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Title: Biology and Demography of Grape Phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Homoptera: Phylloxeridae), in Western Oregon

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Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) development was studied under field and laboratory conditions. The aphid-like insect feeds on *Vitis vinifera* L. roots producing swellings that decay and lead to vine decline. The only long-term economic solution for control of grape phylloxera (GP) is to plant vines grafted onto a resistant rootstock, but over 6000 acres of Oregon's wine grapes are planted with root-susceptible *V. vinifera* cultivars. Ten GP infestations have been verified throughout the state since August 1990. The objective of this research was to characterize GP development and within-site distribution in Western Oregon.

Grape phylloxera were collected from roots dug at two depths (15-30 cm and 30-45 cm) in three infested vineyards in the Willamette Valley during the summers of 1992 and 1993. A sieve centrifugation method was used to extract GP for counting. The number of eggs, nymphs, n/adults (late-instar), and adults per 30 grams of roots were recorded twice a month. Mean population levels were calculated for each site, sampling date and depth.

Population development of GP varied between sites and between dates sampled. Population size appeared to be directly related to soil temperature. The site with the highest overall GP populations had the warmest soil temperatures but the least above-

ground symptomatic vine decline ratings. Nymphal population peaks, identified at one site, may correlate with generation time or changes in root and soil environments.

Similar numbers of GP were found at both depths sampled, but higher populations were collected from vine roots sampled on the downhill side of an infestation and from vines immediately adjacent to satellite infestations. Sticky-trunk-wraps, placed on the base of vine trunks from June to November at the three sites, collected emerging crawlers (nymphs) and winged-alates in July/August of 1993.

Development of GP populations collected from each of the three sites was studied in the laboratory at five constant temperatures (7, 10, 13, 16, and 21 °C). No significant differences in development were observed between the three populations. Grape phylloxera generally did not molt past the nymphal stage at 7, 10, 13, and 16°C. The first adults appeared before day 35 at 21°C and this was followed by a significant increase in first generation eggs.

In contrast to the laboratory results, eggs were first recovered from field extractions in April/May when mean soil temperatures were 10.5 to 12.0°C in 1993. Eggs were not produced from laboratory populations below 16°C. These results indicate that either the field conditions allowed GP to develop at lower temperatures or the laboratory assay did not provide sufficient time to full development. The low temperature threshold calculated for zero development from the laboratory assay was 5°C. A degree day accumulation, which better reflects GP field development, was estimated at 437.5 using maximum field soil temperatures and a vine development threshold of 10°C. Further use of degree day models may help to predict generation time of GP.

# Biology and Demography of Grape Phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Homoptera: Phylloxeridae), in Western Oregon

by

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## Table of Contents

Page

## Table of Contents (cont.)

Page

## List of Figures

## List of Tables

Page

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## CHAPTER 1. Introduction

Oregon's wine grape acreage increased 40% between 1987 and 1993 (Oregon Agriculture Statistics, 1987; 1993). The major grape growing areas of the state are located in the Willamette Valley and Southern Oregon. Most vineyards are planted with own-rooted vines as opposed to vines grafted with grape phylloxera-resistant rootstocks.

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Homoptera: Phylloxeridae), a sucking, aphid-like insect, was discovered in a commercial vineyard in Oregon in August of 1990 (Connelly and Strik, 1993). Ten infestations had been verified throughout the state by the spring of 1995 (Strik et al., 1995). Grape phylloxera (GP) feeds on the roots and leaves of *Vitis* species creating gall-like formations which lead to vine decline. The feeding sites and life-cycle are dependent on host parentage and climatic conditions. Insecticides have not been cost effective in controlling soil-borne GP, because this insect can be deep in the soil profile and out of range of effectiveness of chemicals (Buchanan and Godden, 1989; Coombe, 1963; Smith, 1993; Stevenson, 1968). Grafting susceptible cultivars onto a resistant rootstock has been the only viable method for control (Pongrácz, 1983; Walker, 1991).

The insect has a complex life cycle with eighteen different forms primarily regulated by the host species of *Vitis*. When found on the European winegrape, *V. vinifera*, GP feed on the roots creating gall-like formations. The root gall symptom is a plant response from the aggregate feeding of GP with their long stylet-like proboscis. These abnormal swellings are classified as "tuberosities" when feeding sites are on the mature roots and "nodosities" when sites are located on new feeder roots (Davidson and

Nougaret, 1921). Tuberosities cause greater root damage (and consequently vine damage) than nodosities. Vine decline, due to GP feeding, is caused by the plant's inability to take up nutrients and water from its injured root system. GP can reproduce in small numbers on many resistant rootstocks without causing economic injury. The rootstock AXR#1 (Ganzin 1 and ARG 1), a hybrid between the susceptible *V. vinifera* 'Aramon' and the American species *V. rupestris,* is susceptible to injury by a GP biotype (Granett et al., 1985). GP biotypes will be discussed in further detail later in chapter 2.

As the biology of GP is affected by climate, the demography of this pest needs to be studied under Oregon conditions. A better understanding of biology is essential for growers to forecast vineyard replanting. Detection and monitoring are also pre-requisites to understanding dispersal of GP.

The primary objectives of this research project were:

1) Determine the critical dates of occurrence of specific grape phylloxera life stages including when the over-wintering form comes out of hibernation, when GP emerge above-ground, and when GP enter hibernation.

2) Determine the number of generations per year of GP, when peak population periods occur, and compare field results with laboratory calculations of generation times.

3) Determine the low temperature threshold of a representative population using laboratory and field studies.

# CHAPTER 2. Literature Review

## Biology

### Systematics

Grape phylloxera is classified in the order Homoptera along with cicadids, leaf hoppers, psyllids, white flies, aphids, and scale insects (Borror et al., 1989). Its suborder, Sternorrhyncha, is further divided on the basis of the antennae, tarsal segments (never more than two for Sternorrhyncha), wing structure, wing venation, and rostrum (beak) position. The rostrum arises posterior between the front coxae, allowing the insect to feed on the phloem of vascular plants (Dolling, 1991). It is difficult to identify members of this suborder without mounting specimens on a slide. The next lower taxonomic group is the super family, Adelgoidea, which is characterized by three oblique veins on the forewing and three antennal segments. Included in this super family is the family Adelgidae, the spruce and pine aphids. A distinguishing feature which separates Adelgids from Aphids is that adelgids are "oviparous" (egg layers), while aphids are "viviparous" (give birth to live young). The next lower taxonomic group is the family Phylloxeridae. There are several genera within Phylloxeridae that feed on other vascular plants besides grapes. These include hickory, pecan oak, willow, and poplar (Dolling, 1991).

Grape phylloxera was first described in 1856 by Asa Fitch, New York's first state entomologist. He misnamed it as *Pemphigus vitifolia* (Riley, 1872). Ten years later Shimer and Walsh disagreed as to whether it was a scale insect, Coccidae, or a plant louse, Aphidae (Smith, 1992). Shimer went on to establish the family Dactylosphaeridae and a genera within it, although one already existed. Charles Valentine Riley, a young assertive Missouri State Entomologist, wrote a great deal through the State Board of Agriculture in

hopes of resolving the confusion (Smith, 1992). Riley helped establish the identification of the leaf and root forms which brought resolution to the nomenclature conflict.

Grape phylloxera was introduced to England and France between 1854 and 1860 on American vines which were imported for their resistance to powdery mildew. Riley worked closely with his French colleagues, Planchon and Lichtenstein, on taxonomy. Planchon named it *Phylloxera vastatrix* (Riley, 1874), and in 1868 Lichenstein proposed that the European and American louse were the same insect (Riley, 1874). Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), was renamed several times and can be found in current literature under the alternate names: *Viteus vitifoliae* (Fitch) and *Dactylosphaera vitifolii* (Shimer).

By 1874 this insect had been described with eighteen different life stages including the root-and leaf-feeding forms (Riley, 1874). It was also realized at this time that phylloxera existed in a parthenogenic as well sexual form. In 1872 Laliman, an ampelographer in France, observed that *V. aestivalis* was not destroyed by GP and later proposed the grafting of European stock onto resistant species (Pongrácz, 1983). Attention was then directed towards the native species of grapes in North America which were observed by Riley to have various degrees of resistance to GP. Riley solicited help from George Englemann in the area of botanical classification. Englemann later went on to describe nine species of *Vitis* (Riley, 1874). Significant changes have occurred since then, and eighteen species of *Vitis* are now recognized in North America.

The first evidence of grape phylloxera in California was in 1858, although verification as to the cause of the large number of dying vines was not made until 1873 (Davidson and Nougaret, 1921). This is surprising since European grapes were introduced into California by the Padres of the Roman Catholic Mission in the 1500's. The 'Mission' and 'Catawba' grapes were the primary cultivars grown until the 1860's, when the governor of California commissioned for the introduction of new Asian and European cultivars. Whether GP came directly from the Eastern United States or via

Europe is still unknown. Further infestations in the state were thought to be the result of accidental distribution of contaminated 'Zinfandel' nursery stock from Napa County. Morse reported the observation of the leaf formed gallicolae in 1884, but upon further investigation through laboratory experiments development of the insect could not be forced past the winged form (Davidson and Nougaret, 1921).

It is believed that California's unfavorable climatic conditions prevent the appearance of the "sexuparae" (sexual forms) (Davidson and Nougaret, 1921). However, Lopez Christobal determined in 1943 that alate eggs were common in San Juan, Argentina, but that they developed into radicicoles, not males and females (De Klerk, 1974). In the Ukraine, researchers found alates and nymphs developing on the leaves implying that a complete life cycle may occur above ground (De Klerk, 1974). Foliar GP was found on *V. vinifera* in Italy during a survey of grafted and ungrafted grape vines in five regions of North-Central Italy (Conti et al., 1985). One own-rooted vineyard showed few symptoms, and several vineyards grafted to 'Kober 5BB' were heavily symptomatic of foliar feeding. The association of rootstock/scion was thought to influence the susceptibility of plants.

**Grape Phylloxera's Life Cycle and Biology**

The life cycle of grape phylloxera is complicated and partly dependent on the species of *Vitis*. The following is a description of the insect's biology on *Vitis vinifera* without the influence of a resistant rootstock. This was first described by Davidson and Nougaret (1921) in California from a comprehensive study performed in the early 1900's .

The major over-wintering form of grape phylloxera is an unmolted, brown nymph or "hibernant", while the remaining stages found during the winter months are later instar hibernants (having molted 1-2 times). In the winter, GP are generally found clustered on

older roots. In spring, when soil temperatures warm and vine sap flow commences, hibernants begin development. The initiation of development occurs over a protracted period, and some hibernants may mature to adulthood six weeks in advance of others. Larval hibernants, in general, proceed to adulthood more slowly than summer broods. Both broods are influenced by the quality of food, soil moisture, and soil temperature.

After four molts, the hibernants reach adulthood and begin laying the eggs of the first generation. Many of the newly hatched nymphs from this generation will settle on the fleshy young roots and begin feeding, creating the nodosity swellings. Other nymphs will feed on older roots, creating the tuberosity swellings.

Little is known about the induction of root gall formation. Granett (1990) tested the hypothesis that galls, induced by the application of indoleacetic acid (IAA is found in grape phylloxera saliva), were functionally similar to tuberosities induced by GP feeding. He concluded that galls induced by GP were different than those induced by IAA. Other factors in addition to IAA must be involved in the mechanism of tuberosity formation (Granett, 1990).

Some nymphs will immigrate to new vines through cracks in the soil and establish new colonies (Davidson and Nougaret, 1921). Once fastened to a vine root, the insect is referred to as a "radicicole" and at maturity reproduces parthenogenetically. Resultant offspring mature in four to seven weeks. Under favorable conditions, egg deposition averages 2-3 eggs per day over a 45 day period but can extend to 110 days under optimal conditions. Egg incubation is greatly influenced by temperature and can vary from five days in July to 27 days in April. Grape phylloxera molt four times and, with the exception of the adults, the first instar nymph has the longest stasis.

Above-ground crawlers (mobile early instar nymphs) occur from July to September and can be blown in the wind creating satellite infestations. Again, single insects can start infestations due to their parthenogenesis. Crawlers have been caught in above-ground sticky traps 20 m from the nearest vine (King and Buchanan, 1986). Nymphs with wing

pads appear after the second generation and will emerge as winged adults between June and October (Davidson and Nougaret, 1921). In July and August, incubation and development proceed rapidly and nymphs can mature in 15 days. Active feeding during this period will lead to the subsequent decay of roots. The onset of fall rains and high soil moisture leads to further root decay. From late-September to early-November, newly hatched nymphs become brown-colored hibernants, and by the middle of December hibernants are the predominant form found on roots. Davidson and Nougaret (1921) speculated that there were five generations per year in California, and under certain conditions the number of generations could range between one and nine.

