	0	1.52	0	1.54	0	1.45	0	1.12	0	0.00	-	1.41	0.20	0.08	R	F
0705-094	0	1.79	0	1.83	0	1.64	0	1.55	0	0.00	-	1.70	0.13	0.05	R	M
0705-095	156	1.35	107	1.15	74	1.19	100	1.00	109	34.25	1.64	1.17	0.14	0.07	S	M
0705-096	0	2.04	0	1.90	0	2.59	0	1.93	0	0.00	-	2.12	0.32	0.11	R	F
0705-098	13	1.94	38	1.84	18	1.76	Dead	Dead	23	13.23	1.59	1.85	0.09	0.04	S	M
0705-099	200	2.23	200	1.80	200	1.99	200	2.16	200	0.00	0.00	2.05	0.19	0.07	S	M

Addendum 3. *(cont.)*

Genotype	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Mean gall #	Std Dev	SE	Mean root #	Std Dev	SE	R/S	F/M
	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)								
0705-100	167	1.34	189	2.34	131	1.37	142	1.19	157	25.98	1.04	1.56	0.53	0.21	S	F
0705-101	200	1.75	200	2.33	200	1.66	200	2.46	200	0.00	0.00	2.05	0.40	0.14	S	F
0705-102	115	2.16	193	2.49	79	2.22	200	2.34	147	59.37	2.45	2.30	0.15	0.05	S	M
0705-103	0	2.26	0	2.54	0	2.06	0	2.37	0	0.00	-	2.31	0.20	0.07	R	F
0705-105	0	2.41	0	2.48	0	1.90	0	2.35	0	0.00	-	2.29	0.26	0.09	R	F
0705-108	0	2.45	0	2.92	0	2.20	0	1.49	0	0.00	-	2.27	0.60	0.20	R	M
0705-109	0	2.08	0	2.64	0	2.26	Dead	Dead	0	0.00	-	2.33	0.29	0.11	R	F
0705-111	55	1.88	22	1.66	49	1.64	Dead	Dead	42	17.58	1.57	1.73	0.13	0.06	S	M
0705-113	0	1.70	0	1.97	0	1.30	0	1.68	0	0.00	-	1.66	0.28	0.11	R	M
0705-115	200	1.83	200	2.03	189	1.51	200	1.76	197	5.50	0.20	1.78	0.21	0.08	S	M
0705-119	26	1.58	42	1.49	65	1.54	72	1.57	51	21.16	1.48	1.55	0.04	0.02	S	M
0705-121	0	1.62	0	2.22	0	1.26	0	1.75	0	0.00	-	1.71	0.40	0.15	R	M
0705-122	137	1.63	103	1.24	65	1.08	78	1.12	96	31.70	1.62	1.27	0.25	0.11	S	M
0705-123	0	1.77	0	1.40	0	1.70	0	1.83	0	0.00	-	1.68	0.19	0.07	R	M
0705-125	7	0.98	23	0.97	47	1.00	31	1.09	27	16.65	1.60	1.01	0.05	0.03	S	-
0705-126	0	2.14	0	2.37	0	1.88	0	2.00	0	0.00	-	2.10	0.21	0.07	R	F
0705-127	52	1.39	87	1.31	73	1.27	63	1.13	69	14.89	0.90	1.28	0.11	0.05	S	F
0705-128	0	1.85	0	2.81	0	2.07	0	2.54	0	0.00	-	2.32	0.44	0.14	R	F
0705-129	0	2.05	0	2.03	0	1.88	0	2.37	0	0.00	-	2.08	0.21	0.07	R	M
0705-130	0	1.36	0	2.02	0	1.58	0	1.94	0	0.00	-	1.73	0.31	0.12	R	M
0705-132	200	2.42	200	2.21	200	2.28	200	2.22	200	0.00	0.00	2.28	0.10	0.03	S	M
0705-133	0	1.42	0	1.65	0	1.17	0	1.60	0	0.00	-	1.46	0.22	0.09	R	M
0705-135	12	1.42	Dead	Dead	16	1.91	Dead	Dead	14	2.83	0.53	1.67	0.35	0.19	S	F
0705-136	4	1.23	2	1.40	7	1.29	8	1.05	5	2.75	0.60	1.24	0.15	0.07	S	M
0705-137	96	1.76	67	1.27	85	1.65	104	1.40	88	16.02	0.85	1.52	0.22	0.09	S	F
0705-138	0	1.08	0	1.25	0	1.34	0	1.19	0	0.00	-	1.22	0.11	0.05	R	F
0705-139	200	1.20	200	1.38	200	1.42	200	1.05	200	0.00	0.00	1.26	0.17	0.08	S	M
0705-140	85	1.85	76	1.76	37	1.92	26	2.76	56	28.88	1.93	2.07	0.46	0.16	S	M
0705-141	9	2.56	15	2.81	6	2.83	21	2.99	13	6.65	0.93	2.80	0.18	0.05	S	F

Addendum 3. *(cont.)*

Genotype	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Mean gall #	Std Dev	SE	Mean root #	Std Dev	SE	R/S	F/M
	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)								
0705-142	0	1.06	9	1.05	14	1.08	3	1.00	7	6.24	1.22	1.05	0.03	0.02	S	F
0705-143	25	0.99	43	1.15	15	1.06	23	1.16	27	11.82	1.15	1.09	0.08	0.04	S	M
0705-144	57	1.04	41	1.23	66	1.54	83	1.51	62	17.54	1.12	1.33	0.24	0.10	S	M
0705-145	175	1.81	200	1.76	200	2.68	200	2.76	194	12.50	0.45	2.25	0.54	0.18	S	M
0705-146	89	2.37	126	2.72	99	2.69	76	2.30	98	21.21	1.07	2.52	0.22	0.07	S	M
0705-147	0	1.34	0	1.36	0	1.15	0	0.94	0	0.00	-	1.20	0.20	0.09	R	M
0705-149	Dead	Dead	1	0.98	9	1.05	Dead	Dead	5	5.66	1.79	1.02	0.05	0.03	S	M
0705-150	0	1.21	0	0.98	0	1.32	0	1.15	0	0.00	-	1.17	0.14	0.07	R	-
0705-151	0	1.40	0	1.41	0	1.54	0	1.44	0	0.00	-	1.45	0.06	0.03	R	F
0705-152	0	1.82	0	2.06	0	2.12	Dead	Dead	0	0.00	-	2.00	0.16	0.06	R	F
0705-153	200	2.34	200	2.29	200	1.57	200	1.88	200	0.00	0.00	2.02	0.36	0.13	S	M
0705-154	38	1.55	41	1.65	35	2.08	27	1.63	35	6.02	0.51	1.73	0.24	0.09	S	F
0705-155	0	1.26	0	1.5	0	1.76	0	1.45	0	0.00	-	1.49	0.21	0.08	R	-
0705-156	200	1.42	200	1.62	200	1.31	200	1.15	200	0.00	0.00	1.38	0.20	0.08	S	M
0705-157	47	1.09	16	1.06	54	1.06	33	1.02	38	16.78	1.37	1.06	0.03	0.01	S	M
0705-159	0	1.29	0	1.01	0	0.92	0	1.04	0	0.00	-	1.07	0.16	0.08	R	F
0705-160	0	1.63	0	1.59	0	1.49	0	1.57	0	0.00	-	1.57	0.06	0.02	R	-
0705-161	23	1.20	7	1.64	32	1.51	11	1.28	18	11.41	1.34	1.41	0.20	0.09	S	M
0705-163	36	1.17	21	1.23	54	1.19	49	1.14	40	14.76	1.17	1.18	0.04	0.02	S	F
0705-164	75	2.01	91	1.90	50	2.62	144	1.85	90	39.76	2.10	2.10	0.36	0.12	S	M
0705-165	172	1.90	144	1.73	200	1.72	119	1.64	159	35.00	1.39	1.75	0.11	0.04	S	F
0705-167	61	1.84	96	2.02	45	1.69	107	1.82	77	29.10	1.66	1.84	0.14	0.05	S	-
0705-169	0	2.18	0	1.58	0	1.58	0	1.90	0	0.00	-	1.81	0.29	0.11	R	F
0705-171	156	1.25	101	1.41	200	1.45	200	1.74	164	46.99	1.83	1.46	0.20	0.08	S	M
0705-173	0	1.20	0	1.04	0	0.92	0	1.07	0	0.00	-	1.06	0.11	0.06	R	M
0705-174	0	0.98	0	1.26	0	1.39	0	1.34	0	0.00	-	1.24	0.18	0.08	R	M
0705-175	200	1.21	200	1.62	200	1.15	200	1.39	200	0.00	0.00	1.34	0.21	0.09	S	M

Addendum 3. *(cont.)*

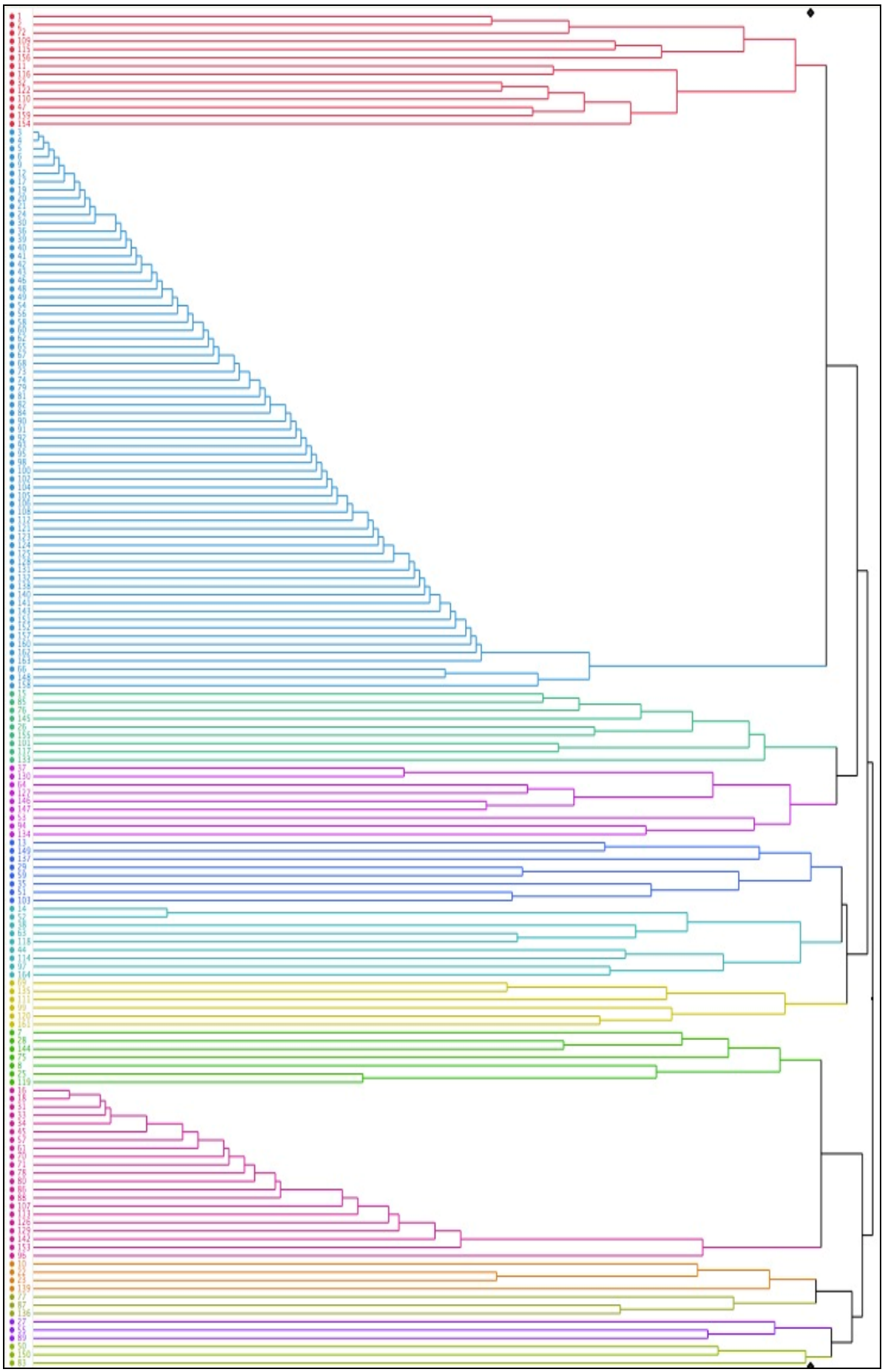
Genotype	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Mean gall #	Std Dev	SE	Mean root #	Std Dev	SE	R/S	F/M
	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)	Galls	Root wgt (g)								
0705-176	0	1.43	0	1.22	0	1.32	0	1.15	0	0.00	-	1.28	0.12	0.05	R	M
0705-177	167	2.31	200	3.14	200	2.29	181	1.65	187	16.06	0.59	2.35	0.61	0.20	S	M
0705-178	Dead	Dead	Dead	Dead	25	1.11	Dead	Dead	25	-	-	1.11	-	-	S	M
0705-179	42	1.15	29	1.10	Dead	Dead	33	1.01	35	6.66	0.65	1.09	0.07	0.04	S	M
0705-181	31	1.48	38	1.99	26	1.80	44	1.84	35	7.89	0.67	1.78	0.21	0.08	S	F
0705-182	1	1.45	0	1.61	0	1.17	1	2.44	1	0.58	0.41	1.67	0.55	0.21	R	M
0705-183	114	1.80	62	2.11	73	2.41	88	1.50	84	22.51	1.23	1.96	0.39	0.14	S	F
0705-184	98	1.45	27	1.39	200	2.04	182	1.35	127	79.99	3.55	1.56	0.32	0.13	S	M
0705-186	0	1.49	0	1.35	0	1.32	0	1.75	0	0.00	-	1.48	0.20	0.08	R	M
0705-188	0	1.62	0	1.48	0	1.74	0	1.47	0	0.00	-	1.58	0.13	0.05	R	-
0705-189	200	2.04	200	1.13	200	1.49	200	1.28	200	0.00	0.00	1.49	0.40	0.16	S	M
0705-190	3	0.99	Dead	Dead	1	1.11	6	1.11	3	2.52	0.80	1.07	0.07	0.04	S	M
0705-191	21	1.23	31	1.51	14	1.20	23	1.22	22	6.99	0.74	1.29	0.15	0.06	S	M
0705-192	13	1.68	7	1.06	15	1.37	10	1.42	11	3.50	0.52	1.38	0.25	0.11	S	M
0705-193	0	1.51	0	2.46	0	1.35	0	1.88	0	0.00	-	1.80	0.49	0.18	R	F
0705-194	1	1.19	0	1.22	0	1.83	0	1.16	0	0.50	0.50	1.35	0.32	0.14	R	M
0705-195	5	1.34	9	1.41	3	1.66	1	1.18	5	3.42	0.81	1.40	0.20	0.08	S	M
0705-196	0	1.98	0	2.16	0	2.19	0	2.31	0	0.00	-	2.16	0.14	0.05	R	M
0705-197	96	2.05	117	2.50	104	2.46	77	1.58	99	16.74	0.84	2.15	0.43	0.15	S	M
0705-198	0	1.42	0	1.34	0	1.41	0	2.32	0	0.00	-	1.62	0.47	0.18	R	M
0705-199	0	1.85	0	1.40	0	2.25	0	2.27	0	0.00	-	1.94	0.41	0.15	R	F
0705-200	46	1.86	71	2.38	31	2.21	62	2.22	53	17.67	1.22	2.17	0.22	0.07	S	F

Addendum 4 to Chapter 5. Phenotypic screening data for the parents and controls of the 0705 population during the 1st part of the study.