The life cycle of grape phylloxera on American species and French-American hybrids becomes increasingly complex with the foliar form predominating. Leaves with GP galls have reduced net photosynthesis (McLeod and Williams, 1992). When foliar GP populations reach high levels, vines undergo premature defoliation and have reduced shoot growth, yield and quality (McLeod and Williams, 1992). This in turn leads to delayed ripening, reduced fruit quality, and winter injury (McLeod and Williams, 1992).

The following is a grape phylloxera life cycle description by McLeod and Williams (1992) from Ohio, where the primary cultivars are French-American hybrids. The over-wintering stage begins as either a winter egg found under the bark of the trunk and older canes, or as nymphs (hibernants) on the roots. The winter egg hatches in early spring giving rise to the "fundatrix" or stem mother which, upon feeding on the newly emerging leaves, creates a gall. She becomes enclosed within the leaf gall and can lay 200 eggs in her lifetime.

The nymphs emerging from leaf galls can either crawl up the shoot to new fleshy plant material, inducing further galls, or they can move below-ground and begin feeding on roots. They cannot feed within the galls in which they hatched; the nymphs must seek a new food source. The subterranean life cycle is much like that found on *Vitis vinifera*. The extent to which GP is in the terrestrial or subterranean form is largely dependent on

the parentage of the grape vine. With the French-American hybrids, the greater the portion of European parentage, the greater the amount of feeding on roots.

From July through October, below-ground nymphs with wing pads appear, giving rise to "alates" (winged adults). The winged female emerges from the soil and lays male and female eggs on branches and trunks of vines. These eggs hatch into beakless sexual forms, molt four times, mate, and the female lays an over-wintering egg. There are three to five foliar generations per year in North America (McLeod and Williams, 1992)

The production of alates verses crawlers is thought to be regulated by the host plant (Hawthorne and Dennehy, 1991). *Vitis labrusca,* 'Concord' sustains root populations without loss of productivity while 'Aurora' [*(V vinifera* x *V. rupestris)* x *V.lincecumii]* is highly susceptible to the leaf form. When the two cultivars are planted in close proximity reciprocal movement of alates and crawlers may occur. Alates emerging from the large ground populations found on 'Concord' flew to 'Aurora' to complete the sexual part of the life-cycle. Crawlers emerging from 'Aurora' leaf galls were then blown in the wind and colonized the 'Concord' root system (Hawthorne and Dennehy, 1991). Some concern exists over the genetic recombination that takes place when GP complete the sexual part of the life cycle. Will the production of new biological races emerge as a result of this phenomenon?

The biology and ecology of grape phylloxera in South Africa has been examined in great detail in greenhouse and field studies (De Klerk, 1974; De Klerk and Loubster, 1988). Root performance under South African sandy, dryland conditions is determined by the volume of soil fully colonized by the roots. The percentage of finer roots becomes increasingly important, because this is the zone of active absorption for nutrients and water. Seasonal root-growth is the factor most influencing the occurrence of GP (De Klerk and Loubster, 1988).

Grape phylloxera has been present in South Africa since 1886, with root inhabiting forms being of primary concern. Seasonal fluctuations of GP under southern hemispheric

conditions are: in September when buds break, no active insect forms are present in the soil; late September to late October, GP populations increase along with root growth; November, populations increase rapidly and, by January, the "summer" populations peak; March, numbers decrease and by April hibernants appear. Leaf drop commences in May and, by June, the hibernant populations peak. From July to October hibernant populations start to decrease; this time, the insects protected from unfavorable conditions (e.g. under the bark) survive, not those found on nodosities and tuberosities (De Klerk, 1974).

The induction to quiescence or hibernation of grape phylloxera by vine root condition and sap flow was further proven by the rearing of insects under glasshouse conditions with a mean temperature of 23°C (De Klerk, 1974). Twenty metal soil cages with sandy loam soil were maintained at 19.8 to 23.0°C for two seasons in a greenhouse. Ungrafted grapes, 'Fairy' and 'Metallica', were infested with insects and nodosities appeared 30 days later. Results indicated that the average incubation period for eggs was 10 days. The development from nymph to adult female averaged 12 to 13 days. The development time to nymphs with wing pads took 12 days and the time from nymph to alate averaged 8 days. The average fecundity was 32 eggs per female (gross reproductive rate) with an average of three eggs per day (De Klerk, 1974). This average was less than that observed by Davidson and Nougaret (1921). The average life span of adults calculated by De Klerk (1974) was 14 days. Hibernants were found on nodosities of dead or weakened roots throughout the year, except in October and November (De Klerk, 1974).

Weekly field sampling of grape phylloxera over a two month period was performed in one infested vineyard in South Africa planted with Jacquez rootstock (De Klerk, 1974). Populations were classed as none, low, high and very high. He maintained in this study that it was impractical to determine the number of individuals on a standard size root piece per vine, because GP numbers differed considerably between roots and between vines.

De Klerk (1974) theoretically determined the number of generations per year from the radicicole activity data, the results of field experiment, and the average generation time in the greenhouse. Calculations were made with an average air temperature of 19.4°C. The period of activity was divided by the generation time with the results indicating 10 generations per year under South African conditions.

Two infested vines were excavated in the field in South Africa with holes dug 1.2 m deep adjacent to plants (De Klerk, 1974). Root infestations were examined microscopically, and populations were rated as; none, low, high, and very high. Grape phylloxera were present at a depth of 1.2 m with the highest infestation found in the top 30 cm of soil.

Twenty vineyards, 10 to 15 years in age, were sampled to assess the influence of soil type on infestation (De Klerk, 1974). All roots were excavated to 30 cm in depth on randomly selected vines. GP populations were highest in soils with a higher percentage of clay and silt and lower percentage of sand.

Alates were recovered in greenhouse cages on American and *V. vinifera* rootstocks during a four month period (De Klerk, 1974). The observance of nymphs with wing pads followed a similar pattern to alates, but the first occurrence of nymphs was one month in advance of alates. Alates were caught in field cages in South Africa from January 1 to February 1 (De Klerk, 1974). De Klerk (1974) concluded that the rarity of gallicolaes (leaf forms) in South Africa was possibly due to the "sporadic occurrence of sexuparae", and this was thought to be due to the minimum temperature thresholds necessary for development. De Klerk found that the commencement and termination of the nymphal stage with wing pads was regulated by air temperatures above 16°C.

**Biotypes of Grape Phylloxera**

Biotypes or races of grape phylloxera may occur in the grape growing regions throughout the world. The criterion for the establishment of GP biotypes is defined as: a group within the same species which performs differently on different *Vitis* species or cultivars. Research in this area was conducted in Germany by Börner (1914) and King and Rilling (1985), in South Africa by Perold (1927) and De Klerk (1974), in Canada by Stevenson (1970), in New Zealand by King and Rilling (1985), and in the United States by Granett et al. (1985, 1987, 1992) and De Benedictis et al. (1992, 1993).

Grape phylloxera biotypes have expressed their traits through a difference in resistance of rootstocks. One such study was performed in New Zealand by King et al. (1982a) with a modified version of De Klerk's (1974) glass-sided pots (for GP observation) under greenhouse conditions. Five rootstocks (1613C, 1202C, AXR#1, SO4, and 5BB), and one-own rooted *V. vinifera*, 'Mueller Thurgau', were planted into replicated observation pots and held between 15 and 25 °C. Grape phylloxera were introduced on nodosities with roots examined weekly for insect and nodosity numbers adjacent to the glass. Nine days after the initial infection nymphs were observed feeding on 'Mueller Thurgau' roots. Nodosities were observed on 1613C and AXR#1, and damage was found on the larger roots on 1613C. 'Mueller Thurgau' had severe tuberosities that had begun to rot. GP did not establish and reproduce on 5BB or SO4, while AXR#1 supported large populations of eggs. Grape phylloxera-root interactions were also observed in this study, and a "birds head" reaction (a doubling back) occurred on the root tips of 'Mueller Thurgau' and AXR#1. It was concluded by De Klerk (1974) and subsequently by King and Rilling (1985) that rootstocks showing resistance to GP in one country might not in another. It was debated whether it was the difference in growing conditions or the occurrence of biologically different races (King et al., 1982a).

The first evidence of biotypes in California occurred in 1983 when a 15-year-old vineyard in Rutherford, grafted onto AXR#1, showed signs of grape phylloxera damage (Granett et al., 1985). AXR#1, a hybrid between *V. vinifera*, 'Aramon' and an American species, *V. rupestris*, was selected as a rootstock that showed sufficient levels of resistance and good viticultural characteristics. The new biotype obtained from the infested AXR 1 vineyard was called type-B, and all previous GP populations were designated as type-A. The standard laboratory protocol was to compare type-B's reproductive capacity against a known population type-A. Granett et al. (1985) calculated life table parameters from fecundity ($m_x$ or eggs per female per day), and survivorship of individuals to next age class ($l_x$). A conventional formula (Birch, 1948) to calculate life-table parameters was used with the objective of determining if type-A and B were distinct biotypes (Granett et al., 1985). Type-B insects were found to mature more rapidly on AXR#1 in the first 35 days than type-A, and type-B laid twice as many eggs. The life-table was calculated using a combination of survivorship, developmental rate, and fecundity. Results indicated that type-B will potentially increase in the field more rapidly than type-A on AXR#1. Granett et al. (1985) identified this as the reason for the Rutherford vineyard's decline. They were not able to explain the nature or source of this virulence. Was it an introduction of a new race and therefore a point source introduction, or was it phylloxera's adaptation to 40 years of AXR#1 rootstock use in California?

Song and Granett (1990) determined the presence of biotype races in France. Colonies were established from three grape cultivars (41B, 3309C, and 'Alicante Bouschet'), collected in three locations. Population data for the developmental time and fecundity were recorded for insects on tuberosities only (Song and Granett, 1990). Damage seen in the field is largely due to the feeding of grape phylloxera on older roots as opposed to feeding on root tips which results in nodosities. A substantial number of GP established on tuberosities with 41B, a widely planted rootstock in France. The hypothesis of a "host-based" biotype was supported by the fact that GP obtained from one

rootstock (e.g. 3309C) performed better on that rootstock than grape phylloxera obtained from another rootstock (e.g. 41B). Survivorship was the most important parameter for determining differences in populations in this study, and second to that was fecundity (Song and Granett, 1990). This was in contrast to the work done in California in which developmental rate and fecundity were the two determining parameters (Granett et al., 1985). Many questions are still unanswered with regards to host-based versus geographically based differences in phylloxera populations. Would population virulence increase with continued exposure to a moderately resistant host or is it fixed?

Granett et al. (1992) used an egg multiplication index (EMI) to test the susceptibility of *V. californica* to biotypes A and B. The algorithm $1/n \sum [ A_i/100 \times (45-D_i) \times F_i ]$ was calculated with A= the percent maturing to adulthood, D= the developmental times from egg to adulthood, F= the fecundity, or eggs per female per day in the first 9 to 12 days, and n= the number of root pieces. Susceptibility of *V. californica* varied considerably and was independent of site or proximity to cultivated plants. The localized host resistance to GP biotypes of *V. californica* could be helpful for plant breeders in selection of parentage. Low phylloxera numbers associated with the riparian *V. californica* was thought to be the result of its sandy habitat (Granett et al. 1992).

## Temperature

### Temperature and Insect Metabolism

Limited ranges of environmental temperature and humidity can influence the enzyme function of insects and since insects are poikilothermic, their body temperature approximates the ambient temperature. Humidity is not as critical as long as a water balance can be maintained through feeding. An insect's response to temperature change

can vary with the previously experienced temperatures (Chapman, 1982). The insect body temperature changes more slowly than the outside temperature and response generally occurs with persistent temperature changes. The body temperature is also regulated by the balance between heat loss and heat gain. Cooling can occur from latent heat of evaporation and a dryer environment will increase evaporation. In this case, an insect's body temperature can be 3-4°C below ambient (Chapman, 1982).

Optimum ranges for diapause are significantly lower than temperature ranges for development. Many temperate species require temperatures below 10°C to complete diapause. Insect tissues do not freeze until temperatures are well below 0°C due to the presence of electrolytes and glycerol in the haemolymph and the effect of super cooling. Diapause and hibernation can occur at a lower temperature when an insect has not fed. Food in the gut can provide a nucleus for ice crystal formation which eventually leads to rapid freezing (Chapman, 1982). It is uncertain whether grape phylloxera undergoes a true diapause during its hibernating period but feeding may influence hibernant survival. Futher research is needed to determine the effect feeding on low temperature survival of GP.

Development is not completed at temperatures below the extreme thresholds. It is important to distinguish between three temperature thresholds that at which: 1) no development takes place, 2) moderate differentiation takes place, and 3) full development takes place. An example of moderate differentiation in grape phylloxera could by the situation in which the insect has not molt past the first instar stage.

**Temperature Models**

Many mathematical models have been constructed to predict insect population growth rates. Population growth rates are dependent on generation time and, in turn,

generation time is influenced by temperature (Southwood, 1978). While it is easier for biologists to track a population with a calendar, the actual physiological time can better describe development. The computing of degree days, which best illustrates physiological time, is calculated by the total accumulated time x temperature (above the threshold). Degree day determination has been done by sine wave computing, by field temperature integrators, and by monitoring insect development at a constant-temperature with growth chambers (Southwood, 1978).

Threshold temperatures for development have been determined with pear rust mites, *Epitrimerus pyri* (Nalepa), using an *x*-intercept method and the reciprocal of the slope to determine the median for degree day development (Bergh, 1994).

In a study by Roltsch et al (1990), linear and nonlinear functions were fitted to data at constant and fluctuating temperatures for western grapeleaf skeletonizer *Harrisina brillian* (Barnes and MacDunnough). The linear degree day model best predicted skeletonizer larval development in the field. They found that functions derived from constant temperatures or the means obtained in fluctuating temperatures can underestimate development rate at low temperatures and overestimate development rate at high temperatures. Current models using constant temperature thus may not account for the time required in the changes in rate controlling mechanisms.

**Temperature Thresholds**

Soil temperature may critically influence the development of grape phylloxera in California (Granett and Timper, 1987). Regional severity of GP-induced vine decline can be influenced by soil temperature once soil type has been accounted for. In an experiment conducted by Granett and Timper (1987) life-table parameters were calculated on laboratory populations of biotype A grape phylloxera, reared on excised *V. vinifera* root

pieces with two ranges of temperatures, 7 to 39°C and 16 to 32°C. Duration of life stages, generation times, and doubling times were greater at 16°C than at higher temperatures up to 32°C, at which no insects survived to the adult stage. Previous work by Davidson and Nougaret (1921) indicated that hibernation began at temperatures below 19°C and activity resumed when temperatures exceeded 15°C. Granett and Timper's (1987) laboratory work confirmed this. They found the greatest fecundity between 21 and 28°C in their laboratory bioassay. Results were then compared with soil temperatures of three grape growing regions in California (Granett and Timper, 1987). In the central valley, where populations grow slowly, winter soil temperatures drop below 16°C, and summer soil temperatures can exceed 32°C. In the northern coastal region, where GP can be quite damaging, soil temperatures are favorable throughout the summer. In this region it is reported that vine mortality due to GP doubles each year. The Salinas Valley has winter temperatures that never drop below the threshold, and reproducing GP can be found throughout the year. In this region phylloxera-induced vine mortality can have a 4-fold increase. Granett and Timper's research (1987) supported field observations, but they also concluded that biotypes may have different temperature optima.

Temperature thresholds were established for foliar grape phylloxera by Belcari and Antonelli (1989). Duration of development of the nymphal stage as well as total developmental time was calculated at constant temperatures of 13, 15, 18, 20, 25, 30, 32, and 35°C. Replicated plants of the hybrid, 'Kober 5BB', were infested with nymphs. At 13°C the first instar (L1) did not complete its development, and at 15°C the duration of development was 45 days. The L1 took 40% of the total developmental time in each of the temperature treatments, and L1 was the most resistant life stage to lower temperatures. Developmental zero, temperatures at which no further development takes place, was established for L1 by three formulae: thermal summation, regression, and Davidson's equation. The developmental zero was 6.4, 5.1, and 3.2°C, respectively, indicating results can be affected by the choice of formula (Belcari and Antonelli, 1989).

Optimal temperatures for foliar phylloxera were from 25 to 30 °C, when mean generation time was 16 days and a low level of mortality occurred (Belcari and Antonelli, 1989).

## Distribution and Detection

### Distribution of Phylloxera

The abundance of grape phylloxera within a grape growing region has been studied by many entomologists. Investigative surveys are necessary to understand the prevalence of phylloxera. Stevenson (1963) reported in a survey conducted in the Niagara Peninsula of Ontario that 72 out of 73 vineyards surveyed had GP present on the roots, and in 28 of the vineyards all of the vines were infested. Stevenson (1963) sampled roots in August and September from 'Concord', 'Deleware' and several French-American hybrids. A "gall index" rating was established on the number of nodosities per unit of rootlets. Many of the vineyards had 90 to 95% infestation, which meant that the insect was found on all but two of the sampled vines.

Stevenson (1963, 1964) also looked at the distribution within soil types and found a considerable variation that he attributed to vine age and cultivar. The number of galls were, however, significantly lower in the sandy loam than in the heavier clay soils. This survey did not attempt to determine the correlation between vine decline and the number of nodosities.

The distribution of grape phylloxera in Southeast Australia was studied in a survey of 281 vineyards in Central and Northeastern Victoria where "Vine Disease Districts" quarantine the movement of plant material (Buchanan, 1987). Vines were sampled for root symptoms at 0.5 m in depth. Vineyards were classified as infested if GP was found on any of the vines. Infested vines were found in 41 of the 281 vineyards surveyed.

Infestations were more often in either older vineyards, vineyards with plant material obtained from a grape phylloxera-infested source, or vineyards grafted to resistant rootstocks.

**Infrared Aerial Photography**

Rate of spread of above-ground symptoms can be difficult to quantify. Other problems such as nematodes, shallow soils, and plant pathogens must also be considered. The typical above-ground symptom of a grape phylloxera infestation is a "lens shaped" weak area, where vines in the middle are dead or in severe decline and vines on the perimeter of the lens appear more healthy. Other vineyard soil or plant borne pathogens do not produce as distinct a symptom. The effect of GP on vine health is a reduction of shoot growth, the discoloration of leaves, and early leaf fall. The best way to detect an infestation early is with the use of infrared aerial photography (Wildman et al., 1988). There is a four to six year lag period between initial GP infestation and the ground observation of vine decline (Wildman et al., 1988). Aerial detection can occur two to three years in advance of ground detection.

Infrared is within the electromagnetic spectrum and has longer wavelengths than those visible to the human eye. Plants naturally reflect green and infrared while absorbing red for photosynthesis. Infrared film introduces an infrared dye layer onto the film replacing blue. The image formed upon exposure and development is a false color (Wildman, 1992). Unhealthy plants will reflect less infrared and appear less red when captured on infrared film.

Infrared can be used to establish a color variation that can be compared to a ground sampling or "truth". In a study conducted in Gisborne, New Zealand, 250 individual vineyard blocks were assessed by an aerial survey for color variation and a

ground survey for vine vigor (King et al., 1982b). Six classes were established from the ground truthing. Changes in the infrared image along a transect were correlated with GP population, root damage, vine health, and fruit yield. Results of the aerial survey indicated a majority of the blocks examined showed less than 15% damage (within block), and 76% of the vineyards showed some vine decline. This high proportion of "first visible" damage alarmed the survey team due to the tremendous potential for spread. Grape phylloxera ground truthing established that 35 out of the 38 transects examined had insects. King et al. (1982b) speculated that the rate of vineyard decline in productivity will be affected by the number of infestations within the vineyard, the rate of spread of the insect, and the rate of individual vine decline. Vine decline was shown to vary considerably between vineyards. This was theorized to be the result of differences in soil micro flora and vineyard cultural practices such as nutrition, irrigation, drainage, and weed control (King et al., 1982b). Biotypes had not been researched in New Zealand at this time.

## Control

### Chemical control

Treatment of grape phylloxera-infested grapevines with insecticides has had limited results. At best, when combined with optimum viticultural practices, insecticidal fumigants and drenches may delay or reduce the decline of vines (Buchanan and Godden, 1989). Cultural practices, cultivars, and soil characteristics can greatly influence the efficacy of chemical control. The following chronological review will illustrate the extensive research in this area and hopefully also show that the ultimate control measure for GP is use of a suitable resistant rootstock.

By 1962, 98 % of the grapes grown in Ontario, Canada were located on the Niagara Peninsula. Most of the cultivars grown were American and French-American hybrids. American species consisted of wild vines and earlier cultivated varieties and, with this diversity in parentage, both the root and the leaf inhabiting forms of GP were found. Stevenson (1968; 1970) examined the use of both soil and foliarly applied chemicals. In his soil experiment Stevenson (1968) used Baygon (o-isopropoxyphenyl methylcarbamate) at three rates, and Zinophos (o,o-diethyl o-2-pyrazinyl phosphorothioate) at two rates. Drenches were applied in a well-drained, clay-loam soil. The Baygon EC reduced GP galling on 'Agawam', 'Delaware', 'Catawba', and 'Elvira', but the treatment was not consistently effective. Previous experiments indicated some phytotoxicity existed with the EC formulation (Stevenson, 1968). Zinophos was less effective, and Stevenson's general conclusion was that, while the drench method was superior to other forms of application, chemical control was of little practical value in Ontario. In the foliar control experiment, summer applications of insecticides showed better control than dormant applications. Endosulfan was effective in reducing foliar GP but, in general, the moderate infestation of this experiment did not warrant the use of insecticides, because no reduction in yield could be attributed to the level of phylloxera found in the controls. Threshold limits for foliarly-applied phylloxericides would be appropriate for French-American hybrids as injury by moderate infestations maybe largely cosmetic.

Coombe (1963) reported that Argentina's use of carbon-bisulfide at 30 to 35 g per square meter was an effective phylloxericide, but at 40 g per square meter the vine dropped leaves. Well aerated soils were necessary, because impermeable soil layers resulted in concentrations of material that became phytotoxic. The level of control established in this application did not appear cost-effective and the author did not report any residue-testing of the subsequent first harvest.

The control of the leaf-galling form of phylloxera with foliar and sub-surface applications of 16 different experimental and commercially available chemicals was done

by Williams (1979). Tests in Ohio were conducted from May to August to coincide with the above-ground activity of the gallicolae. American and French-American hybrids are common to this grape growing region where, in addition to the root-feeding form, growers must contend with defoliation due to the heavy leaf galling. The most effective foliar insecticides were phosalone and endosulfan. Subsurface application of granular aldicarb was effective in years with high rainfall (Williams, 1979).

A granular formation of carbofuran was tested against the root form under a variety of soil conditions in California (Rammer, 1980). A biennial treatment of 11.2 kg ai/ha (24.89 lbs/2.47 acres) post harvest resulted in a 99% reduction of GP. However, no pre-treatment sampling was performed in this study to determine the variance between sample counts. In addition, insect counts were calculated on the root surface area, expressed as $A = \pi dl$ (d = diameter, l = length), where roots were assumed to be cylindrical. In this study, vine pruning weights of 1-year-old wood were used as an indicator of vine treatment response, and no statistical significance was found between treatments. Rammer (1980) did concede the difficulty of correlating plant response with GP reduction due to the great variation between and years.

When carbofuran granules were applied at the same rate in a North Island vineyard in New Zealand reduction of populations was not significant due to a large amount of organic matter from an earlier weed incorporation (King et al., 1981). The insecticide was thought to be bound up in the organic matter and microbial activity associated with organic matter breakdown may have deactivated the carbofuran. At a second test site, carbofuran gave a 100% reduction, but a high level of control was necessary due to the reproductive potential of grape phylloxera (King et al., 1981).

Laboratory testing of insecticides can provide information used in estimating the time of application, the rate of application, the most susceptible insect life stage, the sub lethal dose, the difference in response of clonal populations, and the development of resistance (Granett et al., 1986). Granett et al. (1986) developed laboratory bioassays to

evaluate the toxicity of carbofuran to phylloxera and the effect of repeated exposures of low concentrations to various insect life stages. Life table parameters were also estimated for three chemical concentrations and a control (water). Gross reproductive rate, generation time, finite rate of increase, doubling time, mean survivorship, and time to the adult molt were calculated. First instars were more susceptible to carbofuran than eggs, large nymphs and adults. There was little effect of sub-lethal concentrations of the insecticide on survivorship, fecundity, and developmental rates. There was little variation in susceptibility of clonal colonies, but shifts in the $LC_{50}$ (lethal concentration at which 50% mortality is achieved) value could be detected.

Field applications of granular carbofuran, oxamyl, fenamiphos, and aldicarb were tested for three seasons in Victoria, Australia for their effectiveness against the root form of GP (Buchanan and Godden, 1989). In addition, six foliar applications of oxamyl were tested. Carbofuran was the most effective treatment when applied twice a year, but in the first year of application its effectiveness in reducing phylloxera did not persist through the growing season. Residues of aldicarb and oxamyl were detected in berry samples the first year, but carbofuran was not detected. The improved vine growth and yield observed with carbofuran treatment was not sufficient to restore a grape phylloxera-infested vineyard to its normal yields.