Genotype	Mean gall #	STD DEV	SE	Mean root weight (g)	STD DEV	SE	Resistant or Susceptible	Female or Male
b40-14	0	0.00	-	2.12	0.68	0.19	R	M
161-49C	8	2.71	0.39	1.05	0.18	0.07	S	F
R8916-22	0	0.00	-	1.83	0.38	0.12	R	F
St. George	165	29.50	0.94	2.52	0.45	0.12	S	M
O39-16	0	0.00	-	1.98	0.55	0.16	R	Sterile

Addendum 5 to Chapter 5. Phenotypic screening data for the parents and controls of the 0705 population during the 2nd part of the study.

Genotype	Mean gall #	STD DEV	SE	Mean root weight (g)	STD DEV	SE	Resistant or Susceptible	Female or Male
Wichita Refuge	120	61.00	2.27	2.14	0.65	0.18	S	F
b40-14	0	0.00	-	1.78	0.30	0.07	R	M
161-49C	7	3.76	0.59	1.46	0.29	0.10	S	F
R8916-22	0	0.00	-	2.50	0.23	0.05	R	F
St. George	176	30.78	0.73	2.23	0.39	0.08	S	M
O39-16	0	0.00	-	1.39	0.15	0.04	R	Sterile



Addendum 6. Cluster analysis to create a range of highly resistant to highly susceptible groups in the 0705 population. The different colour groups represent a cluster of a specific degree of resistance or susceptibility to *X. index* feeding. JMPSas (Version 8.0) to perform this multivariate cluster analysis.

Chapter 6

Research results

A framework genetic map for *Xiphinema index* resistance derived from *Vitis arizonica*

Part of this manuscript will be submitted for publication in
BMC Genetics

Chapter 6. A framework genetic map for *Xiphinema index* resistance derived from *Vitis arizonica*

6.1 Introduction

The dagger nematode, *Xiphinema index* is one of the most damaging grape pests. Since *X. index* was first identified in California (Thorne & Allen, 1950), its occurrence has been reported in most grape growing countries around the world (McKenry *et al.,* 2004; Leopold *et al.,* 2007). This nematode is of particular importance because of its ability to vector grapevine fanleaf virus (GFLV) (Hewitt *et al.,* 1958). Nematode feeding causes swollen club-like root tips that become stunted, decay and lead to vine decline. The virus disrupts fruit set and causes severe crop losses (Shurtleff & Averre III, 2000). Even without host plants, *X. index* can survive in the soil for long periods of time while still retaining GFLV (Demangeat *et al.,* 2005). The combined effect of *X. index* feeding and its association with GFLV may even kill grapevines (Nicholas *et al*., 2007). The use of fumigants and nematicides is not always effective, especially in California where the soils are deeper than the average penetration depth of these products (Lear *et al.,* 1981; Raski *et al.,* 1983). Due to the high toxicity levels of nematicides, and because they are unsafe for the environment and human health, their use is becoming highly restricted in the world's vineyards (Bouquet *et al.,* 2000). Resistance to the feeding of this particular nematode remains an important priority in grapevine rootstock breeding as a preventative measure (Jawhar *et al.,* 2006; Leopold *et al.,* 2007).

Several *Vitis* species, such as *V. arizonica* have been identified as resistant to *X. index* feeding (Kunde *et al.,* 1968; Coiro & Brown, 1984). *Muscadinia rotundifolia* has also been found to be resistant to *X. index*, but crossing it with *Vitis* species remains challenging due to the difference in chromosome numbers between the genera (Olmo, 1986). Work done by Kunde *et al.* (1968) on several *Vitis* species showed that *V. arizonica* is resistant to *X. index*. Other *Vitis* species tested include *V. candicans, V. rufotomentosa, V. smalliana* and *V. solonis*. It was later determined that the resistance in *V. arizonica* was inherited as a single dominant gene (Meredith *et al.,* 1982; Walker & Jin, 2000).

Several greenhouse-based screening techniques for *X. index* resistance have been described (Kunde *et al.,* 1968; Meredith *et al.,* 1982; Harris, 1983; McKenry & Anwar, 2006). Nematodes were extracted and collected from infested vineyard or greenhouse soils and used to assess *X. index* resistance. These studies were time consuming and root damage could only be rated 8-18 months after nematode inoculation. Large numbers of progeny are usually required. The lack of a reliable and rapid screening technique has always been a major limitation to the efficiency and success of breeding programs pursuing *X. index* resistance (Xu *et al.,* 2008).

Molecular markers have been used for some years to allow breeders to screen and select plants in the seedling phase and reduce the cost of maintaining large numbers of unwanted plants. Genetic markers are also very useful in the creation of genetic linkage maps and to identify and tag regions that are associated with disease resistance (Riaz *et al.,* 2006). In 1995 the first grapevine genetic map was published (Lodhi *et al.,* 1995). Early molecular maps for grapevine were based on dominant AFLP and RAPD markers (Lodhi *et al.,* 1995; Dalbó *et al.,* 2000), where the major focus of breeding populations and map development was based on disease resistance and phenological traits of grapevine. Since then genetic maps have been based on reproducible and highly polymorphic SSR (Simple Sequence Repeat) markers (Zhang *et al.,* 2009).

Genetic maps for grapevine have been developed for Pierce's disease resistance (Riaz *et al.,* 2006; Krivanek *et al.,* 2006), the detection of QTL's for seedlessness and berry weight (Doligez *et al.,* 2002; Striem *et al.,* 1996; Cabezas *et al.,* 2006; Mejía *et al.,* 2011) as well as berry size (Mejía *et al.,* 2007), the detection of QTL's affecting fungal disease resistance and leaf morphology (Welter *et al.,* 2007), downy mildew resistance (Merdinoglu *et al.,* 2003; Marguerit *et al.,* 2009; Bellin *et al.,* 2009), powdery mildew resistance (Pauquet *et al.,* 2001; Barker *et al.,* 2005; Hoffmann *et al.,* 2008; Riaz *et al.,* 2011), phylloxera resistance (Zhang *et al.,* 2009) and nutrient deficiencies (Mandl *et al.,* 2006). Genetic maps have also been created to exploit the qualities of interspecific grapevine crosses such as the rootstock cross, Ramsey (*V. champinii*) x Riparia Gloire (*V. riparia*) (Lowe & Walker, 2006), a *V. rupestris* x *V. arizonica* cross (Doucleff *et al.,* 2004) and a *V. vinifera* x *V. riparia* cross (Grando *et al.,* 2003). Recent maps include a physical contig map of the *V. vinifera* cultivar Pinot noir (Scalabrin *et al.,* 2010), and characterization and physical mapping of *XiR1*, a gene controlling resistance to *X. index* (Hwang *et al.,* 2010).

Previously, an AFLP-based genetic map was created to position *X. index* resistance (Doucleff *et. al*., 2004). The mapping population named 9621 was derived from a cross between D8909-15 (*V. rupestris* A. de Serres x *V. arizonica/girdiana* b42-26) and F8909-17 (*V. rupestris* A. de Serres x *V. arizonica/candicans* b43-17 (*V. arizonica*)). A moderately dense map of this population was created for the initial mapping of genes and QTL's for resistance to *X. index* and *Xylella fastidiosa* (the bacterial agent that causes Pierce's disease). The 9621 population was mapped with highly informative and co-dominant SSR markers further positioning and placing a major quantitative trait locus (QTL) for *X. index* resistance (*XiR1*) on chromosome 19 (Xu *et al.*, 2008). They found *XiR1* to be tightly linked to three markers, M4F3F, VMCNg3a10 and VMC5a10. However, the 9621 population was not derived from a pure form of *V. arizonica*.

In the study presented here, *X. index* resistance from b40-14, a pure form of *V. arizonica*, was genetically mapped in a population that used the susceptible commercial rootstock, 161-49 C was used as a female parent (Pongrácz, 1983). The aim of the study was to develop a better understanding of the inheritance of b40-14's resistance, and create a SSR based genetic map to enable the identification of genetic markers linked to this resistance.

6.2 Materials and Methods

6.2.1 Plant material

The 0705 population originated from a 2007 cross between 161-49C (*V. riparia* x *V. berlandieri)*, a commercial rootstock with susceptibility to *X. index* feeding, and R8916-22 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14), which is highly resistant to *X. index* feeding and derives its resistance from b40-14 – collected by. H.P. Olmo collected in 1961 from Chihuahua, Mexico (Riaz *et al*. 2007). Wichita Refuge is susceptible and b40-14 is homozygous resistant to *X. index* feeding (see Chapter 4). In the fall of 2007 the crosses were harvested and the berries were removed from the rachis and pedicels where after the berries were crushed in plastic bags and then poured onto different sized mesh screens to separate the seeds from the pulp and skins. The floating seeds were separated from the sinking (viable) seeds by placing them in water. The viable seeds were air-dried and then placed in cold storage (4°C) for three months. After cold stratification the seeds were germinated and grown under greenhouse conditions. In total, 195 plants from the 0705 population were transferred to the field on their own roots. All

seedling and parental plants are maintained in the vineyards of the Department of Viticulture and Enology, University of California, Davis.

6.2.2 Screening of plant material for resistance to *Xiphinema index*

A total of 164 plants in the 0705 population were tested under greenhouse conditions for *X. index* resistance. Four replicates of each genotype, including the parents were tested using a completely randomized design. Saint George and O39-16 were included as susceptible and resistant genotypes, respectively. Greenhouse controls were set for a temperature range of between 24 and 28°C, and during the summer months the greenhouse was white washed to moderate high temperatures. The plants were hand watered as needed throughout the six-week test period. The soil surface was kept moist prevent the plants from drying out, but care was taken to avoid over-watering, which damages grape roots and can wash nematodes out of the pots. After six weeks all plants were removed from the pots, soil was removed and the roots were examined for *X. index* feeding symptoms in the form of swollen club-like galls on the root tips.

6.2.3 DNA extraction

Young leaf tissue was collected from the greenhouse grown plants for DNA extraction. A modified CTAB (cetyltrimethylammonium bromide) as described by Lodhi *et al.,* (1994), was used to extract the DNA form leaf tissue. Approximately 0.5 g of leaf tissue were placed in Bioreba grinding bags and frozen until needed. Five mL extraction buffer containing ß-mercaptoethanol (0.5% v/v) and polyvinylpyrollidone (PVP) (5% w/v) was pipetted into each bag, and samples were ground with a Homes 6 mechanical homogenizer (Bioreba, Longmont, CO). Two mL of each homogenized sample was pipetted into a 2 mL eppendorff tube and centrifuged at 8500 rpm for five minutes. The supernatant was discarded without disturbing the pellet. The pellet was resuspended in 0.8 mL high salt lysis buffer and 0.2 mL 5% sarcocyl solution where after the samples were incubated at 65°C for 45 minutes. Extraneous material was denatured by adding 0.8 mL chloroform:isoamyl alcohol (24:1). The DNA was then precipitated with 90 µL 3M sodium acetate and 900 µL isopropanol where after the DNA pellet was washed with 70% ethanol and resuspended in 100 µL 1X TE buffer where after it was stored at -20°C.

6.2.4 Microsatellite markers and marker amplification

In total, 431 SSR markers were tested for amplification on the 0705 population. A small subset of eight genotypes including parents was used was tested with the 431 SSR markers. One unpublished SSR marker, developed in Prof. Andrew Walker's lab at the Department of Viticulture and Enology, University of California, Davis, also proved to be polymorphic. The majority of the SSR markers used were from *Vitis* Microsatellite Consortium (VMC). Others were from the VVI and UDV marker series. These markers are available as NCBI uni-STS sequences (http://www.ncbi.nlm.nih.gov/). All these markers used in the study and their sources are referenced in Table 6.1.

Table 6.1. The sources and references of the SSR markers used for map construction in the 0705 population.

Marker symbol	Sources	Reference
CTG, CB, AF	University of California, Davis, USA	http://www.cgf.ucdavis.edu/
SC8-0071-014	University of Udine	Coleman *et al.,* 2009
S65I24SP6L	University of California, Davis, USA	Unpublished
SCU	Southern Cross University, Australia	Scott *et al.,* 2000
UDV	University of Udine	Di Gaspero *et al.,* 2005
VChr	University of Udine	Cipriani *et al.,* 2008
VMC, VMCNg	*Vitis* Microsatellite Consortium	Agrogene SA (Moissy Cramayel, France), now Eurofins (http://www.eurofins.com)
VMC	University of Madrid, Spain	Arroyo-Garcia & Martinez-Zapater, 2004
VMC	University of Udine	Di Gaspero *et al.,* 2000
VrZag	University of Agriculture, Vienna, Austria	Sefc *et al.,* 1999
VVI	NCBI uni-STS	Merdinoglu *et al.,* 2005
VVMD	University of California, Davis, USA	Bowers *et al.,* 1996; 1999
VVS	CSIRO, Australia	Thomas & Scott, 1993

The PCR conditions described by Riaz *et al.* (2004) were used for amplification. All amplifications were done in 10 µl reactions of 10 ng genomic DNA with 10 pmol of each of the forward and reverse primer, 2.5 nM of each NTP, 2 µl 10x gold PCR buffer, 2 nM $MgCl_2$ and 0.5 units AmpliTaq Gold DNA polymerase. The small subsets of eight DNA samples were run at a temperature of 56°C. If the reaction was not successful, the samples were tested at 60°C and 52°C for optimization. Amplification conditions were kept the same for all primer pairs, which were 10 min at 95°C, then 35 cycles of 30 sec denaturation at 94°C, 45 sec annealing at 52, 56 or 60°C, 1 min extension at 72°C, followed by an additional 10 min extension and finally cool down to 4°C. Three microliters of the PCR product was visualized on a 2% agrose gel prepared with 1x TBE buffer to determine the amplification success. All marker test gels were run on the 5% denaturing polyacrylamide gels; gels were developed and visualized by silver staining with a commercial kit provided by Promega, Madison, WI, USA.

Smaller sized markers (< 130 bp) were run on a 5% polyacrylamide gel as described above. The larger sized markers were run on an ABI PRISM 377 DNA sequencer (Applied Biosystems). The forward primers were labelled at the 5'-end with ABI fluorescent dyes (6-FAM, HEX and TET) for detection of the SSR fragments. Analysis was done visually for both the gels run on 5% polyacrylamide plates and the DNA sequencer for consistency. Four gel plates were used to run markers on the entire 0705 population. Forty-six genotypes and the two parents were run per plate for the first three plates. The fourth plate contained the remaining 57 genotypes and the two parents. In total, 195 genotypes from the 0705 population were screened, but only the 164 true-to-type genotypes were used in this study. To save time and resources, markers were combined when sizing differences allowed it, and 2-3 markers were run together. Size standards were not used due to the high cost. The ABI PRISM GeneScan® analysis software (Applied Biosystems) was used to create gel images for markers run on the ABI PRISM 377 DNA sequencer. Images of all plates were converted to JPEG images, archived and visually scored. All scoring data was double-checked.

6.2.5 Scoring and map construction

All the marker types were scored visually for the presence (1) or absence (0) of the bands. The visual scoring was done for each parent independently and later combined into one file. The scoring for each marker was double-checked. The data was collected into Excel spreadsheets and later converted to JoinMap 3.0. Segregation patterns were assigned to each marker (abxcd, abxac, abxaa, aaxab, abxab).

Linkage analysis was performed with JoinMap 3.0 (Van Ooijen & Voorrips, 2001) and final female, male and consensus maps were aligned. Map units (cM) were derived from the Kosambi (K) mapping function (Kosambi, 1944). The individual parental maps were created where alleles for each marker were scored separately and segregating markers were paired with a dummy locus. All the female marker data was entered as <abxaa> and the male data was entered as <aaxab> following the double pseudo-testcross strategy (Grattapaglia & Sederoff, 1994). Calculation parameters were set for a minimum LOD threshold of 4.0 and a maximum of 8.0. The female, male and consensus maps for all 19 linkage groups were constructed with MapChart 2.1 software (Voorrips, 2002).

6.2.6 QTL analysis

The gall numbers observed on the individual plants were used directly as the quantitative score of susceptibility. The average data for the four replicates was log-transformed to homogenize variances. The average data was also categorized in four categories for comparison with the average and log-transformed data sets (Table 6.2). The data for the individual female and male parents as well as the consensus data were converted to MapQTL 6.0 (Van Ooijen, 2009) file formats.

Kruskal-Wallis analysis and interval mapping (IM) in 1 cM intervals was performed to identify regions with QTL.

Table 6.2. The categorized average data set based on gall numbers for the 0705 population.

Category	Average gall number	Definition
1	0-1.0	Resistant
2	1.1-10.0	Moderately susceptible
3	10.1-50.0	Susceptible
4	>50.1	Highly susceptible

6.3 Results

6.3.1 Segregation analysis for *Xiphinema index* resistance

One hundred and sixty four genotypes from the 0705 population were evaluated for *X. index* resistance. Of these genotypes, 94 were susceptible with two or more galls. The remaining 70 genotypes were considered resistant with no or one galls. The average gall numbers were categorized into four groups where 70 genotypes had no or one gall, nine genotypes had 2-10 galls, 21 had 11-50 galls, and the remaining 64 genotypes had 51-200 galls. These gall numbers were log transformed and used in the QTL analysis for *X. index* resistance. O39-16, the resistant control genotype, R8916-22 and b40-14 had no galls after six weeks. The susceptible St. George had high numbers of galls on all ten of its replicates. The root damage was severe on the highly susceptible genotypes, which made it difficult to accurately count

galls. The female parent, 161-49C was difficult to propagate and had low gall numbers with an average of 6.83. A total of 88 susceptible genotypes had a higher average gall numbers than 161-49C. The susceptible grandparent, *V. rupestris* cv. Wichita Refuge had high gall numbers on all roots. Statistical analysis was performed to create resistant, susceptible and intermediate groupings of genotypes. Analysis of variance found significant differences among the mean values for the 0705 population with $P < 0.0001$.

Chi-square tests were used to test the hypothesized inheritance model for the 0705 population. The population was expected to segregate 1:1 (R:S) based on a proposed model of a rr X Rr cross, which the data fit loosely, but the X^2-value of 3.52 was borderline significant at the 5% probability level. Nine genotypes had an average gall number below 10. When these genotypes were excluded from the analysis, the X^2-value changed to 1.86 with a p-value of >0.10. However, these nine genotypes were included in the final analysis since the female parent showed low average gall numbers. Screening data determined a clear 1:1 segregation ratio with a X^2 value of 1.56 and a p-value of >0.20 (See Chapter 5 of this dissertation for the data analysis).

6.3.2 Off-type screening

Two hundred seedlings of the 0705 population were first tested with four SSR markers; VMC2c3, VMC2f10, VMC2h10 and CTG1010193, to eliminate off-types. Of these, 31 genotypes tested as off-types and five genotypes died before they were transferred to the field. One hundred and sixty four true-to-type genotypes were eventually used to create a framework genetic map for *X. index* resistance.

6.3.3 Molecular markers

Covering all 19 linkage groups, 172 SSR markers out of 431 tested were polymorphic for either the male parent (R8916-22), female parent (161-49C) or both (Table 6.3). Several markers from the CTG and the VMCNg groups showed no or poor amplification, even when tested at different annealing temperatures. The consensus genetic linkage map was constructed using 172 markers of which 125 segregated for both parents. They represented the most informative segregation types with four alleles (<abxcd>) and three alleles (<abxac>). Thirteen markers segregated for the female 161-49C (<abxaa>) and 35 segregated for the male R8916-22 (<aaxab>) only. Three markers segregated <abxab>. The two markers, VMC6f5 and VMC9f4 showed amplification of multiple genomic regions by producing three bands for the male parent. These pairs were scored and given the suffixes “a”, “b” and “c”. Except for VMC6f5b and VMC6f5c, all these loci were placed on different linkage groups in the male and consensus maps.

Table 6.3. Segregation pattern and polymorphism for SSR marker categories used on the 0705 population.

SSR Marker	Number tested	Segregation type				
		<aaxab>	<abxaa>	<abxab>	<abxac>	<abxcd>
VMC, VMCNg	69	12	3	2	22	30
VVI	39	9	6	1	8	15
UDV	36	8	-	-	11	17
CTG, CB, AF	11	1	2	-	3	5
VChr	5	1	1	-	2	1
VVMD	3	-	1	-	2	-
VrZag	3	1	-	-	-	2
VVS	3	-	-	-	-	3
SCU	1	-	-	-	-	1
SC8-0071-014	1	-	-	-	1	-
Unpublished	1	1	-	-	-	-

The 'locus genotype frequency' command in JoinMap was used to test all markers for deviation from the expected Mendelian segregation. Based on the Chi-square values the markers were sorted and placed into three categories. These categories are denoted 0-5.0, 5.0-10.0 and greater than 10.0 for easy visual denotation (Figure 6.1) as shown in Lowe and Walker (2006). For the 161-49C map 15% of the markers showed segregation distortion. For the 0705 and R8916-22 maps, 29% and 25% showed segregation distortion. Linkage groups 5, 13, 14 and 15 showed higher numbers of distorted markers spread out along the entire length of the linkage groups. Clusters of distorted markers were found on linkage groups 3, 9, 10 and 19.

6.3.4 Parental and consensus map construction

The parental and consensus maps were all constructed at LOD 4.0. These maps showed no change in marker order and marker distances when tested at LOD 8.0.

For the female map, 161-49C, 136 markers were placed in 19 linkage groups with an average of 7.2 markers per linkage group. In total, eight markers were linked but unmapped. The linkage group sizes ranged from 10.9 cM on linkage group 12 to 87.6 cM on linkage group 19. The average map size was 51.4 cM with an average of 7.2 cM between markers (Table 6.3). The marker order on the female map was mostly consistent in comparison with the 0705 consensus map. Marker inversions were found on linkage groups 2 (VMC5g7), 4 (VMCNg2e1), 6 (UDV022 and VMC3f12), 9 (VMC2e11 and VVIN37), 13 (VMC3d12 and VVIC51), 14 (VMC2c3, UDV095 and VVIS70), 15 (VVS16), 18 (VMC2a3, VMC2g6 and VMCNg1e3) and 19 (VVIN04 and VMC5d11).

The male map, R8916-22, was developed with 157 markers with 21 unmapped markers. Linkage group 18 was split into two groups. The average distance between markers was 6.3 cM, and linkage group sizes ranged from 21.0 cM on linkage group 17 to 81.5 cM on linkage group 19. The average linkage group size was 52.3 cM. The marker order on the R8916-22 map was also mostly consistent compared to the consensus map with minor inversions on linkage groups 2 (VMC7g3, VChr2a, VVIB23 and VMC3b10), 4 (VMCNg2e1 and VMC7h3), 8 (VVIV15.2), 9 (UDV132), 13 (VMC9f4b, UDV124, Sc08-0071-014, VMCNg4e10.1 and VMC9h4.2) and 14 (VVIN64 and UDV095), 15 (VVIV67), 17 (VVIQ22.2 and VVIN73). Linkage

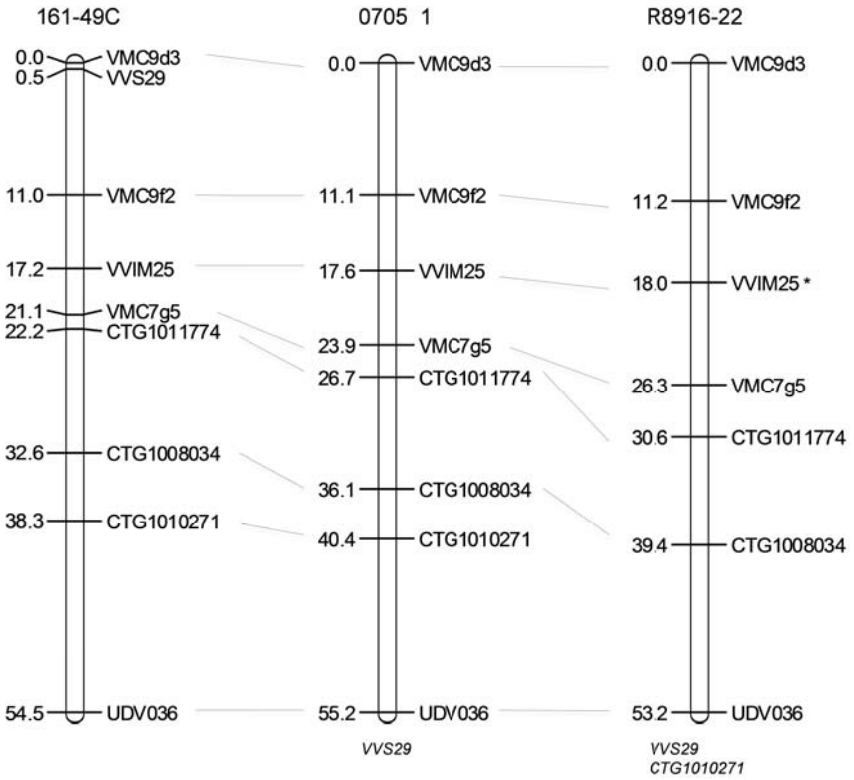
groups 15 and 17 had more prominent rearrangements between markers on the male and consensus maps.

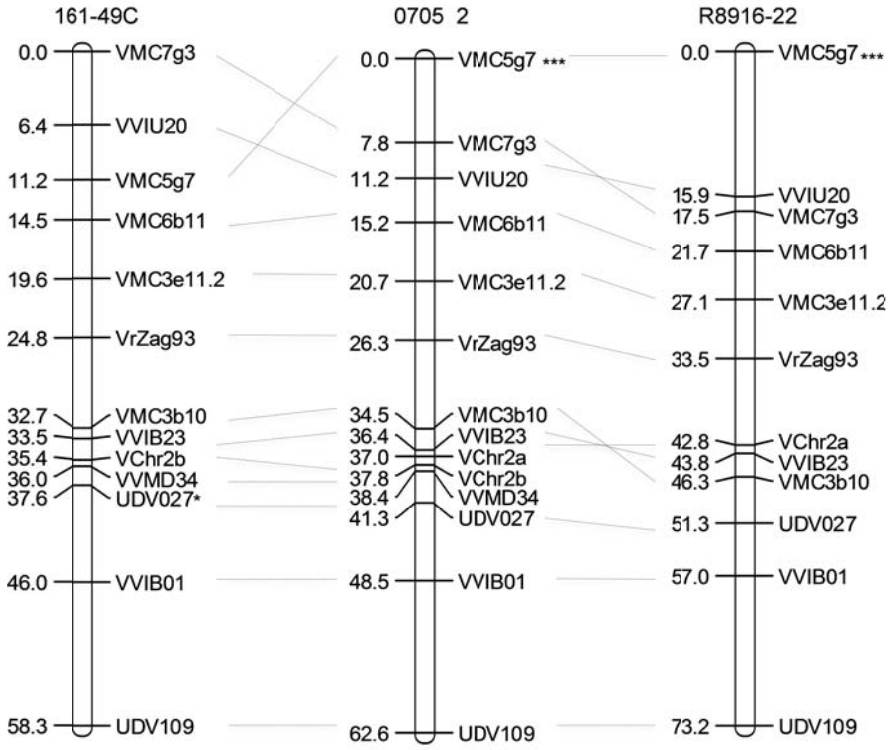
The consensus 0705 map consisted of 19 linkage groups with 172 markers. The average number of markers per linkage group was 9.1. Four markers were unmapped but linked. The map covered 1233.0 cM with an average size of 64.9 cM per linkage group. The linkage group sizes ranged from 42.2 cM (group 4) to 94.2 cM (group 18) with an average distance of 7.2 cM between markers (Table 6.4).