**Grape Phylloxera-resistant Rootstocks**

The tolerance of some species of *Vitis* to root feeding by grape phylloxera is what led European viticulturists in the late 1800's to the breeding of resistant rootstocks using native American species (Galet, 1979). Grape phylloxera were imported to Europe from its native range in North America in the mid 1800's, and by 1900 four million acres of grapes were destroyed in France alone (Vielvoye, 1992). American species, including *V.*

*rupestris*, *V. riparia*, and *V. berlandieri*, were then collected throughout North America and imported to Europe where selections were made to suit European conditions. A majority of those rootstocks are still used throughout the world today.

The hereditary resistance to phylloxera is the most important factor in deciding the suitability of a rootstock. Characteristics in rootstocks that favor resistance are thought to be: rapid growth, early differentiation of root endodermis, early development of secondary vascular tissue, narrow secondary vascular rays, and development of peridermis (Pongrácz, 1983). Millardet stated in the late 1800's that the formation of nodosities only weakens the vine, while tuberosities have proved to be far more debilitating. He maintained that *V. vinifera* did not produce the secondary periderm until the third or fourth year, while *V. riparia* and *V. rupestris* produced the protective layer within the first year. The periderm isolates the destructive tuberosity from the rest of the vascular system (Pongrácz, 1983).

Viala and Ravaz (1903) published the following scale of resistance of GP on one- and two-year-old roots. This 0 to 20 scale summarizes the authors' recommendation that vines with value ratings of 16 to 20 are sufficiently resistant in all soils, values of 14 and 15 are sufficient in sandy soils, and those under 13 should be disregarded completely (Anonymous 1991).

| | | | |
|---|---|---|---|
| 20 | Immunity | | |
| 19 | *V. rotundifolia* | | |
| 18 | *V. cordifolia, rupestris, arizonica, riparia, rubra, riparia x rupestris* | | |
| 17 | *V. berlandieri, monticola, berlandieri x riparia, berlandieri x rupestris* | | |
| 16 | Rupestris du Lot (St. George),*V. aestivalis*, | | |
| 15 | *V. mustangensis (candicans), cinerea* | | |
| 14 | *V. lincecumii, champinii, logii (solonis)* | | |
| 13 | *V. doaniana* | 12 | Glabrous champinii, Jacquez (Lenoir) |
| 10 | Elvira | 5 | *V. labrusca* |
| 4 | Catawba | 3 | Delaware, Concord, coignetiae |
| 2 | Rulander (Pinot noir),*V. amurensis* | | |
| 0 | Cabernet Sauvignon (*V. vinifera*) | | |

Boubals (1966) published a scale of grape phylloxera-resistant rootstocks with potted vines. He assessed the formation of tuberosities on roots and their subsequent decay. From this list variability in resistance can be seen within *V. rupestris*. His scale was based on a class of 0 to 3 and he recommended that only rootstocks with a 0 to 1 rating be used.

> Class 0- no indication of phylloxera activity on roots of the plant. Examples- *V. rotundifolia*, some *V. riparia*, *V. rupestris*, *V. berlandieri*, and *V. cordifolia*, 420 A, 779P, 1616C.
>
> Class 1- from a series of inoculated plants one or two tuberosities appear, and signs of necrosis on the roots. Examples- *V. riparia*, *V. rupestris*, *V. berlandieri*, *V. cordifolia*, 3306C, 3309C, 101-14 Mgt, 33Em, 5BB, 5C, SO4, 8B, 157-11, 161-49, 775P, 1103P, 140Ru, 99R, 110R, 41B, AXR#2, Catawba, Dog Ridge.
>
> Class 2- several tuberosities in chains, or a few tuberosities but with definite necrosis of the root. Examples- *V. labrusca*, *V. monticola*, some selections of *V. riparia*, *V. rupestris*, *V. berlandieri*, 333EM, AXR#1, AXR#9, Catawba, Dog Ridge.
>
> Class 3- large portion of roots are destroyed, decomposed as a result of phylloxera feeding. Examples- *V. arizonica*, *V girdiana*, *V. vinifera*, some *V. labrusca*, 1613C.

Pastena (1976) published the results of his research in Italy with GP on potted vines. He scored root damage as: 10= absolute resistance; 5= tolerable; and 0= completely susceptible. He also scored the variability of resistance of many *V. vinifera* cultivars as well as some French-American hybrids on their own roots (Pongrácz 1983).

| | |
|---|---|
| 10 | *V. rotundifolia* |
| 9 | *V. riparia, V. berlandieri, and V. cordifolia* |
| 8 | Several *V. rupestris* (e.g. Rupestris Martin), 779P, 420A, 57R |
| 7 | Rupestris du Lot (St. George ?), 1103P, 140Ru, 41B, 1447P |
| 6 | Golia, 3306C, 225Ru, SO4, 161-49C, 157-11, 1045P, 2A, 44-53M |
| 5 | Solonis, Jacquez, Baco 1, Seibel 1000, 1506, 8745, S.V. 15.505, 15.151 |
| 4 | AXR#1; several hybrid direct producers: Landot 244, Oberlin 595, Seibel 6740, 7053, S.V.12309, 12390, 23657, 5.276 |
| 3 | Several *V. vinifera*: Tannant, Nocera, Olivetta, Carignan, Sangiovese, Barbera, Trebbiano dorato, Sauvignon, Grecanico |
| 2 | Direct producers: Courdec 7120, Galibert-Coulondre 115-22, 238-35, Kuhlman 188-2, Seibel 10096, 11803, S.V. 12.327, 23, 410, 39.639 |
| 1 | *V. vinifera, vinifera x labrusca* |

Granett et al. (1987) evaluated grape rootstocks resistant to the California biotypes A and B. Grape phylloxera were reared on excised root pieces held in petri dishes. Values were contrasted to previous literature as well as field evaluations (Granett et. al., 1987). Doubling time (DT) of insects was used to establish a rating system. The evaluation of resistance of rootstocks to biotype A and B is given below and is defined by the rating : R= resistant (DT> 12 days); S= susceptible (DT<12 days); I= Immune (no DT).

| | Parentage[a] | Type A | Type B |
|---|---|---|---|
| *V. rotundifolia* | | I | No Data (N D) |
| *V. rupestris* (St. George) | | I | R |
| *V. champini* (Salt Creek) | | I | R |
| 1613 C | complex | I | I |
| *V. riparia* | | I | N D |
| *V. arizonica* | | I | N D |
| AXR#1 | V × Ru | R | S |
| *V. californica* | | S | N D |
| *V. candicans* | | S | N D |
| Cabernet Sauvignon | V | S | S |
| Elvira | | S | N D |
| Jacquez | | S | N D |
| Dogridge | C | N D | R |
| R. Gloire | | N D | I |
| 1202 C | V × Ru | N D | S |
| 93-5 C | V × Ru | N D | S |
| Harmony | complex | N D | S |
| O43-43 | V × Ro | N D | S |
| O39-16 | V × Ro | N D | I |
| O44-4 | V × Ro | N D | I |
| 171-6 | V × Rufo | N D | R |
| Freedom | complex | N D | I |
| 110R | B × Ru | N D | R |
| 99R | B × Ru | N D | R |
| 5 A | B × Ri | N D | I |
| 5BB | B × Ri | N D | I |
| SO4 | B × Ri | N D | I |
| 3306 C | Ri × Ru | N D | R |
| 3309 C | Ri × Ru | N D | I |

[a] V=*Vitis vinifera*, C=*V. champini*, Ri=*V. riparia*, Ru=*V. rupestris*, Ro=*V. rotundifolia*, Rufo=*V. rufotomentosa*, B=*V. berlandieri*

De Benedictis and Granett (1993) repeated the EMI growth rate indicator for biotype A and B on 26 rootstocks. This was contrasted with previous evaluation systems of rootstocks. Populations grew at approximately equal rates on all rootstocks except AXR#1, 41B, and VR043-43.

The current attention focused on grape phylloxera-resistant rootstocks in the United States has largely been due to the AXR#1 crisis in California (Walker, 1991). Breeding programs must now include not only resistance to phylloxera but also resistance to nematodes, grapevine fan leaf virus (vectored by *Xiphinema index*),as well as good viticultural characteristics. Little is known about the mechanisms of resistance to GP. The resistance to many pathogens and the tolerance to abiotic stresses such as drought are traits likely to be found in rootstocks other than *V. riparia, rupestris, and berlandieri* (Walker and Wolpert, 1992).

Many of Oregon's growers must now face major decisions regarding rootstock selection. Rootstock performance is extremely site dependent (Morton and Jackson, 1988) and decisions must be made a year or more in advance to ensure plant availability. In the interim, growers must strengthen their sanitation practices and make long range plans that include planting with grafted vines. It is my hope that this thesis will provide some biological information that may assist in the understanding of grape phylloxera's progression under Oregon conditions.

## History in Oregon

The history of grape phylloxera in Oregon dates back to 1923 when the Oregon Agricultural College recommended the grafting of susceptible cultivars onto resistant rootstocks (Price, 1986). In 1936 a circular (Duruz, 1936) was published suggesting the use of hot water treatment for vines received from infested districts, and a bulletin

published in 1948 (Schuh, 1948) stated that GP was intercepted at the Oregon border on nursery stock. The cultivars grown at this time were primarily selections of the American species, *Vitis labrusca*. By the 1950's interest in growing these cultivars declined, but many of the original plantings could still be found in the 1960's. The first official siting of GP was reported in 1969 by the USDA (Anonymous, 1969). The Oregon Department of Agriculture verified 14 infestations that same year (Anonymous, 1969). None of the original plantings of American species are in production today.

The winegrape industry in Oregon was resurrected in the early 1960's with the planting of European winegrape cultivars self-rooted (without resistant rootstocks). The ten known grape phylloxera infestations verified by the spring of 1995 were in vineyards planted within the last twenty years (Strik et al., 1995). Two of these infestations were found near what may have been the old American type cultivars, but it is still uncertain how GP was introduced to these infested sites.

Over 95% of Oregon's vineyards are planted with susceptible, own-rooted European winegrapes (*Vitis vinifera* L.). The spread of grape phylloxera could lead to economic hardship for many growers in Oregon because they pay $3.00 per dormant, bench-grafted vine (Satouf et al., 1993). The estimated cost, including lost revenues, for replanting an infested vineyard with 870 plants per acre, is $11,000 (Connelly and Strik, 1993).

# CHAPTER 3. FIELD SAMPLING GRAPE PHYLLOXERA POPULATIONS

## Introduction

Grape Phylloxera, *Daktulosphaira vitifoliae* (Fitch) was first detected in an Oregon commercial vineyard in August of 1990. Nine more infestations have been verified throughout the state as of April 1995 (Strik et al., 1995). The only long term, cost effective control for GP is to initially plant susceptible European wine grape (*Vitis vinifera* L.) cultivars as vines grafted to resistant/tolerant rootstocks (Buchanan and Godden, 1989; Galet, 1979; King et al., 1981). Over 95% of Oregon's 6000 acres of wine grapes are planted to root-susceptible, non-grafted *V. vinifera*.

Grape phylloxera's complicated life cycle is governed by the host-plant material, climatic conditions, and soil type. 'Nodosities' and 'tuberosities' are formed by the feeding of GP on *V. vinifera* roots (Davidson and Nougaret, 1921). These gall-like formations lead to secondary decay from pathogens entering the feeding sites and rendering the roots non-functional (Smith, 1993). Some cultivars tolerate or resist GP infestation or root degradation by producing a secondary periderm that can wall off the infection site (Pongrácz, 1983). *V. vinifera* does not produce this protective layer until the third or fourth year of growth (Pongrácz, 1983).

The above-ground symptoms of grape phylloxera feeding in the vineyard can be described as a "lens-shaped" area with dead vines in the center and stunted vines on the periphery (Wildman, 1992). Each year the infested area gets larger and new satellite infestations appear throughout the vineyard. This increase in size of above-ground symptoms is referred to as the "rate of spread". When vineyards reach low economic returns, replanting with vines grafted to resistant rootstocks becomes neccessary to continue production.

The rootstock AXR#1's susceptibility to biotype-B grape phylloxera has caused great concern for many growers in California (Granett et al., 1991). This crisis has focused greater attention on rootstock selection and breeding in California (see Chapter 2). California growers must select new rootstocks which have greater resistance to GP and other pathogens in addition to possessing good viticultural characteristics (Walker, 1991). Oregon is undergoing a similar transition. Growers establishing new plantings or replanting existing vines must select rootstocks which will better suit their needs (Morton and Jackson, 1988). Growers in Oregon need to know how fast GP moves through a vineyard once it becomes infested, to better predict loss of productivity and replanting dates.

Oregon is considered a cool climate viticultural region and is influenced by the Pacific's maritime weather patterns (Connelly et al., 1991). Cool soil temperatures in combination with dry summers and, in most cases, non-irrigated vineyards may influence grape phylloxera's rate of spread. Most of the biological research on grape phylloxera in the United States has been conducted in laboratory experiments using California's GP populations, and plant material (De Benedictis and Granett, 1992 and 1993; Granett et al., 1985, 1987, and 1991). In a laboratory bioassay Granett and Timper (1987) calculated the generation time of GP at four temperatures, 16, 21, 24, and 28°C, to be 80, 37, 36, and 32 days, respectively.

The severity of grape phylloxera infestations has been researched by Stevenson (1963, 1964), DeKlerk (1974), Buchanan (1987), and Hawthorne and Dennehy (1991). In these studies, the factor used to measure the degree of infestation was an index of root symptoms. The relative number of nodosities was equal to the level of severity. GP populations were not quantified in these surveys.

The dispersal of above-ground crawlers and alates was studied by King and Buchanan (1986), and Hawthorne and Dennehy (1991) and the spread of wind-borne crawlers was recorded at 103 m from an infested area (King and Buchanan, 1986).

It is important to understand the demographics of grape phylloxera under Oregon's climatic conditions. Preliminary field work by Connelly and Strik (1992) estimated the number of generations of GP per year to be 2 to 3, but further research was necessary to verify this. A higher number of generations per year would increase GP populations and possibly infestation rates.

## Field Objectives

In June, 1992, a field sampling program was initiated to address four specific objectives: 1) determine the critical periods of activity of grape phylloxera life-stages, including when hibernants begin molting in the spring, above-ground crawlers and alates emerge above-ground, and GP enters hibernation; 2) determine the number of generations per year; 3) correlate soil temperatures with periods of activity and development of the insect; and 4) quantify the GP population level variance within the lens of infestation.

## Materials and Methods

### Site Description

Three infested vineyards were selected in the Willamette Valley with two sites located in Yamhill County (Yamhill-1 and Yamhill-2) and one site in Marion County (Marion). The three vineyards were selected based on the willingness of the owners to participate and their proximity to OSU. They differed in topographic features and cultural practices.

Ratings of vine decline were recorded from 1990 through 1993 at Yamhill-1 and in 1992 and 1993 at the Marion and Yamhill-2 sites. Vines were rated from zero through five based on vigor and leaf chlorosis with: zero=dead, one=nearly dead, two=low vigor, three=yellow leaves, four=pale green leaves, and five=healthy/vigorous. Vine decline was rated by averaging two individual ground examinations in August of 1992 and 1993 at each site. Figure 3.1 illustrates topography and an example of a July sampling layout at each site. Contours represent a gradation of vine decline recorded in 1992.

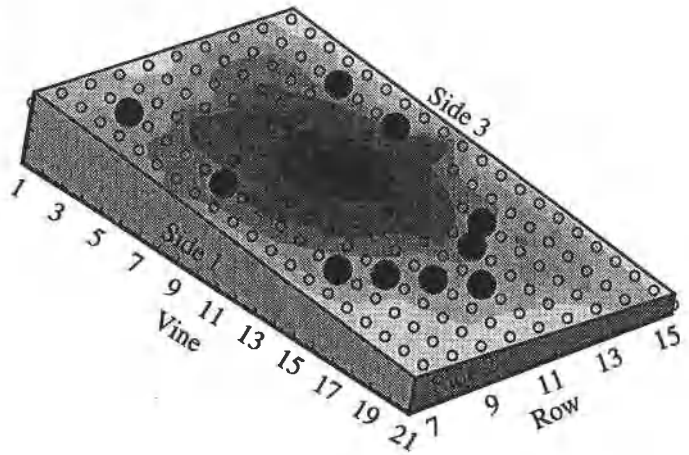
Yamhill-1 was the warmest site with a gravely, clay loam soil, between row sod, and drip irrigation during establishment years (first three years). This 15-year-old, own-rooted 'White Riesling' block was trained to a single canopy in a standard spacing (6' × 9'). This vineyard block was productive and well-managed.

Marion was the coolest site with poor air and water drainage, particularly in the infested area. The clay loam soil was clean cultivated during the growing season. This ten-year-old, own-rooted 'Chardonnay' block, spaced at 6' × 9' was not irrigated.

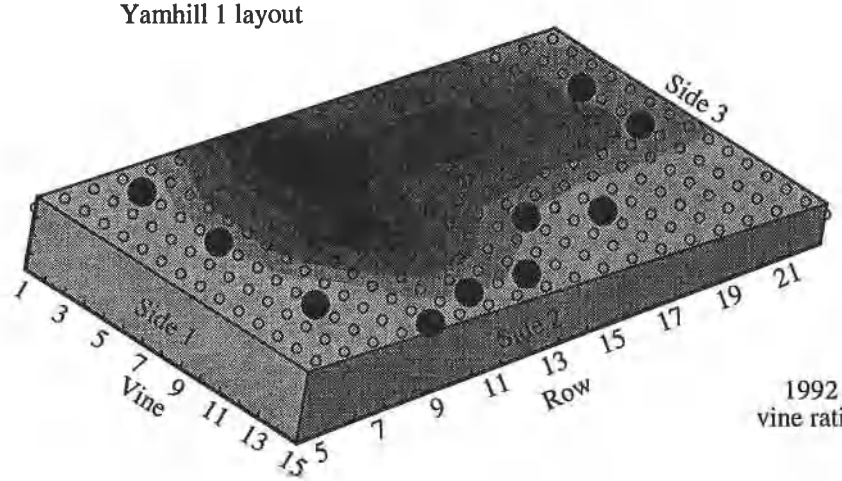
The Yamhill-2 site was intermediate in temperature with good air drainage. This 20-year-old, own-rooted 'Pinot Noir' block had clean cultivated rows and a clay loam soil. Vines were spaced at 6' × 9' and were unirrigated.

Rainy periods during the spring and fall limited field sampling due to the danger of spreading GP throughout the growers' fields. The three infested vineyard sites were sampled every two weeks from July to November in 1992 and 1993 and four to six additional times from March to June in 1992. At each sampling date, ten new vines were selected from the periphery of the lens of infestation (Figure 3.1).

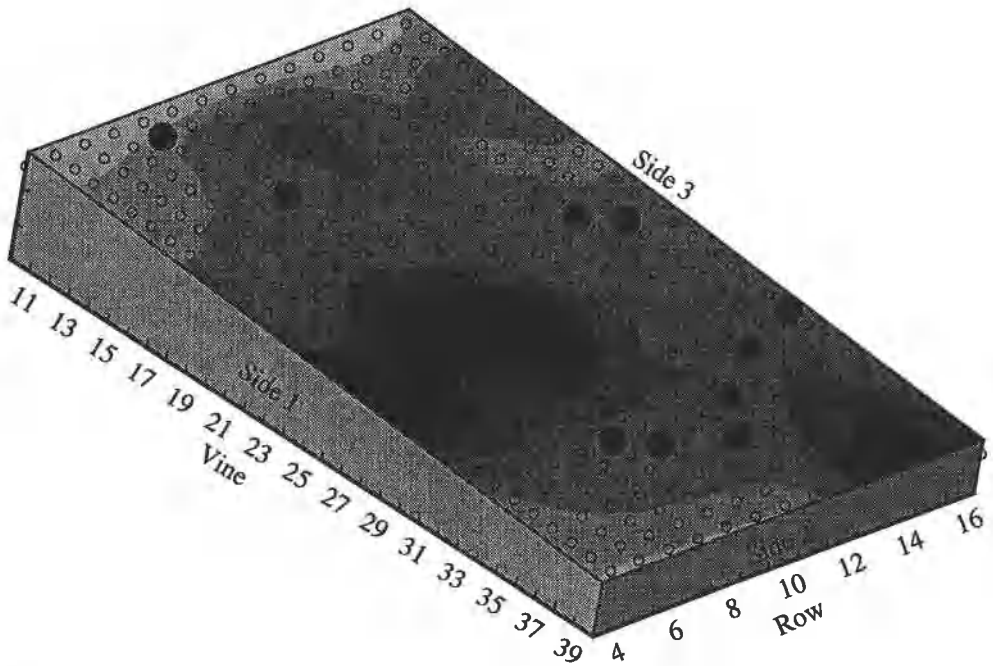
Figure 3.1 Layout of the infested sites where grape phylloxera sampling was conducted. Vine and row numbers are indicated; rows were planted north to south, with vine number increasing to the south. An example a sampling is denoted by the 10 darkened circles on each layout. The three sampling sides of each infestation lens are identified. The slope on each layout is a relative representation of the drainage. The contour shading indicates vine health ratings as descibed in the legend. Ratings were assigned to individual vines in the fall, based on visual inspection in the field.



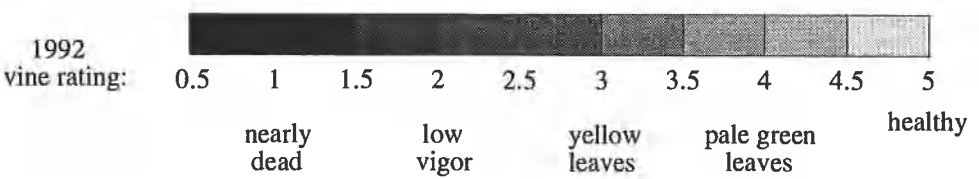

Yamhill 1 layout




Yamhill 2 layout




Marion layout



Sides of the infestation areas were considered "blocks" when evaluating the effect of vine location on population size. Three-vine replicates were blocked for each of three sides at each site. The tenth vine on each sample date was not analyzed when balancing the number of experimental units in blocking for sides. Otherwise, ten vines for each site were evaluated when examing all other effects.

Holes were dug by hand within 20 cm of the vine truck. Root samples, averaging 30 grams per sample with varying diameters, were collected at two depths, 15-30 cm and 30-45 cm. Bagged roots and associated soil samples were transported in a cooler back to the laboratory at OSU where root measurements and extractions were performed.

A basic sieve centrifugation extraction procedure was performed with some modification for the size and weight of grape phylloxera (Griesbach personal communication). Low speed sucrose centrifugation settles out large particles and fragments, thereby cleaning samples for accurate counting. Roots and associated soil were sprayed at high pressure with tap water for approximately 15 seconds to send any insects and debris through a 500 micrometer mesh screen to be collected on a 106 micrometer mesh screen (U.S.A. Standard Testing Sieves, W.S. Tyler Inc.). The GP sample collected on the 106 micrometer screen was rinsed with distilled water into a 100 ml centrifuge tube and left to settle out for one hour. The supernatant was poured off and a mixed sucrose solution of 450 g /liter was added to pellets with insects. The pellet and sucrose solution was stirred and centrifuged at 2800 rpm for 3 minutes with a lab top swinging bucket type centrifuge (Marathon 6k, Fisher Scientific, Pittsburgh, PA.). The supernatant was poured off onto a 45 micrometer mesh screen suspended in water. GP collected on the fine screen was rinsed with distilled water into a 5 cm petri dish for counting with a 70x dissecting scope.

Grape phylloxera were categorized into four life stages during counting, eggs, nymphs (early-instar nymphs), nymph/adults (late-instar nymphs), and adults. The distinction between nymph, nymph/adult, and adult was based on size and morphology

(Davidson and Nougaret, 1921). The n/adult life stage was constructed in order to accommodate several late-instar nymph stages which are difficult to distinguish. Early-instar nymphs tend to be more mobile while late-instar nymphs remain more sedentary. Dividing the nymphs into these two catagories allowed for a better description of population trends. Life stages were counted for each aforementioned sampling date. Peak periods of population growth were monitored and generation times predicted based on the duration between peaks and the behavior of GP in the lab. (Belcari and Antonelli, 1989; Raspi et al., 1987)

Soil temperatures were taken once per hour with a two-channel field recorder (Datapod, Omnidata International Inc. Logan, Utah) at each of the three sites at 15 cm in 1992 and at 15 cm and 30 cm in 1993. The daily temperature means were averaged over the two week period preceding each sampling date.

Beginning and cessation of activity of grape phylloxera in the spring and fall were correlated to field temperatures and low temperature laboratory experiments performed at Oregon State University (Chapter 4) and University California Davis (Granett and Timper, 1987).

Above-ground emergence of crawlers (nymphs) and alates was monitored in 1993 with sticky trunk-wraps placed on 15 vines at the periphery of the infestation lens. Preliminary trunk-wrap tests were run during the summer of 1992 to establish methodology. Traps consisted of 6-cm wide bands of standard laboratory tape wrapped around trunks 6 cm above the soil line. Tape was coated with insect stickem (Fullbright Enterprises, Emmeryville, California). Traps were changed every two weeks during the summer season in 1993 and inspected with a 70x dissecting scope for the presence of GP. Other insect groups were noted if found on trunk wraps but species identification was not made due to time constraints.