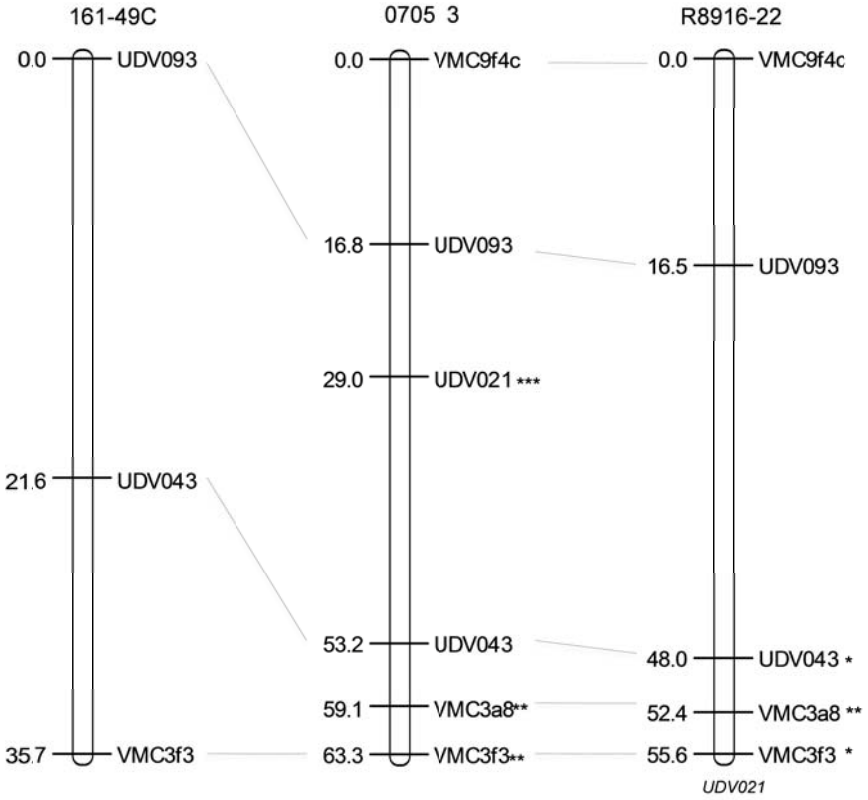
The marker order between the parental maps and the consensus maps were relatively consistent. This map was compared to four other published maps (Doligez *et al.,* 2006; Riaz *et al.,* 2006; Lowe & Walker, 2006; Adam-Blondon *et al.,* 2004) with high numbers of marker order similarities on 12 linkage groups.

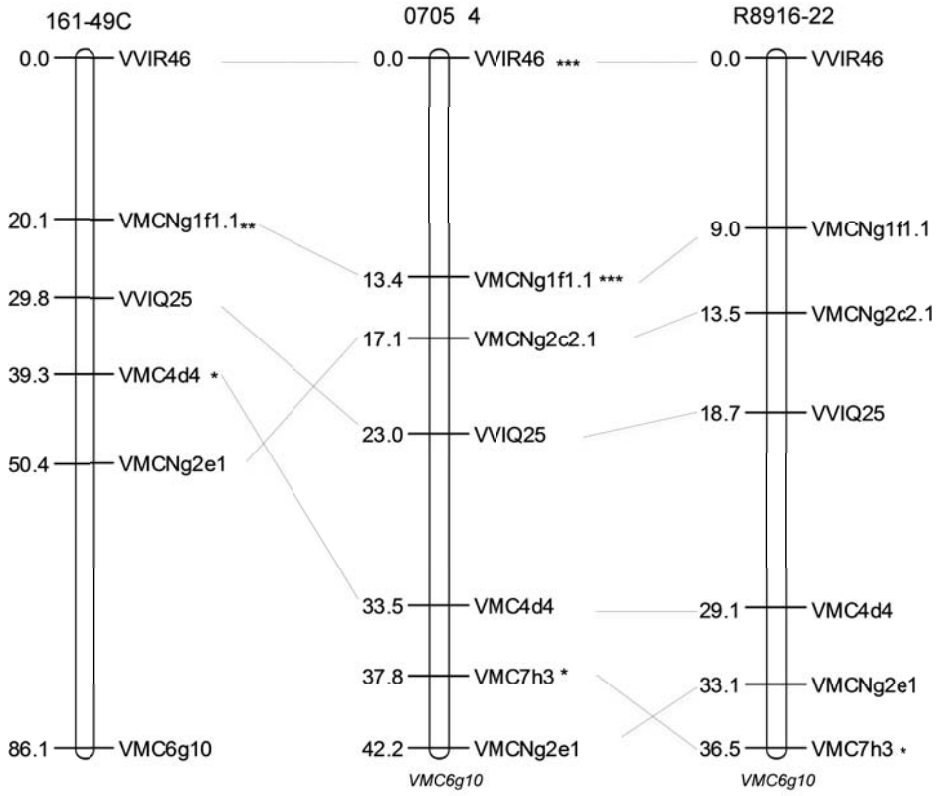
Table 6.4. Linkage map characteristics of the 161-49C, 0705 and R8916-22 populations.

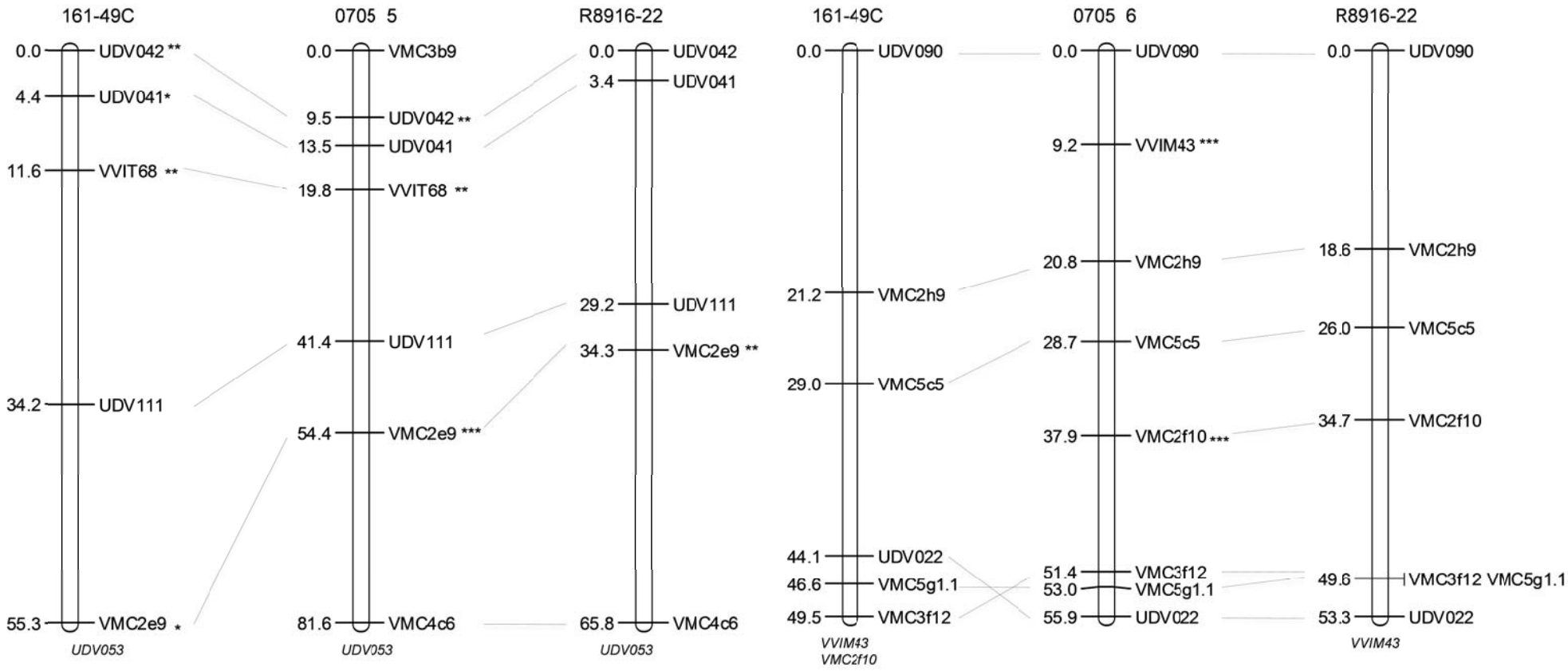
LG	161-49C			0705			R8916-22		
	Size (cM)	Markers	Distorted markers	Size (cM)	Markers	Distorted markers	Size (cM)	Markers	Distorted markers
1	54.5	9	0	55.2	8	0	53.2	7	1
2	58.3	13	1	62.6	14	1	73.2	12	1
3	35.7	3	0	63.3	6	3	55.6	5	3
4	86.1	6	2	42.2	7	3	36.5	7	1
5	55.3	5	4	81.6	7	3	65.8	5	1
6	49.4	7	0	55.9	8	2	53.3	7	0
7	64.8	6	1	77.5	9	1	80.8	9	0
8	28.3	4	0	74.1	9	2	31.5	7	1
9	42.8	8	5	54.8	12	3	46.0	9	2
10	60.1	5	0	47.5	7	6	41.9	7	6
11	81.1	4	2	67.1	4	1	56.3	4	0
12	10.9	2	0	42.3	3	0	39.6	3	0
13	39.0	10	3	42.8	13	6	46.6	12	5
14	71.4	11	0	72.7	14	5	64.5	8	6
15	27.8	3	0	69.5	8	3	70.2	7	3
16	13.5	3	2	78.3	7	2	48.8	5	1
17	49.5	6	0	67.8	9	1	21.0	8	1
18	61.0	9	0	94.2	11	1	28.3	4	0
19	87.6	15	1	83.6	18	8	81.5	16	8
Average	51.4	6.8	1.1	64.9	9.2	2.7	52.3	7.5	2.1
Total	977.1	129.0	21.0	1233.0	174.0	51.0	994.6	142.0	40.0

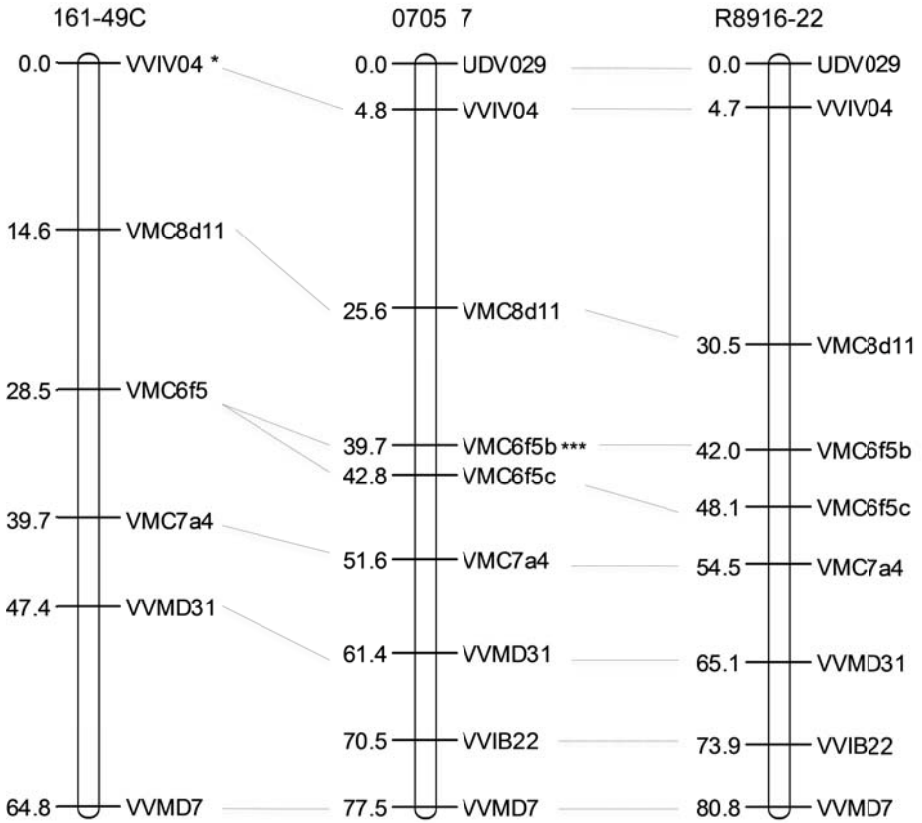

161-49C
0.0 VMC9d3
0.5 VVS29
11.0 VMC9f2
17.2 VVIM25
21.1 VMC7g5
22.2 CTG1011774
32.6 CTG1008034
38.3 CTG1010271
54.5 UDV036
0705 1
0.0 VMC9d3
11.1 VMC9f2
17.6 VVIM25
23.9 VMC7g5
26.7 CTG1011774
36.1 CTG1008034
40.4 CTG1010271
55.2 UDV036
VVS29
R8916-22
0.0 VMC9d3
11.2 VMC9f2
18.0 VVIM25 *
26.3 VMC7g5
30.6 CTG1011774
39.4 CTG1008034
53.2 UDV036
VVS29
CTG1010271

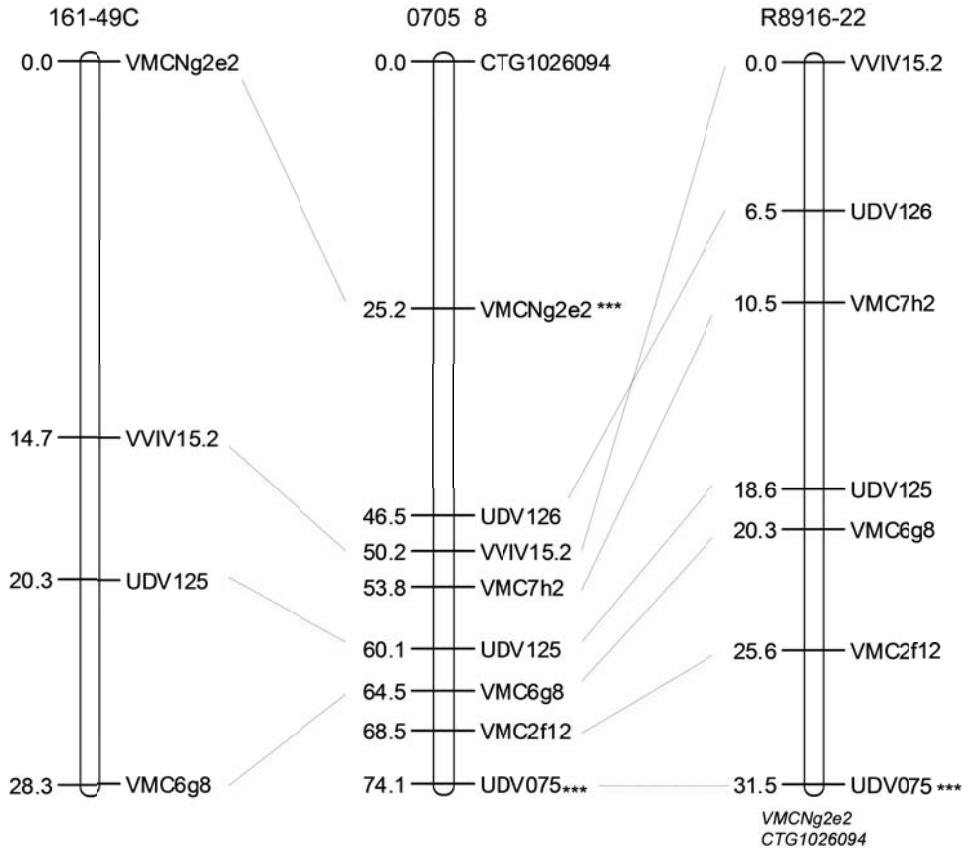

161-49C
0.0 VMC7g3
6.4 VVIU20
11.2 VMC5g7
14.5 VMC6b11
19.6 VMC3e11.2
24.8 VrZag93
32.7 VMC3b10
33.5 VVIB23
35.4 VChr2b
36.0 VVMD34
37.6 UDV027*
46.0 VVIB01
58.3 UDV109
0705 2
0.0 VMC5g7 ***
7.8 VMC7g3
11.2 VVIU20
15.2 VMC6b11
20.7 VMC3e11.2
26.3 VrZag93
34.5 VMC3b10
36.4 VVIB23
37.0 VChr2a
37.8 VChr2b
38.4 VVMD34
41.3 UDV027
48.5 VVIB01
62.6 UDV109
R8916-22
0.0 VMC5g7 ***
15.9 VVIU20
17.5 VMC7g3
21.7 VMC6b11
27.1 VMC3e11.2
33.5 VrZag93
42.8 VChr2a
43.8 VVIB23
46.3 VMC3b10
51.3 UDV027
57.0 VVIB01
73.2 UDV109