A plant pathogenic nematode survey was performed at the three GP infested sites from June 15 to September 21 in 1993. Soil, collected with roots at these dates, was run

through a 5-day Baermann funnel procedure for nematode extraction. Root extractions for nematodes were performed in a 7-day mist chamber on roots 0 to 5 mm in diameter. The laboratory nematode extraction protocols described (e.g. roots versus soil) are designed to trap species that live inside and outside of roots.

Preliminary grape phylloxera sampling was conducted at the three sites prior to the commencement of this masters thesis project. Roots (standardized to 100 grams) and 400 ml of soil associated with roots were collected from 15 individual vines at one depth (15 to 30 cm). Sampling was performed four times in May and June of 1992 at the two Yamhill sites and six times, from March through June, at the Marion site.

Comparisons between years were done from mid-July to mid-October when the eight sample dates matched (± one week) from July to October. Population densities of each life stage were calculated for the ten vines sampled at each depth and standardized to 30 grams of root. Transformations [log(n+1)] were performed on the data of insect life stages for homogeneity of variance; untransformed/unstandardized field counts ranged from 0 to 1000. LS mean comparisons were used to compare populations between dates.

Analysis of variance was used to examine the effect of year, date, site, and interactions on insect populations (SAS Institute, Cary, NC). A nested, repeated measures analysis of variance was used to evaluate the effect of sampling side within site. Paired mean differences of the log of insects collected at the two depths were examined.

## Results and Discussion

A year by date by site interaction was found for population of eggs (P=0.0093), nymphs (P=0.0542), and adults (P=0.0104); and a year by site interaction for n/adults (P=0.0229). Due to the complexity of these interactions many of the figures in this chapter represent site, year, and date effects. The three sides of the lens of infestation that

were sampled approached significance for insect population levels. Eggs, nymphs, and adults were significantly different (at the $P<0.10$ level ) between sides of the lens ($P=0.0587$, 0.0510, 0.0578, respectively) while n/adults were not affected by the sampling location.

The mean difference in populations between soil depths across all sites was not significant for any of the life stages, therefore insect populations were pooled across the two depths. However, if GP is present at a shallow depth, there is a greater risk of spreading the pest through cultural practices. Tables 3.1 and 3.2 represent the mean (and standard errors) number of eggs, nymphs, n/adults, and adults standardized to 30 grams of roots and averaged over the two depths. A level of separation exists between the number of eggs and nymphs, and nymphs and adults. This may reflect the length of stasis for eggs and first instar nymphs versus that of n/adults and adults (Davidson and Nougaret, 1921). This in combination with mobility and mortality may have lead to the decrease in numbers of n/adults and adults.

**Appearance of life stages**

One egg was recovered from Marion soil extractions in early-April, 1992, and 91 eggs were recovered from Yamhill-1 root extractions on May 7 (Table 3.3). These eggs are from matured hibernants of the over-wintering generation. The monitoring of soil temperatures did not begin until July of 1992, however, soil temperatures at the 15-cm depth in late-March to early-May, 1993 ranged from 8.5 to 12.0°C (Figure 3.2). Soil temperatures in early-May, 1993 at the 30 cm depth ranged from 10.5 to 12.5°C (Figure 3.3). Laboratory results from a 45 day bioassay at OSU (see Chapter 4) indicated that egg laying did not occur between 7 and 16°C.

Table 3.1 Grape phylloxera populations of four life stages, averaged over two depths, for three sites sampled from July to November, 1992.

| 1992 | Eggs | | Nymphs | | N/Adults | | Adults | |
|---|---|---|---|---|---|---|---|---|
| Date | Mean* | SE** | Mean* | SE** | Mean* | SE** | Mean* | SE** |
| **Yamhill-1** | | | | | | | | |
| 6-Jul | 25.4 | 8.9 | 24.7 | 10.2 | 4.8 | 1.8 | 2.7 | 1.1 |
| 21-Jul | 17.6 | 7.3 | 17.4 | 7.0 | 1.5 | 0.6 | 1.3 | 0.5 |
| 4-Aug | 15.6 | 8.5 | 57.1 | 26.1 | 6.4 | 2.8 | 5.4 | 3.7 |
| 20-Aug | 43.2 | 18.2 | 90.3 | 27.1 | 23.5 | 11.3 | 9.0 | 3.3 |
| 1-Sep | 63.9 | 25.9 | 83.0 | 38.0 | 16.5 | 8.9 | 11.7 | 5.3 |
| 17-Sep | 47.0 | 19.1 | 107.2 | 36.8 | 10.9 | 3.8 | 8.5 | 3.5 |
| 29-Sep | 68.7 | 27.5 | 206.4 | 97.1 | 7.2 | 2.9 | 12.9 | 5.3 |
| 5-Nov | 14.4 | 6.5 | 61.5 | 24.6 | 1.0 | 0.3 | 1.1 | 0.6 |
| **Marion** | | | | | | | | |
| 6-Jul | 1.0 | 0.5 | 1.0 | 0.7 | 0.0 | 0.0 | 0.1 | 0.1 |
| 21-Jul | 41.3 | 15.4 | 18.5 | 5.8 | 5.2 | 2.1 | 3.8 | 1.7 |
| 4-Aug | 10.6 | 6.0 | 23.1 | 10.3 | 4.1 | 1.8 | 2.6 | 1.3 |
| 20-Aug | 0.0 | 0.0 | 0.4 | 0.4 | 0.1 | 0.1 | 0.0 | 0.0 |
| 1-Sep | 55.0 | 18.4 | 158.6 | 73.2 | 14.8 | 4.9 | 16.8 | 6.2 |
| 17-Sep | 45.1 | 27.3 | 47.6 | 32.5 | 3.2 | 1.8 | 2.7 | 1.4 |
| 29-Sep | 12.3 | 6.2 | 17.9 | 8.5 | 0.1 | 0.1 | 1.1 | 0.7 |
| 5-Nov | 14.8 | 6.9 | 165.0 | 65.8 | 1.8 | 0.9 | 1.3 | 0.6 |
| **Yamhill-2** | | | | | | | | |
| 6-Jul | 34.7 | 13.5 | 53.7 | 17.7 | 6.2 | 2.6 | 3.5 | 1.2 |
| 21-Jul | 22.0 | 13.0 | 22.2 | 12.8 | 4.3 | 2.9 | 1.8 | 1.2 |
| 4-Aug | 16.6 | 10.3 | 36.0 | 26.6 | 3.2 | 2.1 | 2.8 | 1.6 |
| 20-Aug | 119.7 | 50.4 | 282.9 | 138.8 | 29.7 | 13.5 | 30.2 | 17.0 |
| 1-Sep | 10.9 | 4.5 | 15.7 | 6.2 | 1.4 | 0.6 | 2.0 | 0.9 |
| 17-Sep | 6.5 | 3.8 | 8.6 | 6.2 | 0.6 | 0.3 | 0.8 | 0.5 |
| 29-Sep | 20.4 | 12.3 | 10.9 | 5.0 | 0.4 | 0.3 | 0.8 | 0.5 |
| 5-Nov | 4.4 | 2.0 | 65.2 | 30.9 | 1.1 | 0.6 | 0.2 | 0.2 |

* Means are the average of 10 vines standardized to 30 grams of roots.

** SE = Standard error of the mean.

Table 3.2 Grape phylloxera populations of four life stages, averaged over two depths, for three sites sampled from July to October, 1993.

| 1993 | Eggs | | Nymphs | | N/Adults | | Adults | |
|---|---|---|---|---|---|---|---|---|
| Date | Mean* | SE** | Mean* | SE** | Mean* | SE** | Mean* | SE** |
| **Yamhill-1** | | | | | | | | |
| 13-Jul | 156.3 | 104.1 | 88.2 | 58.5 | 21.5 | 14.2 | 23.8 | 15.8 |
| 27-Jul | 107.7 | 45.4 | 53.1 | 20.4 | 17.9 | 7.6 | 10.1 | 4.2 |
| 10-Aug | 84.7 | 19.2 | 43.8 | 12.3 | 14.7 | 5.1 | 13.1 | 3.6 |
| 24-Aug | 68.0 | 23.7 | 154.9 | 65.9 | 17.9 | 8.2 | 16.2 | 7.0 |
| 7-Sep | 42.1 | 12.2 | 65.6 | 20.4 | 19.3 | 6.4 | 10.6 | 3.3 |
| 21-Sep | 164.0 | 94.1 | 171.7 | 78.4 | 46.2 | 27.5 | 24.9 | 16.4 |
| 5-Oct | 31.7 | 17.3 | 99.2 | 30.2 | 11.1 | 4.7 | 6.3 | 2.3 |
| 19-Oct | 64.1 | 28.4 | 117.2 | 50.4 | 4.2 | 1.7 | 2.9 | 1.9 |
| **Marion** | | | | | | | | |
| 13-Jul | 7.9 | 7.5 | 5.0 | 4.2 | 1.1 | 1.0 | 1.1 | 1.1 |
| 27-Jul | 6.3 | 4.0 | 6.3 | 3.8 | 1.2 | 0.9 | 0.5 | 0.3 |
| 10-Aug | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24-Aug | 17.3 | 8.4 | 17.7 | 9.3 | 2.4 | 1.2 | 1.2 | 0.7 |
| 7-Sep | 39.9 | 16.3 | 32.4 | 14.4 | 10.1 | 4.4 | 4.4 | 1.8 |
| 21-Sep | 5.2 | 4.4 | 6.9 | 3.6 | 1.1 | 0.5 | 0.4 | 0.2 |
| 5-Oct | 0.2 | 0.1 | 0.4 | 0.4 | 0.0 | 0.0 | 0.1 | 0.1 |
| 19-Oct | 4.2 | 1.6 | 31.5 | 12.1 | 1.1 | 0.7 | 1.2 | 0.6 |
| **Yamhill-2** | | | | | | | | |
| 13-Jul | 42.6 | 34.8 | 8.6 | 5.0 | 4.9 | 4.0 | 4.4 | 3.9 |
| 27-Jul | 168.0 | 150.1 | 26.6 | 22.7 | 17.7 | 15.6 | 17.8 | 15.4 |
| 10-Aug | 26.1 | 11.2 | 21.8 | 10.1 | 6.7 | 2.9 | 7.6 | 4.3 |
| 24-Aug | 43.0 | 24.7 | 40.3 | 18.5 | 7.4 | 4.3 | 5.0 | 2.5 |
| 7-Sep | 21.1 | 8.0 | 17.1 | 8.0 | 3.2 | 1.4 | 1.4 | 0.7 |
| 21-Sep | 122.8 | 96.6 | 52.8 | 22.6 | 8.9 | 3.9 | 6.4 | 4.2 |
| 5-Oct | 11.5 | 4.0 | 14.2 | 5.4 | 1.4 | 0.6 | 1.2 | 0.5 |
| 19-Oct | 48.5 | 17.1 | 68.8 | 32.0 | 1.8 | 0.7 | 1.4 | 0.6 |

* Means are the average of 10 vines standardized to 30 grams of roots.

** SE = Standard error of the mean.

Table 3.3 Grape phylloxera populations of four life stages extracted from roots and soil, at 15 cm depth, at three sites from March to June, 1992.

| 1992 | | Eggs | | Nymphs | | N/Adults | | Adults | |
|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | Mean* | Total | Mean* | Total | Mean* | Total | Mean* | Total |
| **Yamhill-1** | | | | | | | | | |
| 5\7 | Roots | 6.1 | 91.0 | 0.9 | 14.0 | 0.5 | 7.0 | 0.0 | 0.0 |
| | Soil | 0.1 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5\21 | Roots | 0.5 | 7.0 | 0.4 | 6.0 | 0.2 | 3.0 | 0.0 | 0.0 |
| | Soil | 0.0 | 0.0 | 0.1 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6\3 | Roots | 0.5 | 7.0 | 0.6 | 9.0 | 0.1 | 2.0 | 0.1 | 1.0 |
| | Soil | 0.5 | 7.0 | 0.1 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6\23 | Roots | 2.9 | 43.0 | 1.5 | 23.0 | 0.9 | 13.0 | 0.6 | 9.0 |
| | Soil | 3.0 | 45.0 | 1.5 | 23.0 | 0.1 | 1.0 | 0.0 | 0.0 |
| **Marion** | | | | | | | | | |
| 3\13 | Roots | 0.0 | 0.0 | 0.2 | 3.0 | 0.1 | 1.0 | 0.0 | 0.0 |
| | Soil | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4\9 | Roots | 0.0 | 0.0 | 0.1 | 2.0 | 0.2 | 3.0 | 0.0 | 0.0 |
| | Soil | 0.1 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5\7 | Roots | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Soil | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5\21 | Roots | 0.1 | 1.0 | 0.3 | 5.0 | 0.1 | 1.0 | 0.0 | 0.0 |
| | Soil | 0.1 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6\3 | Roots | 0.0 | 0.0 | 0.3 | 4.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Soil | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6\23 | Roots | 1.1 | 17.0 | 4.3 | 64.0 | 0.3 | 5.0 | 0.4 | 6.0 |
| | Soil | 1.0 | 15.0 | 0.2 | 3.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Yamhill-2** | | | | | | | | | |
| 5\7 | Roots | 0.1 | 2.0 | 0.3 | 5.0 | 0.1 | 1.0 | 0.0 | 0.0 |
| | Soil | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5\21 | Roots | 0.0 | 0.0 | 0.1 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Soil | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6\3 | Roots | 3.4 | 51.0 | 2.0 | 30.0 | 0.5 | 7.0 | 0.4 | 6.0 |
| | Soil | 2.3 | 34.0 | 0.1 | 1.0 | 0.1 | 1.0 | 0.0 | 0.0 |
| 6\23 | Roots | 1.6 | 24.0 | 2.5 | 37.0 | 1.9 | 29.0 | 0.8 | 12.0 |
| | Soil | 2.9 | 44.0 | 1.9 | 28.0 | 0.1 | 2.0 | 0.2 | 3.0 |

* Means are the average of 15 vines standardized to 100g of roots and 400ml of soil.

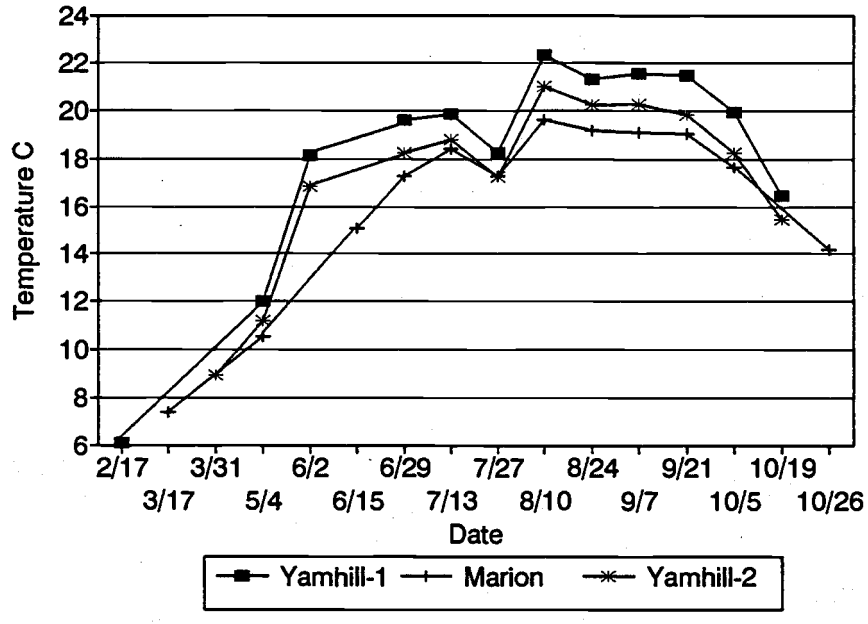



Figure 3.2 Mean soil temperatures at 15 cm two weeks preceding dates 1993.

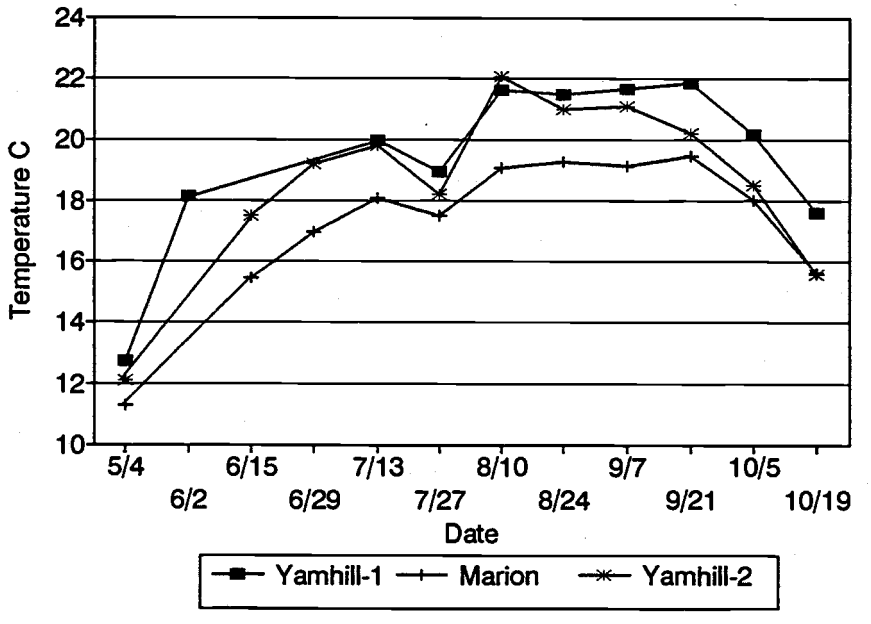



Figure 3.3 Mean soil temperatures at 30 cm two weeks preceding dates 1993.

The contrasting results between the field sampling and laboratory study (Chapter 4) may indicate that 'time to fecundity' was not accurately estimated in the laboratory. Granett and Timper (1987) calculated generation time at 80 days for GP reared at 16°C and a net reproductive rate of 9 eggs.

The appearance of darkened nymphs in late-July, 1993 in extracted samples may have marked the beginning of hibernation (data not shown). Soil temperatures at 15 cm were 17.3 to 18.8°C at this time. Davidson and Nougaret (1921) found nymphs entering hibernation at 19°C. The quality of root substrate may also play an important role in hibernation. De Klerk (1974) found hibernants on decayed roots at temperatures of 20°C. He believed root growth was the dominant factor in GP regulation, but he considered ambient air temperature (De Klerk, 1974), when soil temperature might be a more appropriate factor when tracking a subterranean insect.

Nymphs with wing pads, the pre-aerial stage to the alate, were collected from extractions in Oregon in September, 1992 and in July and August, 1993 (data not shown). After sexual reproduction and overwintering , this can lead to above-ground leaf feeding in the spring by nymphs. No official documentation has been made of above-ground feeding occurring on *V. vinifera* cultivars in the Western United States, but researchers in Italy found leaf galls on *V. vinifera* grafted on American rootstocks (Conti et al., 1985).

Several above-ground crawlers were recovered on sticky trunk-wraps from early-July to early-September and alates were recovered in August and September, 1993 (Table 3.4). Alates were also trapped on trunk-wraps in August of 1992 (data not shown). There was difficulty in identifying grape phylloxera on trunk-wraps because the tape was soiled from cultivation. We did not find as high a number of GP on sticky trunk-wraps as found by researchers in New Zealand (Smith personal communication). However, the above-ground presence was significant in that it alerted Oregon growers that above-ground activity does take place under our conditions.

Table 3.4 Sticky trunk wrap catches at three vineyard sites sampled from July to October in 1993.

| Date | Site | G Phylloxera | Thrips | Mites | Aphids | F.Knats | Other |
|---|---|---|---|---|---|---|---|
| 1993 | | | | | | | |
| 7/6 | Yamhill-1 | | Y+* | Y** | Y | Y+ | Spiders |
| | Marion | | Y | Y | Y | Y+ | |
| | Yamhill-2 | 1-Crawler | Y | Y | Y | Y+ | Ants |
| 7/20 | Yamhill-1 | | Y+ | Y | Y | Y+ | Leafhopper |
| | Marion | 2-Crawlers | Y+ | Y | Y | Y+ | |
| | Yamhill-2 | ?*** | Y+ | Y | Y | Y+ | Tachinids |
| 8/3 | Yamhill-1 | ? | Y+ | Y+ | Y | Y+ | Spiders |
| | Marion | ? | Y+ | Y+ | Y | Y+ | Flies |
| | Yamhill-2 | ? | Y+ | Y+ | Y | Y+ | Beetles |
| 8/17 | Yamhill-1 | 1-Alate | Y | Y | Y | Y | Oribatids |
| | Marion | 1-Crawler | | Y | | Y | |
| | Yamhill-2 | | Y | Y | | Y | |
| 9/2 | Yamhill-1 | 1-Crawler | Y+ | Y+ | Y | Y+ | Leafhoppers |
| | | 1-Alate | | | | | Oribatids |
| | Marion | | Y+ | Y | Y | Y | Leafhoppers |
| | Yamhill-2 | 1-Alate | Y | Y+ | Y | Y+ | Weevils |
| 9/14 | Yamhill-1 | 1-Alate | | Y+ | | Y+ | Oribatids |
| | | | | | | | Leafhoppers |
| | Marion | 2-Alates | | Y | | Y | Ants |
| | Yamhill-2 | | Y | | | Y | Psocids |
| 9/28 | Yamhill-1 | | Y | Y | | Y | Oribatids |
| | Marion | | Y | Y | Y | Y | |
| | Yamhill-2 | | Y+ | Y | Y | Y+ | Psocids |
| 10/12 | Yamhill-1 | | Y+ | Y | Y | Y+ | Oribatids |
| | | | | | | | Leafhoppers |
| | Marion | | Y+ | Y | Y | Y+ | Psocids |
| | | | | | | | Leafhoppers |
| | Yamhill-2 | | Y+ | Y | Y | Y+ | Leafhoppers |

G Phylloxera = Above-ground forms of grape phylloxera in sticky trunk-wraps.
Y+* = Insects found in large numbers.
Y** = Insects found in fewer numbers.
?*** = Identification could not be confirmed due to damaged sample.
Oribatids = Predacious mite population found in large numbers.

**Populations and generation times**

The Yamhill-1 site had a higher number of grape phylloxera eggs in early July, 1993 relative to July, 1992 ($P=0.0018$). Mean soil temperatures at 15-30 cm, were two degrees cooler in 1993 (19.8°C) than in 1992 (22.1°C) (Figures 3.4 and 3.5).

The effect of date on nymph populations was significant at Yamhill-1 in 1992 and 1993 ($P=0.011$). A significant difference ($P<0.05$) in nymphal populations between dates in 1992 was found for the combinations 7/21 and 8/20, 7/21 and 9/17, 7/21 and 9/29, and 8/4 and 8/20. These differences represent an increase in nymphal populations over time. Distinct population peaks and valleys (or a drop in populations) could not be proven statistically at the Yamhill-1 site. Laboratory bioassays performed in California by Granett and Timper (1987) showed that mean generation time was 37 days for insects raised at a constant temperature of 21°C and 36 days for insects at 24°C. The mean soil temperatures at Yamhill-1 ranged from 19 to 25°C during the above dates (Figure 3.4).

Differences ($P<0.05$) in nymphal population for Yamhill-1 in 1993 were found between 7/13 and 9/21, 7/13 and 10/5, and 7/13 and 10/19 (Figure 3.7). The calculation of generation peaks could not be determined for this site in 1993 due to the lack of significance between population peaks and valleys. However, a general increase in nymphal population over time was experienced.

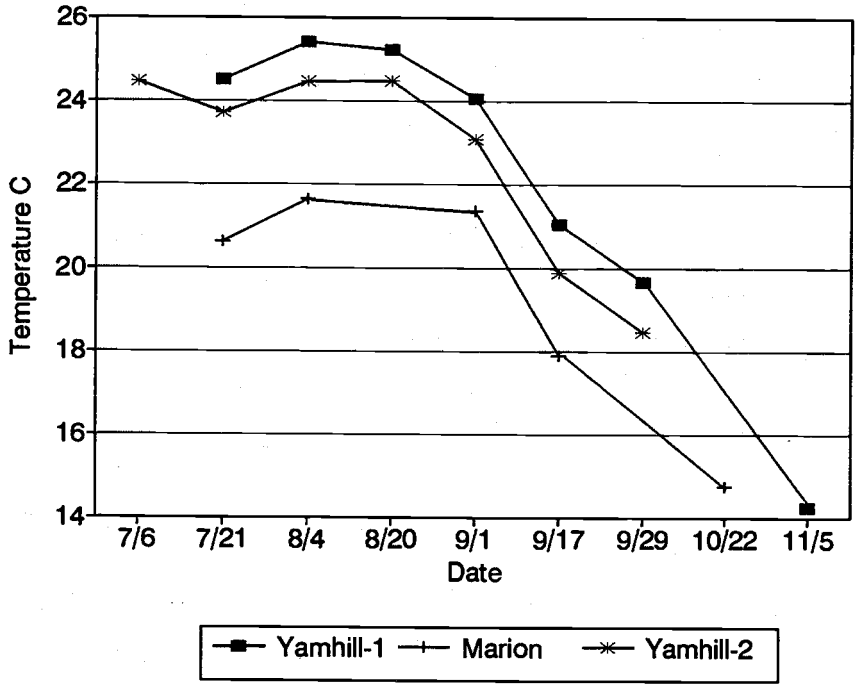



Figure 3.4 Mean soil temperatures at 15 cm two weeks preceding dates 1992.