161-49C
0705 3
R8916-22
0.0 UDV093
21.6 UDV043
35.7 VMC3f3
0.0 VMC9f4c
16.8 UDV093
29.0 UDV021 ***
53.2 UDV043
59.1 VMC3a8**
63.3 VMC3f3**
0.0 VMC9f4c
16.5 UDV093
48.0 UDV043 *
52.4 VMC3a8 **
55.6 VMC3f3 *
UDV021


161-49C
0705 4
R8916-22
0.0 VVIR46
20.1 VMCNg1f1.1**
29.8 VVIQ25
39.3 VMC4d4 *
50.4 VMCNg2e1
86.1 VMC6g10
0.0 VVIR46 ***
13.4 VMCNg1f1.1 ***
17.1 VMCNg2c2.1
23.0 VVIQ25
33.5 VMC4d4
37.8 VMC7h3 *
42.2 VMCNg2e1
VMC6g10
0.0 VVIR46
9.0 VMCNg1f1.1
13.5 VMCNg2c2.1
18.7 VVIQ25
29.1 VMC4d4
33.1 VMCNg2e1
36.5 VMC7h3 *
VMC6g10


161-49C
0.0 UDV042 **
4.4 UDV041*
11.6 VVIT68 **
34.2 UDV111
55.3 VMC2e9 *
UDV053
0705 5
0.0 VMC3b9
9.5 UDV042 **
13.5 UDV041
19.8 VVIT68 **
41.4 UDV111
54.4 VMC2e9 ***
81.6 VMC4c6
UDV053
R8916-22
0.0 UDV042
3.4 UDV041
29.2 UDV111
34.3 VMC2e9 **
65.8 VMC4c6
UDV053
161-49C
0.0 UDV090
21.2 VMC2h9
29.0 VMC5c5
44.1 UDV022
46.6 VMC5g1.1
49.5 VMC3f12
VVIM43
VMC2f10
0705 6
0.0 UDV090
9.2 VVIM43 ***
20.8 VMC2h9
28.7 VMC5c5
37.9 VMC2f10 ***
51.4 VMC3f12
53.0 VMC5g1.1
55.9 UDV022
R8916-22
0.0 UDV090
18.6 VMC2h9
26.0 VMC5c5
34.7 VMC2f10
49.6 VMC3f12 VMC5g1.1
53.3 UDV022
VVIM43


161-49C
0705 7
R8916-22
0.0 VVIV04 *
14.6 VMC8d11
28.5 VMC6f5
39.7 VMC7a4
47.4 VVMD31
64.8 VVMD7
0.0 UDV029
4.8 VVIV04
25.6 VMC8d11
39.7 VMC6f5b ***
42.8 VMC6f5c
51.6 VMC7a4
61.4 VVMD31
70.5 VVIB22
77.5 VVMD7
0.0 UDV029
4.7 VVIV04
30.5 VMC8d11
42.0 VMC6f5b
48.1 VMC6f5c
54.5 VMC7a4
65.1 VVMD31
73.9 VVIB22
80.8 VVMD7


161-49C
0705 8
R8916-22
0.0 VMCNg2e2
14.7 VVIV15.2
20.3 UDV125
28.3 VMC6g8
0.0 CTG1026094
25.2 VMCNg2e2 ***
46.5 UDV126
50.2 VVIV15.2
53.8 VMC7h2
60.1 UDV125
64.5 VMC6g8
68.5 VMC2f12
74.1 UDV075***
0.0 VVIV15.2
6.5 UDV126
10.5 VMC7h2
18.6 UDV125
20.3 VMC6g8
25.6 VMC2f12
31.5 UDV075 ***
VMCNg2e2
CTG1026094

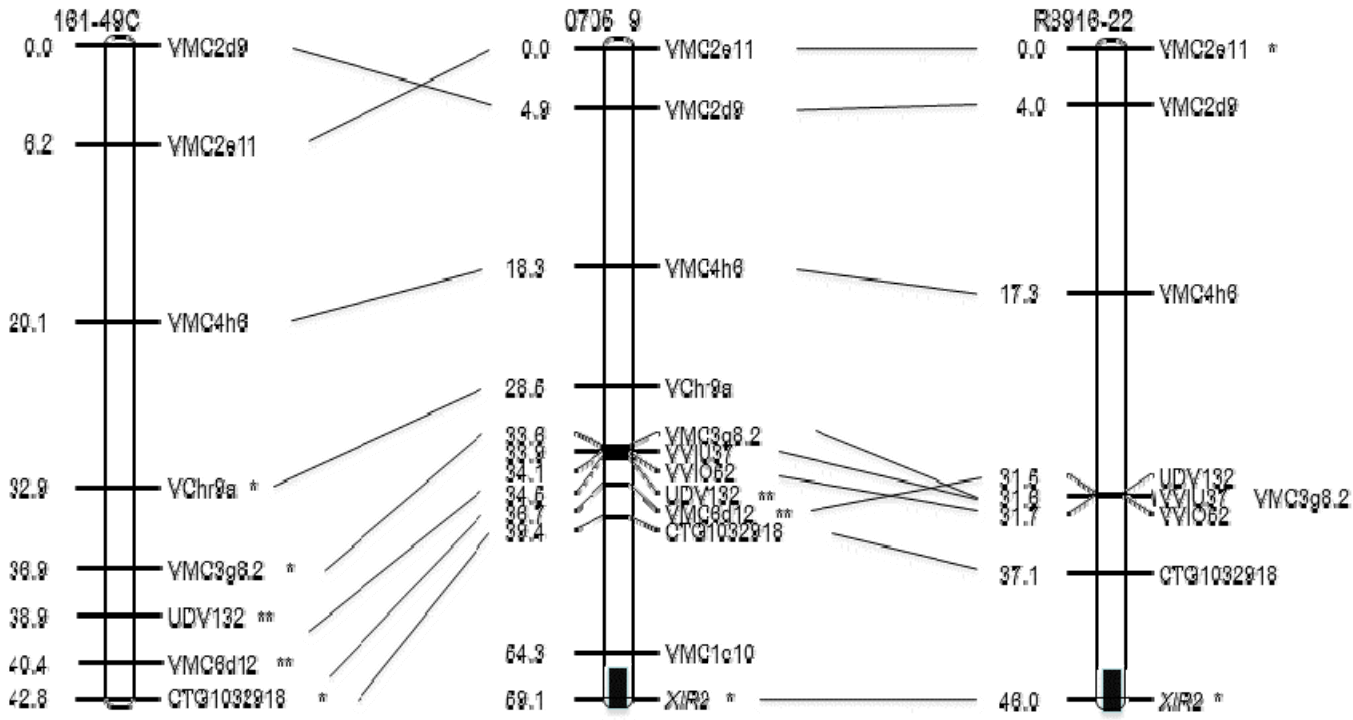
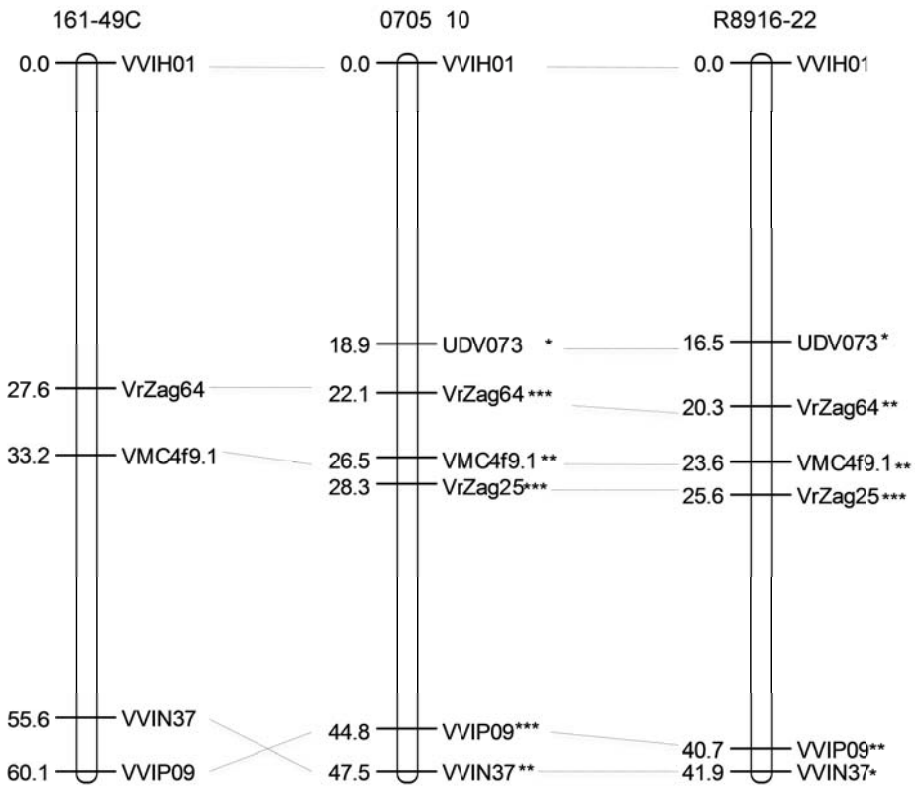
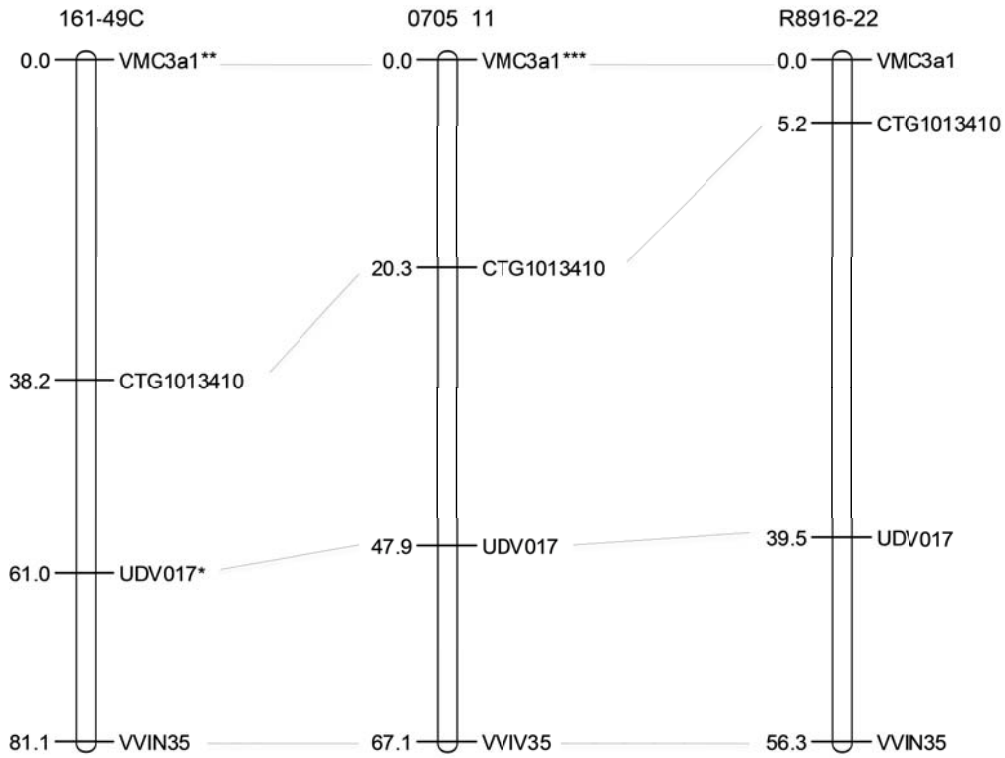
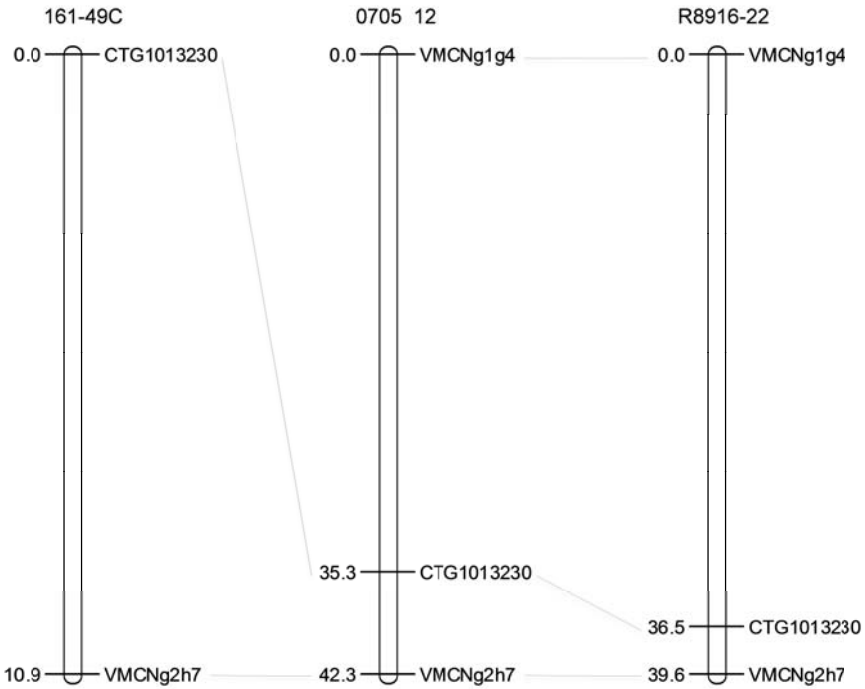
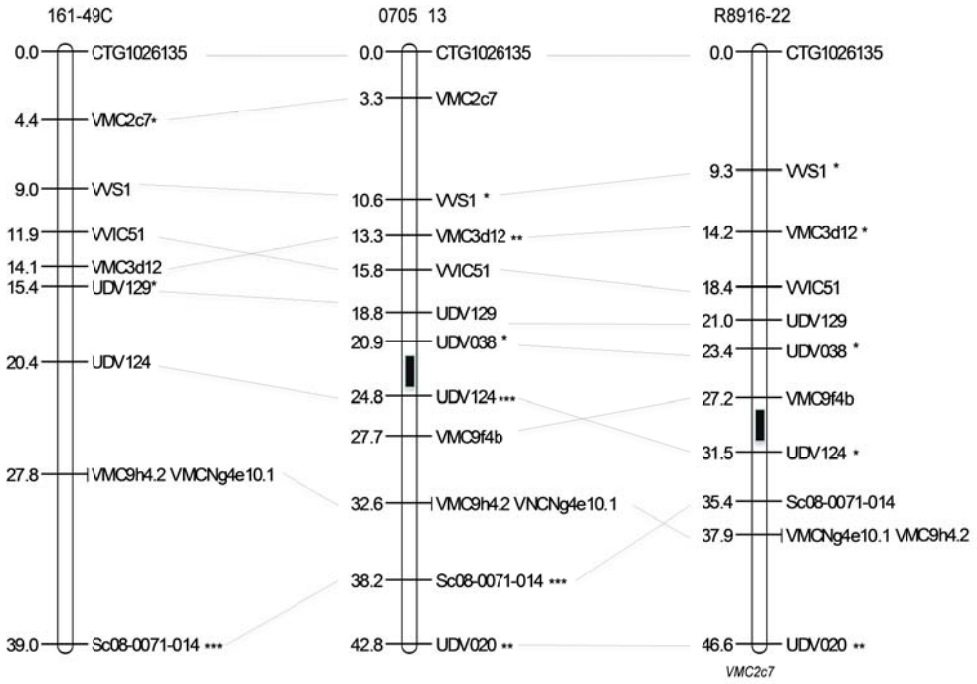


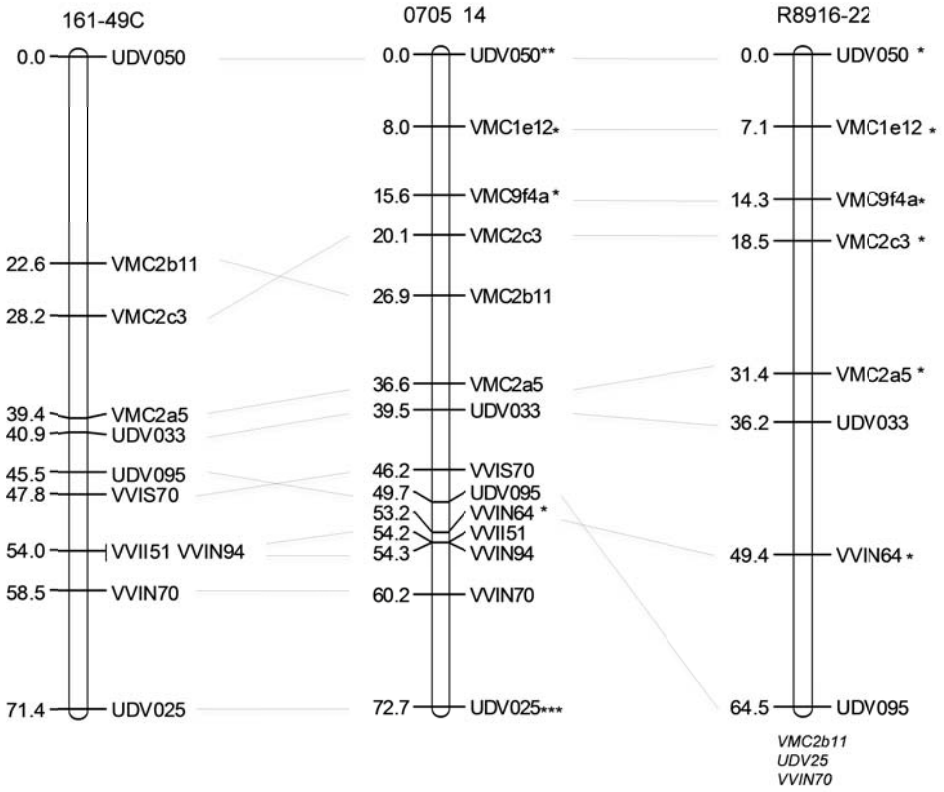
Figure 6.1. (*cont.*)

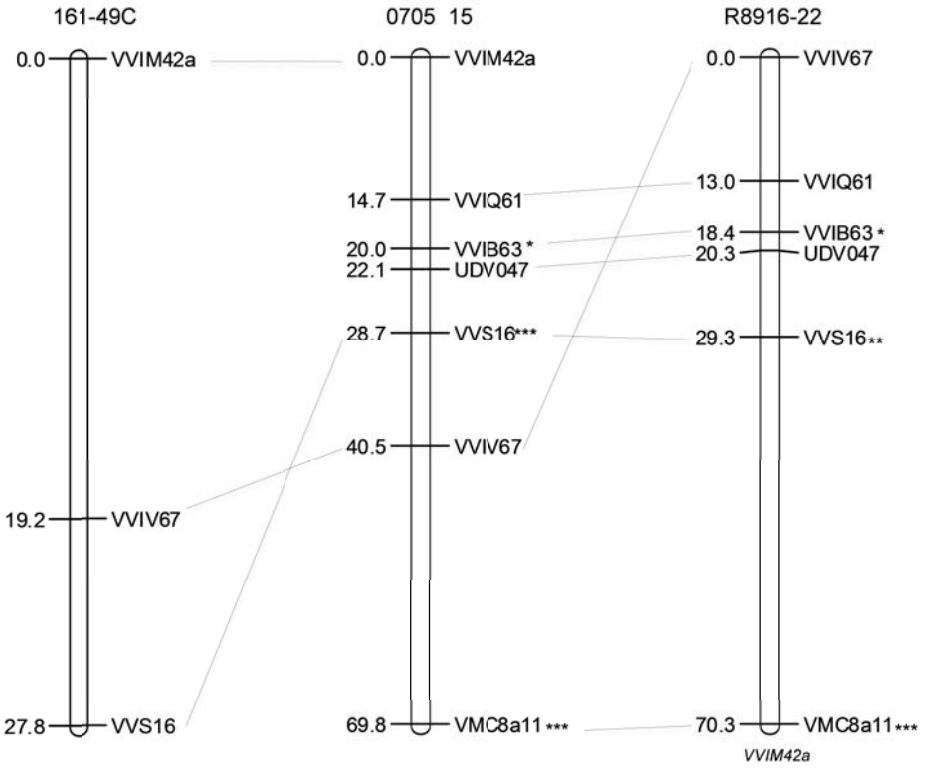

161-49C
0705 10
R8916-22
0.0 VVIH01
0.0 VVIH01
0.0 VVIH01
18.9 UDV073 *
16.5 UDV073*
27.6 VrZag64
22.1 VrZag64***
20.3 VrZag64**
33.2 VMC4f9.1
26.5 VMC4f9.1**
23.6 VMC4f9.1**
28.3 VrZag25***
25.6 VrZag25***
55.6 VVIN37
44.8 VVIP09***
40.7 VVIP09**
60.1 VVIP09
47.5 VVIN37**
41.9 VVIN37*

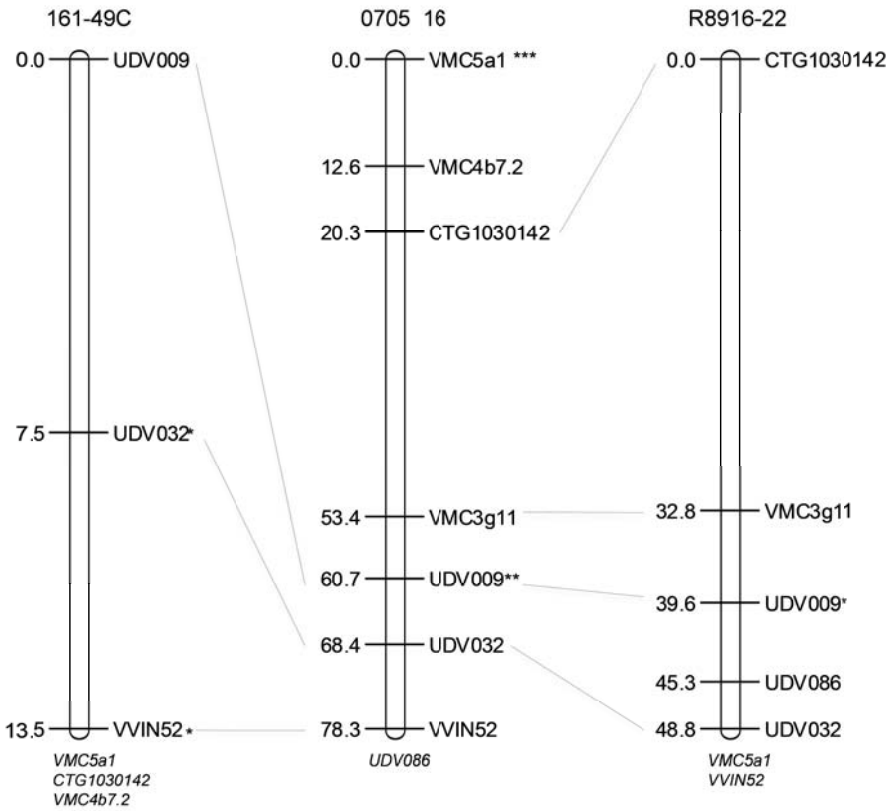

161-49C
0705 11
R8916-22
0.0 VMC3a1**
0.0 VMC3a1***
0.0 VMC3a1
5.2 CTG1013410
20.3 CTG1013410
38.2 CTG1013410
47.9 UDV017
39.5 UDV017
61.0 UDV017*
81.1 VVIN35
67.1 VVIV35
56.3 VVIN35

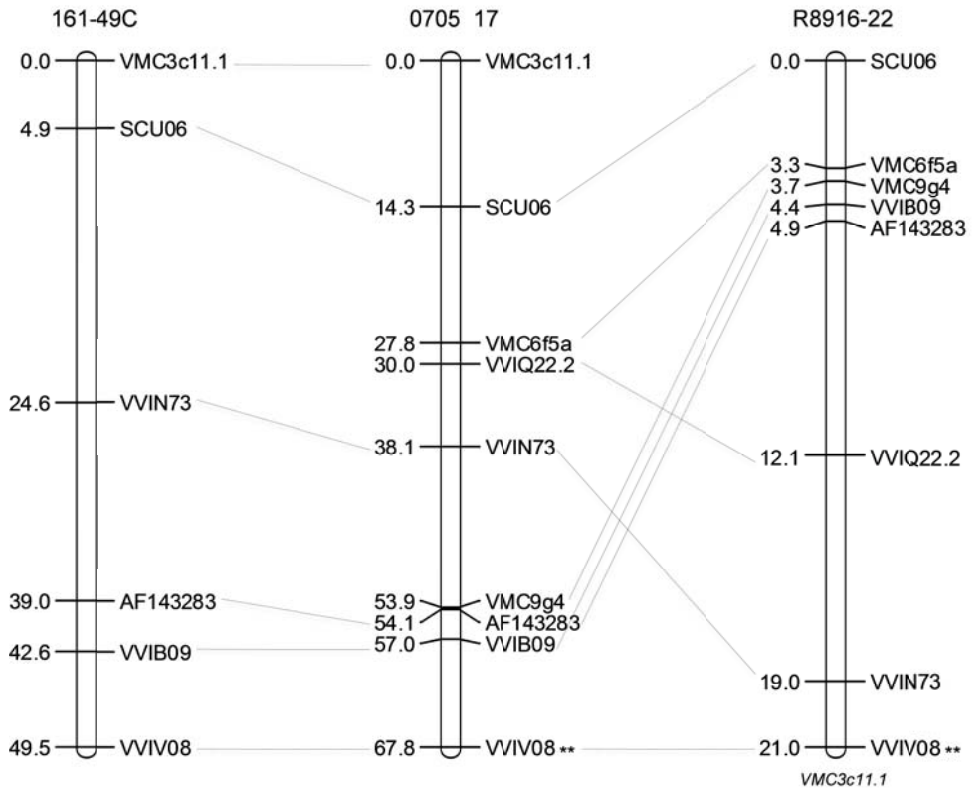

161-49C
0705 12
R8916-22
0.0 CTG1013230
10.9 VMCNg2h7
0.0 VMCNg1g4
35.3 CTG1013230
42.3 VMCNg2h7
0.0 VMCNg1g4
36.5 CTG1013230
39.6 VMCNg2h7


161-49C
0705 13
R8916-22
0.0 CTG1026135
4.4 VMC2c7*
9.0 VVS1
11.9 VVIC51
14.1 VMC3d12
15.4 UDV129*
20.4 UDV124
27.8 VMC9h4.2 VMCNg4e10.1
39.0 Sc08-0071-014 ***
0.0 CTG1026135
3.3 VMC2c7
10.6 VVS1 *
13.3 VMC3d12 **
15.8 VVIC51
18.8 UDV129
20.9 UDV038 *
24.8 UDV124 ***
27.7 VMC9f4b
32.6 VMC9h4.2 VNCNg4e10.1
38.2 Sc08-0071-014 ***
42.8 UDV020 **
0.0 CTG1026135
9.3 VVS1 *
14.2 VMC3d12 *
18.4 VVIC51
21.0 UDV129
23.4 UDV038 *
27.2 VMC9f4b
31.5 UDV124 *
35.4 Sc08-0071-014
37.9 VMCNg4e10.1 VMC9h4.2
46.6 UDV020 **
VMC2c7


161-49C
0705 14
R8916-22
0.0 UDV050
22.6 VMC2b11
28.2 VMC2c3
39.4 VMC2a5
40.9 UDV033
45.5 UDV095
47.8 VVIS70
54.0 VVII51 VVIN94
58.5 VVIN70
71.4 UDV025
0.0 UDV050**
8.0 VMC1e12*
15.6 VMC9f4a*
20.1 VMC2c3
26.9 VMC2b11
36.6 VMC2a5
39.5 UDV033
46.2 VVIS70
49.7 UDV095
53.2 VVIN64 *
54.2 VVII51
54.3 VVIN94
60.2 VVIN70
72.7 UDV025***
0.0 UDV050 *
7.1 VMC1e12 *
14.3 VMC9f4a*
18.5 VMC2c3 *
31.4 VMC2a5 *
36.2 UDV033
49.4 VVIN64 *
64.5 UDV095
VMC2b11
UDV25
VVIN70


161-49C
0705 15
R8916-22
0.0 VVIM42a
19.2 VVIV67
27.8 VVS16
0.0 VVIM42a
14.7 VVIQ61
20.0 VVIB63 *
22.1 UDV047
28.7 VVS16***
40.5 VVIV67
69.8 VMC8a11 ***
0.0 VVIV67
13.0 VVIQ61
18.4 VVIB63 *
20.3 UDV047
29.3 VVS16**
70.3 VMC8a11***
VVIM42a


161-49C
0705 16
R8916-22
0.0 UDV009
7.5 UDV032*
13.5 VVIN52*
VMC5a1
CTG1030142
VMC4b7.2
0.0 VMC5a1 ***
12.6 VMC4b7.2
20.3 CTG1030142
53.4 VMC3g11
60.7 UDV009**
68.4 UDV032
78.3 VVIN52
UDV086
0.0 CTG1030142
32.8 VMC3g11
39.6 UDV009*
45.3 UDV086
48.8 UDV032
VMC5a1
VVIN52


161-49C
0705 17
R8916-22
0.0 VMC3c11.1
4.9 SCU06
24.6 VVIN73
39.0 AF143283
42.6 VVIB09
49.5 VVIV08
0.0 VMC3c11.1
14.3 SCU06
27.8 VMC6f5a
30.0 VVIQ22.2
38.1 VVIN73
53.9 VMC9g4
54.1 AF143283
57.0 VVIB09
67.8 VVIV08**
0.0 SCU06
3.3 VMC6f5a
3.7 VMC9g4
4.4 VVIB09
4.9 AF143283
12.1 VVIQ22.2
19.0 VVIN73
21.0 VVIV08**
VMC3c11.1

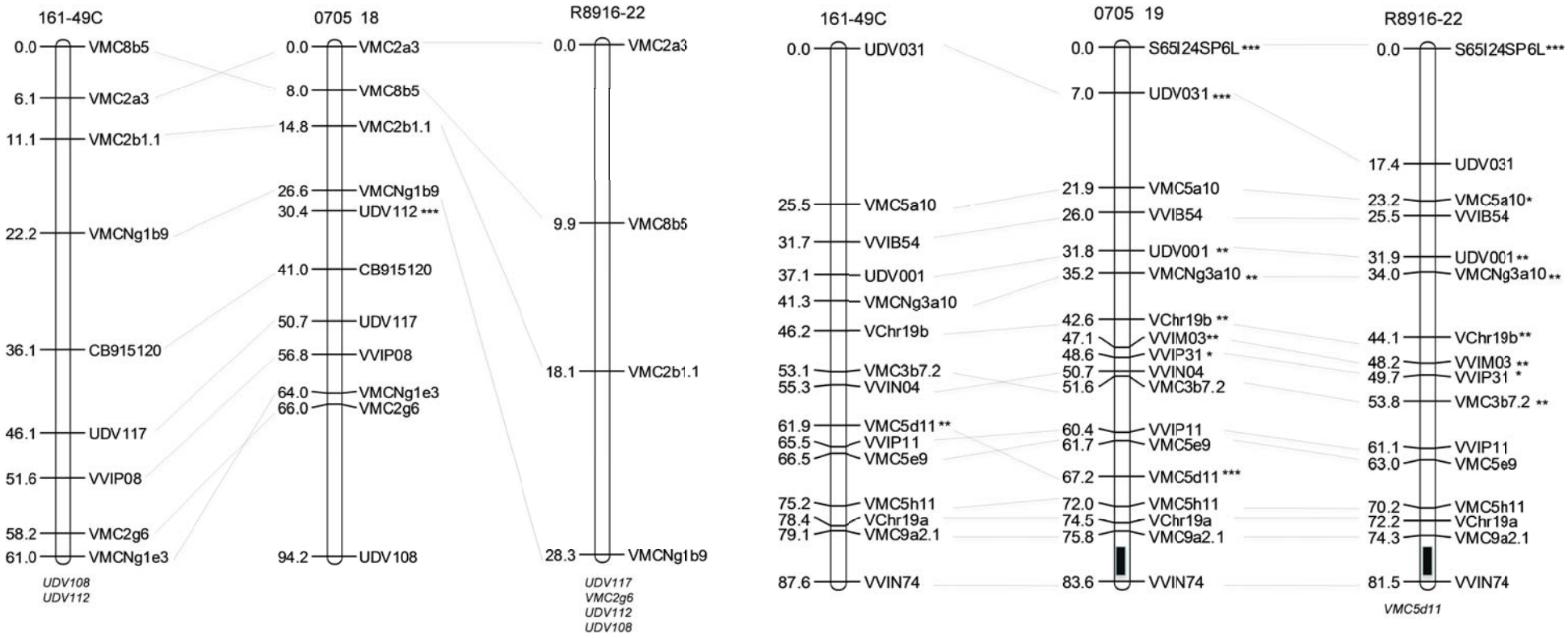


Figure 6.1. The linkage maps of 161-49C (left), R8916-22 (right) and the F1 population, 0705 (middle). The linkage groups are named according to the international consensus map. Markers showing segregation distortion are depicted with: one asterisk - X^2 value of 0-5, two asterisks – X^2 value of 5-10 and three asterisks – X^2 value of >10. Markers linked to the group but are unmapped are italicized at the bottom of the linkage group. A number follows the 0705 code on the consensus map. This number refers to the specific linkage group.

6.3.5 Placement of the *Xiphinema index* resistance locus

QTL analysis was performed on the log-transformed data of all 164 genotypes of the 0705 population. In a previous study (Xu *et al.*, 2008), the marker CD009354 was located at 14.6 cM on linkage group 19. This marker is the same as CTG1032918, which was placed on linkage group 9 in the 0705 population. The analysis showed a strong QTL on linkage group 9 closest to CTG1032918 on the R8916-22 map with a LOD score of 32.06 explaining 59.7% of the phenotypic variance with fewer markers than for the consensus map. The QTL was designated *X. index Resistance 2 (XiR2).* The *XiR2* locus mapped 8.9 cM from CTG1032918 on this male map. Analysis done on the consensus map data also placed the QTL on linkage group 9 closest to the marker VMC1c10 at the end of the linkage group. The marker VMC1c10 was heterozygous for the same alleles on both parents and it was placed only on the consensus map next to the CTG1032918 marker, which represents a LOD score of 33.36 explaining 70.5% of the phenotypic variance (Figure 6.2).

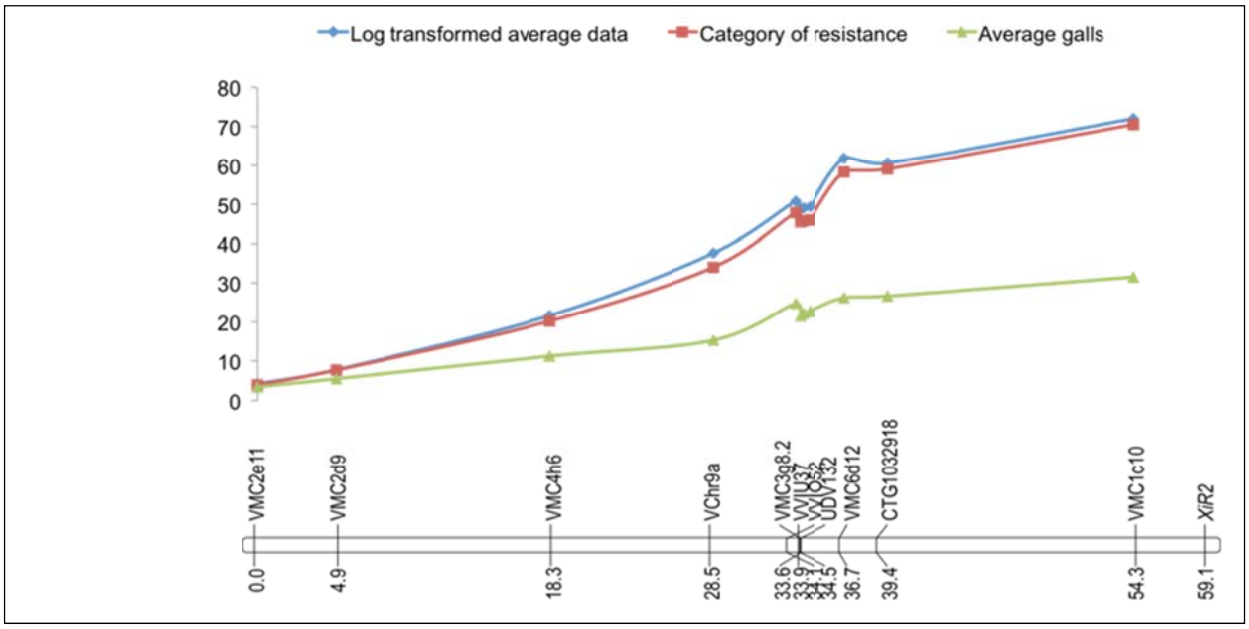


Figure 6.2. The placement of the *XiR2* QTL on linkage group 9. The QTL was detected using the log-transformed data of the average gall numbers. The numbers along linkage group 9 are the genetic distances in cM.

Two other minor QTL's were found in addition to *XiR2*. The first was detected on linkage group 13 with a LOD value of 3.15 explaining 8.7% of the phenotypic variance on the 0705 consensus map. The markers UDV038 and UDV124 were identified as flanking markers and they mapped 3.9 cM apart. Both of these markers showed segregation distortion. On the R8916-22 map the location for the possible minor QTL was closest to UDV124 with a LOD value of 3.13 explaining 8.6% of the phenotypic variance. The second possible QTL mapped on linkage group 19 with a LOD value of 2.16 with 6.0% of the phenotypic variance explained for the consensus map. The LOD threshold for both these linkage groups was 4.0. Both these QTL's mapped on the R8916-22 and 0705 consensus maps, but not on the female map (161-49C). No other QTL's were detected in either the female, male or consensus map data. The QTL analysis was performed on each individual parental map and then on the consensus map.

6.4 Discussion

6.4.1 Segregation analysis

Counting actual root galls was the measure of a given genotype's resistance to *X. index* resistance in this study. The 0705 population was divided into two groups to screen for *X. index* resistance due to limited greenhouse space. The screening was done under controlled greenhouse conditions (Chapter 5) over six weeks. Control plants were used throughout the study with O39-16 as a resistant control and St. George as a susceptible control. Many St. George plants were included in the trial and were used to check the root damage on a weekly basis to determine the optimal screening date. The expected segregation was 1:1 (R:S) based on a proposed model of a rr X Rr cross. The data did fit the model loosely, but the X^2-value of 3.52 was borderline significant at the 5% probability level. The molecular markers associated with *X. Index* resistance in the 0705 population segregated with a clear 1:1 ratio with a X^2 value of 1.56 and a p-value of >0.20. The possibility exists that a small percentage of the genotypes might be off-types due to errors made during the collections of the green and hardwood cuttings. When the nine genotypes with an average gall number below 10 are excluded from the segregation analysis, the expected 1:1 segregation ratio is observed, but since the female parent, 161-49C, had low gall numbers in general, these nine genotypes were not excluded from the analysis. It is suggested that these genotypes should be tested again to determine whether they were useful recombinants for positioning potential resistance loci, or possible mistakes.

The female parent, 161-49C exhibited poor root and plant quality throughout all the replicates used in this study, even when cuttings were treated with rooting hormones. When rooted cuttings are weak, nematodes cannot feed effectively and result in low gall numbers. It is possible that better quality plant material would result in higher feeding rates, thus higher gall numbers. When the root mass and the quality of the plant material were compared with the 161-49C data, all the plants with low gall numbers (2-20) were weak cuttings with poor roots.

Eighty-eight of the 94 susceptible genotypes had higher average gall numbers than 161-49C. Although the 0705 study was conducted in two parts, the environmental and watering conditions in the greenhouse were kept constant and resulted in consistent results for the parents and controls in the two-part study. The plants were hand watered when needed, but care was taken not to over or under water the genotypes. Thus, greenhouse conditions should not have had an effect on the higher gall numbers for the susceptible genotypes. 161-49C was used as the female parent in both this population and in another inheritance study with the same result. It seems possible that 161-49C is contributing to the high gall numbers in the susceptible genotypes perhaps with a second gene for susceptibility interacting with the *X. index* resistance gene. The 0705 population showed more concrete evidence that two major genes might be interacting while a susceptible gene is interfering with the alleles from the resistance gene from b40-14. A seedling population from a selfed 161-49C would be useful to study this effect, but it cannot be made because 161-49C has pistillate flowers. The results obtained with the 0705 crosses have provided evidence that 161-49C is only moderately susceptible to *X. index* feeding, and it may accentuate the level of susceptibility when it is used as a parent. However, given that the quality of the 161-49C plants was sub-optimal, which may have affected its susceptibility, it is hard to draw concrete conclusions. Crosses between some of the 0705 genotypes in different combinations of resistant and susceptible genotypes, and also backcrosses to the parents and grandparents might help to find the origin of the proposed susceptibility gene in 161-49C.

6.4.2 Molecular markers and genetic maps

The 0705 population was derived from a complex non-*V. vinifera* background where the female parent (161-49C) was derived from a *V. riparia x V. berlandieri* cross and the male parent (R8916-22) was derived from a *V. rupestris x V. arizonica* cross. SSR-based *V. vinifera* markers proved to be highly polymorphic and reproducible in the 0705 population with *V. arizonica* in the background.

Single inversions of markers exist on 9 of the 19 linkage groups for the female map and 8 linkage groups for the male map. Of these, 14 inversions on nine linkage groups might be due to the markers segregating for only one parent. The marker order on these linkage groups can also be affected by possible errors in the data sets, which might result in inversions where none exist. The population size and the saturation of the genetic map may also have an influence on the marker order and differences in marker scoring can also contribute. The 0705 map was presented as a framework map only, and adding more markers to the population will result in a more reliable fixed map order, especially if more individuals in the population can be tested. The complexity of this hybrid might also contribute to the differences in map order and marker inversions. b40-14 is from an isolated population in a remote area of Mexico, which may have resulted in uneven recombination rates and greater representation for one allele over another.

When the map order of the 0705 consensus map was compared to other published maps (Doligez *et al.,* 2006; Riaz *et al.,* 2006; Lowe & Walker, 2006; Adam-Blondon *et al.,* 2004) it was consistent at 12 linkage groups. The other seven linkage groups showed minor inconsistencies or inversions when compared to published maps. On linkage group 1 VMC7g5 and CTG1011774 were inverted when compared to Riaz *et al*. (2006). Linkage group 2 on the 0705 map had VMC5g7 at the end of the chromosome but compared to Adam-Blondon *et al.* (2004) it was placed between VMC6b11 and VrZag93. The marker VMC9f4c (linkage group 3) on the 0705 consensus map was in a different position than other published maps. This particular marker segregated for multiple regions and showed different locations on the published maps. UDV075 on linkage group 8 maps at the end in the 0705 population but is located between VMC6g8 and UDV125 when compared to Doligez *et al.* (2006). The markers VVIV15.2 and VMC7h2 were inverted when the 0705 map was compared with Doligez *et al*. (2006) and the marker VMC2c7 on linkage group 13 placed in a different location when compared the same published map. The marker order of linkage group 14 is consistent when compared to Adam-Blondon *et al.* (2004) but VVIS70 and VVIN64 was inverted when compared with Doligez *et al.* (2006) and Riaz *et al.* (2006). VVIV67 on linkage group 15 placed in a different location when compared to Doligez *et al*. (2006). These inconsistencies might be due to scoring errors and the lack of markers to create a denser linkage map. The UDV markers showed inversions on the 0705 map, but these markers were only included on the map created by Doligez *et al.* (2006) for comparison. The 0705 marker order compared well with genetic maps developed by Riaz *et al*. (2006), Lowe & Walker, (2006) and Adam-Blondon *et al.* (2004).

When the marker order was compared with the Pinot noir map (Jaillon, *et al*., 2007; Velasco *et al.,* 2007), marker order differed on linkage groups 2, 6 and 13 for only one marker on each group. On linkage group 2, VMC7g3 and VMC5g7 was inverted on the 0705 and male map. VVIM43 placed higher on the 0705 map compared to the Pinot noir map and was highly distorted. On linkage group VMC3d12 and VMC9h4.2 was inverted in comparison with the Pinot noir map where VMC3d12 was distorted. These inversions might be due to scoring errors.

The 0705 genetic map identified genomic regions associated with segregation distortion. The female map showed 21 (15%) distorted markers and the male map showed 40 (25%). On

the consensus map, 51 (30%) of the markers showed segregation distortion. The percentage of distorted markers on the 0705 map was higher than the percentage found on a previously published *V. rupestris* x *V. arizonica* map (9%) (Doucleff, *et al.,* 2004). The higher segregation distortion ratios on the 0705 map could be due to the nature of the cross, i.e. the complex background inherited from the parents – as 161-49C introduces both *V. riparia* and *V. berlandieri* into the 0705 population. The majority of the distorted markers were on linkage groups 9, 10, 13, 14 and 19, which includes the three linkage groups where QTL's were found for *X. index* resistance. The markers close to the QTL regions were not distorted except for UDV124 on linkage group 13. When distorted markers were compared to other *Vitis* maps, similarities were found on linkage group 14, but linkage groups 3, 5, 14, 15 and 19 had different distorted markers compared to the consensus maps of Ramsey x Riparia Gloire (Lowe & Walker, 2006) and the 9621 population (Riaz *et al.,* 2006). If the same markers were used in both studies and the same rate of segregation distortion was observed, then factors like gametophytic selection might be considered. The distorted markers were mostly grouped on linkage groups 9, 10, 13, 14 and 19 on the 0705 map and excluding these distorted markers resulted in missing parts of the linkage groups.

6.4.3 Placement of the *Xiphinema index* resistance locus

Previous work done on a hybrid form of *V. arizonica*, b42-26, showed a major QTL on linkage group 19 (Xu *et al*., 2008). In the 0705 population, this marker was closely linked with *XiR2* on linkage group 9. Results indicate that the *X. index* resistance in b40-14 (0705 population) differs from that previously published using a different accession of *V. arizonica.* The, b42-26 accession, appears to be a *V. arizonica* x *V. girdiana* hybrid, but the b40-14 form appears to be a typical *V. arizonica* selection with small cordate and tomentose leaves. The resistance locus also mapped on a different linkage group; linkage group 9 on the 0705 map compared to linkage group 19 on the map created from the b42-26 background. In the Xu *et al.* (2008) study, the marker CD009354 was incorrectly named and placed. The correct marker is CTG1032918 as it was called in this study and was closely associated with *X. index* resistance on the 0705 map.

MapQTL analysis was done on all 19 linkage groups and revealed a major QTL on linkage group 9 and two minor QTL's on linkage groups 13 and 19. The log–transformed data was used in the QTL analysis and compared to a categorized dataset of the average gall numbers where four categories were used. QTL analysis was done on both these data sets but only minor differences of less than 1% were detected for the major and two minor QTL's. The major QTL (*XiR2*) on linkage group 9 (Figure 6.2) was placed between markers CTG1032918 and VMC1c10 on the consensus map, supported by a LOD score of 33.4 explaining 70.5% of the phenotypic variance for VMC1c10 and a LOD score of 29.83 explaining 59.1% of the phenotypic variance for CTG1032918. This *XiR2* QTL explains *X. index* resistance derived from the male parent, R8916-22 with a pure *V. arizonica* background. No QTL's were detected when the same analysis was done on the female parent. When this QTL was compared with Xu *et al.* (2008), the major QTL was detected on a different linkage group (19). The QTL, *XiR1* was located near the markers VMC5a10 and M4F3F with a LOD score of 36.9 explaining 59.9% of the phenotypic variance.

It is possible that a link exists between the *X. index* resistance from the work done by Xu *et al.* (2008) on linkage group 19 and the work done in this study on linkage group 9, 13 and 19. The minor QTL on linkage group 19 in the 0705 population was detected on the opposite side of the linkage group than *XiR1*, suggesting that the minor QTL in 0705 is not the same as

XiR1. The two minor QTL's on linkage groups 13 and 19 might not be entirely accurate due to a lack of markers. If these areas were better covered, the minor QTL on linkage group 19 might position more accurately. The distance of the *XiR2* locus from the flanking markers in the 0705 population was relatively far, which might skew the effect of the QTL. On linkage group 13 the flanking markers of the minor QTL were 3.9 cM apart, and on linkage group 19, they were 7.7 cM. More makers need to be placed on these linkage groups to further explain linkages to resistance QTL's. Then, additional seedlings will need to be screened to discover recombinants capable of shortening the genetic distance between flanking markers, and leading to map-based positional cloning of *X. index* resistance genes. The population will be retested prior to any submission of this framework map.

6.4.4 Future use of the map

In addition to *V. arizonica* other sources like O39-16 (*Muscadinia rotundifolia* x *V. vinifera* hybrid) and Börner (Becker, 1989) also exhibit resistance to *X. index* feeding. The rootstock, Börner (*V. riparia* x *V. cinerea*) is also highly resistant to phylloxera feeding (Zhang *et al*., 2009). In previous studies done on different rootstocks and their resistance to *X. index* feeding, tolerance and possible resistance was found, but never total immunity (Kunde *et al.,* 1968). By exploring these additional sources, a better understanding of the different mechanisms for *X. index* resistance will be gained, and in addition, the cloning of both *XiR1* and *XiR2* will provide insight into understanding the genetic mechanisms for *X. index* resistance.

The 0705 population is the only known population where a commercial rootstock (161-49C) was crossed with a typical form of *V. arizonica*. The 0705 map can be a valuable contribution towards understanding the inheritance of specific traits in complex inter-specific grapevine crosses. This map in addition to the male and female maps can be a valuable tool to research phenotypic characterization and rootstock traits for the wine, table and raisin grape industries. *XiR2* can be used to integrate *X. index* resistance with other traits, such as drought and salt tolerance, and/or phylloxera resistance into a single rootstock background with marker-assisted breeding efforts.

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Chapter 7

General discussion and conclusions

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Grapevine, *Vitis vinifera*, is a well known horticultural crop throughout the world. Because of its outstanding fruit quality this species is used for wine, table and raisin grape production. *V. vinifera* grapes are also unusual because of their susceptibility to a wide variety of pests and diseases (Riaz *et al.,* 2004). Soil-borne pests such as nematodes have been studied to develop proper control measures in grapevine management. The dagger nematode, *Xiphinema index* is of particular importance for the grape industry due to its ability to vector grapevine fanleaf virus (GFLV), the causal agent of the severe grape disease fanleaf degeneration (Hewitt *et al.,* 1958). There is a strong body of literature available on *X. index* anatomy and morphology, and its habitat, range and biology. Its interaction with grapevines in terms of damage, feeding, virus transmission and management has also been well documented.

Previous research done at the University of California, Davis laid a good basis for the work described in this dissertation. The aim was to broaden breeding efforts and better understand the genetics of grapevine rootstocks that are resistant to *X. index* feeding. All the work presented is based on the assumption that at least one accession of *V. arizonica* shows resistance to *X. index* as a single dominant gene.

Several *X. index* resistant *Vitis* species have been identified over the years using different screening techniques in the field and under greenhouse conditions. Greenhouse screens begin with the collection of nematode infested soil from which only the adult nematodes are extracted in a laboratory using sieving methods in combination with the Baermann funnel technique. These nematodes are then inoculated into potted plants to screen vines for their resistance or susceptibility. This procedure is labor intensive and requires long periods of time and large numbers of nematodes to fully assess nematode resistance. Because few nematodes survive the mechanical process of extraction and die after inoculation, only a handful of nematodes are responsible for feeding on grapevine roots. Thus, if all the nematodes die before feeding, a susceptible variety tested might be mistaken for resistant.

In this study, the tested genotypes were planted into a *X. index* infested soil mixture, as an alternative screening method. The method proved to be highly effective, possibly due to the presence of juvenile nematodes and eggs in the infested soil. The nematodes are minimally handled and thus are less damaged than in the traditional methods and have better feeding capability. The average gall numbers on roots of tested plants were significantly higher and damage occurred quicker with the infested soil inoculum compared to pipette inoculation of extracted nematodes. However, the soil must be thoroughly and gently mixed before planting vines in the infested soil to ensure even nematode distribution. Other nematode species are often present in field soils, so maintaining pure cultures in the greenhouse or shadehouse is recommended. This method may also prove to work well with other nematode species and crops.

Several grapevine rootstocks have been tested and classified as susceptible, moderately resistant or fully resistant to *X. index* feeding (Kunde *et al.,* 1968; Meredith *et al*., 1982). Research has also found that some grapevine species are resistant to GFLV, but the resistance to both the nematode and the virus in one genotype still needs to be found. The commercial rootstock, O39-16 (*V. vinifera* x *M. rotundifolia*), is resistant to *X. index* feeding and does not form galls on the roots, but GFLV is still transmitted into the scion. *V. arizonica* was previously found to be resistant to *X. index* feeding, but was never genetically exploited for this ability. Some of its accessions are also resistant to Pierce's disease, which further promotes its use as a breeding source with multiple resistances. The pure *V. arizonica* accession, b40-14, inherited

resistance to both *X. index* feeding and Pierce's disease. Other *V. arizonica* accessions and their progenies were tested for *X. index* resistance. The results were compared to two previous studies that used a different inoculation method. The *V. arizonica* genotypes tested were collected from areas in northern Mexico. Of the 18 genotypes tested, six had a different response to *X. index* feeding than found in previous tests. It is possible that these genotypes were incorrectly collected or labeled in the past, or that *X. index* resistance varies in *V. arizonica*, perhaps because of the multiple forms or hybrid complexes this species possesses. Genetic markers might be useful in determining the relationships and origins within this species. There is no record of *X. index* in Mexico, which makes the occurrence of the strong resistance noted in *V. arizonica* genotypes interesting. Since *X. americanum* is present in Mexico, as well as several other Longidorid nematodes, it is possible that *V. arizonica*'s resistance to *X. index* is a result of their resistance to Mexican *Xiphinema* or related species.

Three grapevine populations with *V. arizonica* parentage were tested for resistance to *X. index* to study the genetics of resistance to this nematode and to develop resistant rootstocks. The genotype, b40-14, a pure form of *V. arizonica* and its progeny from the R-series were used in these crosses. Previous work found that the *X. index* resistance in b40-14 was derived from a single homozygous locus which would result in all progeny from crosses with this genotype being resistant. The 0701 (R8916-07 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14) x R8916-32 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14)) and 0704 (161-49C (*V. riparia* x *V. berlandieri*) x *V. arizonica* b40-14) populations were created to confirm the homozygous nature of b40-14. The 0705 population (161-49C x R8916-22 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14)) was created to develop a genetic map of resistance in a pure *V. arizonica* line. The 0701 population held true to the expected segregation ratio of 3:1 (R:S), but the pistillate parent allowed *X. index* feeding. Previous studies suggested this genotype was resistant, but repeated testing found it to be susceptible. It is possible that the screening method in this study with the higher nematode numbers and more intense feeding pressure resulted in the susceptibility. The 0704 population also showed a different segregation pattern than expected. All the progeny should have been resistant, but a 1:1 (R:S) segregation ratio was observed. In this population the gall numbers were fairly low, not only for the progeny, but also for the susceptible parent, 161-49C. The 0705 mapping population segregated 1:1 (R:S) as expected, but was only significant at the 5% level. For both the 0704 and 0705 populations care was taken to ensure that the nematodes were distributed evenly in the soil; the control vines and parents used in the study confirmed that a well-mixed soil inoculum was used for the evaluations. The female parent for these populations was the same (161-49C) and it had low gall numbers in both cases, while some of the progeny sustained very high gall numbers. It is possible that some of these genotypes were off-types either by uncontrolled crosses or mistakes in plant material collection. The segregation ratio was peculiar for both these populations, which might be due to the interference of a second, susceptibility gene inherited from 161-49C. Further investigation is needed into the nature of 161-49C's response to *X. index* feeding to fully understand the segregation patterns observed in the 0704 and 0705 populations. The study confirmed that a single dominant gene might control the inheritance of *X. index* resistance derived from *V. arizonica*, which holds true for the 0701 population. However, the resistance inheritance might be more complex if there is a second gene from a different source interacting with the resistance gene derived from *V. arizonica*.

Molecular markers are used in grapevine breeding efforts to create genetic maps that associate resistance regions with specific genetic markers (Xu *et al.,* 2008; Zhang *et al.,* 2009). The 0705 population was used to create a genetic framework map for *X. index* resistance. Previous research found a major QTL region segregating for *X. index* resistance on linkage

group 19. Resistance in this population was based on a different form of *V. arizonica* (b42-26), which appears to be a natural hybrid with *V. girdiana*, while b40-14 appears to be pure *V. arizonica*. The *X. index* resistance in the 0705 population was placed on linkage group 9. Two smaller possible QTL regions were discovered on linkage groups 13 and 19. These two minor regions need further investigation. For this study the major QTL on linkage group 9 mapped close to the marker VMC1c10, indicating it was a different source of *X. index* resistance derived from *V. arizonica*.

The discovery of another strong QTL for resistance to *X. index* now allows the complexing or pyramiding of the two types of resistance. This will allow the simultaneous expression of more than one resistance gene, and promote a more durable resistance. The *X. index* resistance discovered in *V. arizonica* b40-14 is complemented by its strong resistance to Pierce's disease. This selection may possess other important traits and it is currently being screened for resistance to drought and salinity. Work is now beginning to verify the location of the second *X. index* resistance gene, *XiR2*, both on an expanded genetic map and through physical mapping. The analysis of the grapevine populations created in this study, and of the evaluated genotypes and traits will further our knowledge of the breadth and durability *X. index* resistance. It will also help characterize useful alleles and mutations that may be important in the future for both forward and reverse genetic approaches in the study of grapevine biology.

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