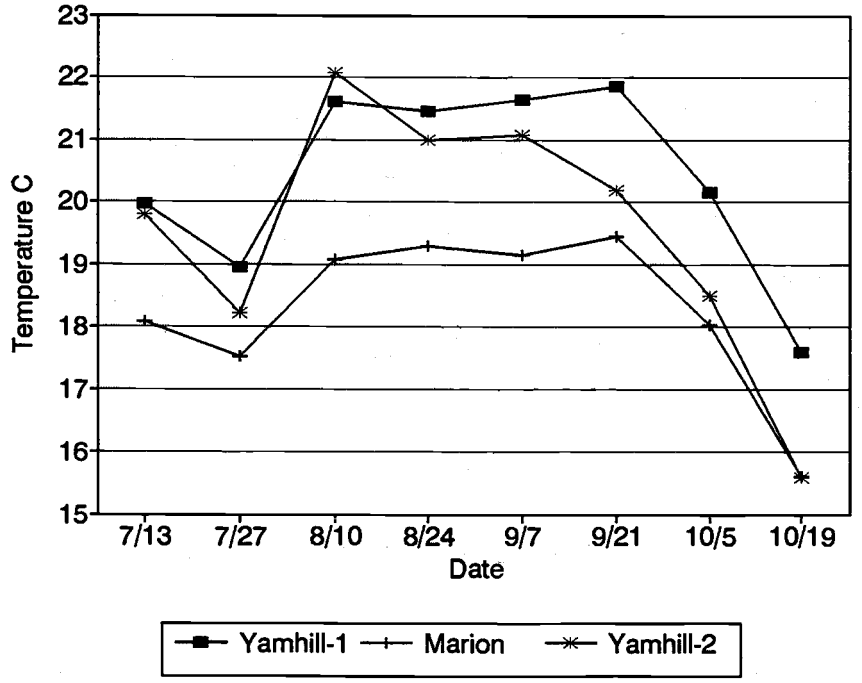



Figure 3.5 Mean soil temperatures at 15 cm two weeks preceding dates 1993.

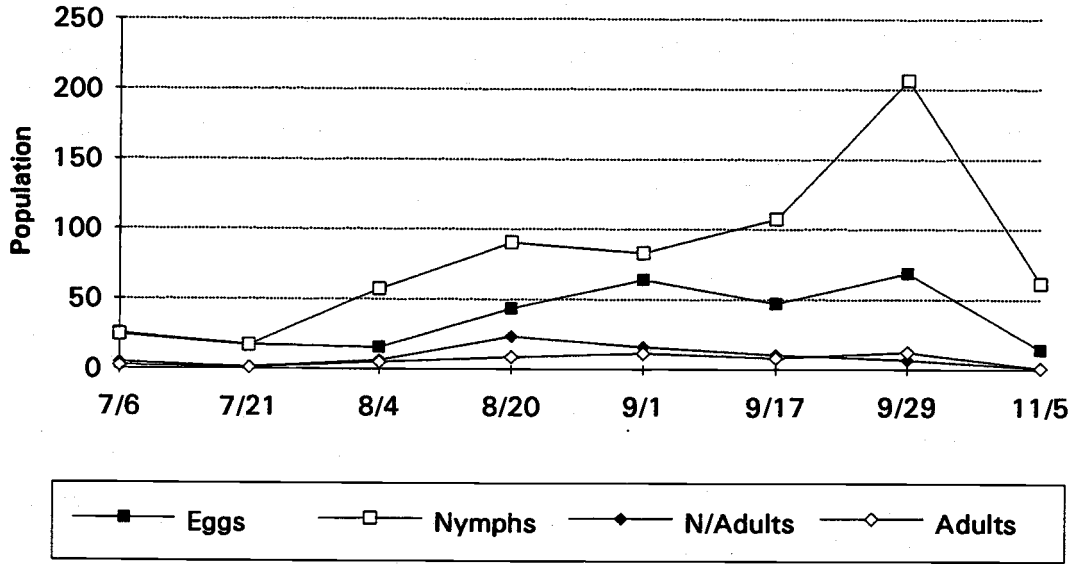



Figure 3.6 Yamhill-1 grape phylloxera populations sampled in 1992.
Mean number of insects from 10 vines, pooled across two depths (15 to 30 and 30 to 45 cm), standardized to 30 grams of roots.

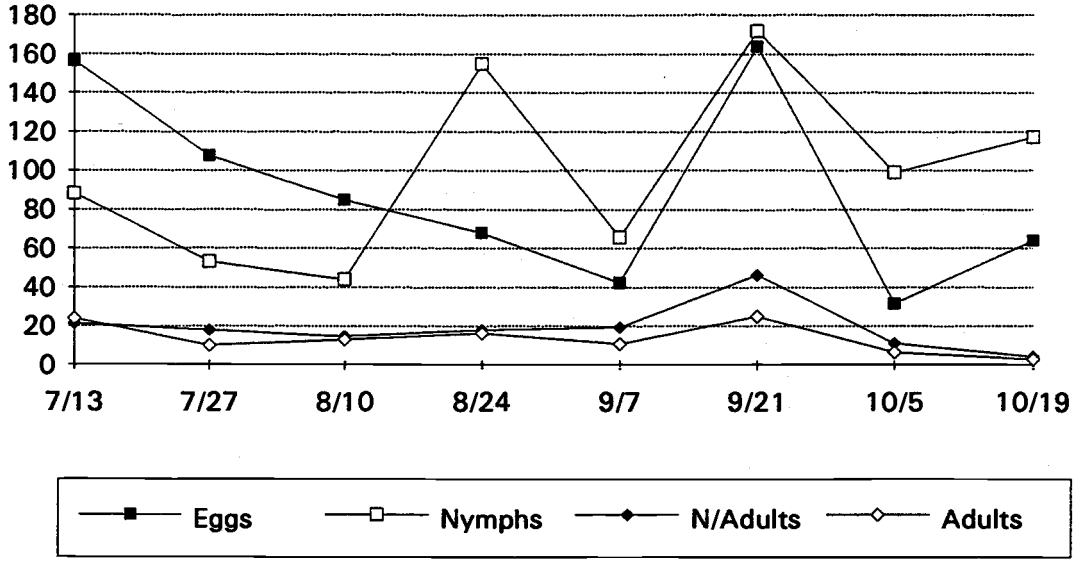



Figure 3.7 Yamhill-1 grape phylloxera populations sampled in 1993.
Mean number of insects from 10 vines, pooled across two depths (15 to 30 and 30 to 45 cm), standardized to 30 grams of roots.

There were no significant differences between nymphal populations in 1993 and 1992 (Figures 3.6 and 3.7), and soil temperatures in early summer of 1993 were 2 to 4°C cooler than in 1992 (Figures 3.4 and 3.5). The general trend for both years was an increase over time. The surviving nymphs of the last generation become the over-wintering hibernants, demonstrated by the high nymphal populations in late fall.

The sampling layout of the three sides at the Yamhill-1 infestation were, side one went down two rows of vines 10 to 15 vines long, side two went across eight vine rows and, side three went up two rows the same length as side one (Figure 3.1). Sides one and two were significantly higher in nymphal populations than side three (P=0.0001 and 0.0019 respectively) (Table 3.5). Side one juxtaposed a satellite infestation that was discovered in late 1992 potentially influencing the number of GP. The higher population in side two may have been due to GP moving down the slope.

Yamhill-1 had the highest total number of grape phylloxera over the two-year period, yet the above-ground symptoms were contained within a smaller area (Figure 3.1). The infestation was thought to be a point source introduction. The Yamhill-1 site also had a high number of nematodes, *Criconemella sp.*, but it is uncertain what economic impact this species has on grapes. Sampling done by our group in 1993 produced 626 nematodes per quart of soil on September 21 at this site (Table 3.6). A comprehensive nematode survey is currently under way in Oregon's wine grape region to determine nematode species present and impact.

The Marion site had a significantly higher number of nymphs and adults in 1992 (Figure 3.8) than in 1993 (Figure 3.9) (P=0.036 and 0.0083, respectively). A year by date interaction was found for the number of eggs (P=0.0188). There were five times more nymphs at the end of 1992 than in 1993 (Figures 3.8 and 3.9).

Table 3.5 Comparison of means from 1992 and 1993 of grape phylloxera nymphs sampled from three sides of each of three infestations, standardized to 30 grams of root fresh weight.

| 1992 | Nymphs | | | 1993 | Nymphs | | |
|---|---|---|---|---|---|---|---|
| Date | Side 1 | Side 2 | Side 3 | Date | Side 1 | Side 2 | Side 3 |
| Yamhill-1* | | | | | | | |
| 7/6 | 64 | 12 | 0 | 7/13 | 284 | 3 | 7 |
| 7/21 | 34 | 13 | 10 | 7/27 | 82 | 86 | 0 |
| 8/4 | 76 | 114 | 0 | 8/10 | 87 | 7 | 18 |
| 8/20 | 87 | 19 | 143 | 8/24 | 154 | 288 | 74 |
| 9/1 | 111 | 25 | 2 | 9/7 | 99 | 5 | 85 |
| 9/17 | 149 | 200 | 9 | 9/21 | 59 | 84 | 65 |
| 9/29 | 577 | 68 | 8 | 10/5 | 93 | 123 | 38 |
| 11/5 | 71 | 6 | 70 | 10/19 | 5 | 332 | 32 |
| Marion** | | | | | | | |
| 7/6 | 0 | 2 | 0 | 7/13 | 0 | 16 | 0 |
| 7/21 | 35 | 0 | 26 | 7/27 | 18 | 2 | 0 |
| 8/4 | 1 | 41 | 36 | 8/10 | 0 | 0 | 0 |
| 8/20 | 1 | 0 | 0 | 8/24 | 57 | 1 | 0 |
| 9/1 | 411 | 117 | 1 | 9/7 | 68 | 8 | 31 |
| 9/17 | 158 | 0 | 1 | 9/21 | 21 | 1 | 0 |
| 10/6 | 0 | 42 | 17 | 10/5 | 1 | 0 | 0 |
| 10/22 | 279 | 205 | 66 | 10/19 | 70 | 32 | 3 |
| Yamhill-2*** | | | | | | | |
| 7/6 | 10 | 23 | 86 | 7/13 | 0 | 18 | 10 |
| 7/21 | 60 | 5 | 8 | 7/27 | 0 | 85 | 3 |
| 8/4 | 1 | 0 | 119 | 8/10 | 0 | 2 | 59 |
| 8/20 | 0 | 376 | 534 | 8/24 | 4 | 2 | 103 |
| 9/1 | 0 | 6 | 26 | 9/7 | 16 | 2 | 34 |
| 9/17 | 2 | 0 | 2 | 9/21 | 0 | 120 | 47 |
| 10/6 | 17 | 1 | 19 | 10/5 | 0 | 3 | 35 |
| 10/22 | 15 | 0 | 193 | 10/19 | 0 | 3 | 146 |

Analysis was performed on the means of three vines for each side standardized and tranformed to log(n+1).

*Yamhill-1 Side 1 and Side 2 significantly different than Side 3 (P=0.0001 and 0.0019 respectively).

**Marion Side 1 significantly different than Side 3 (P=0.0002).

**Yamhill-2 Side 1 and Side 2 significantly different than Side 3 (P=0.0001 for both comparisons).

Table 3.6 Nematode populations in three vinevards in Western Oregon, summer 1993.

| Date | Xiphinema | Pratylenchus | Criconemella* | Paratylenchus | Meloidogyne |
|---|---|---|---|---|---|
| **Soil** | Yamhill-2 | | | | |
| 6/15 | 13 | 4 | 29 | 17 | 0 |
| 7/13 | 25 | 4 | 46 | 8 | 0 |
| 8/10 | 17 | 193 | 38 | 0 | 29 |
| 9/21 | 8 | 0 | 42 | 147 | 361 |
| **Soil** | Marion | | | | |
| 6/15 | 0 | 54 | 50 | 34 | 76 |
| 7/13 | 4 | 260 | 34 | 4 | 141 |
| 8/10 | 13 | 298 | 139 | 273 | 53306 |
| 9/21 | 0 | 706 | 550 | 235 | 878 |
| **Soil** | Yamhill-1 | | | | |
| 6/15 | 8 | 0 | 202 | 0 | 0 |
| 7/13 | 8 | 109 | 256 | 63 | 0 |
| 8/10 | 4 | 8 | 101 | 0 | 5 |
| 9/21 | 8 | 0 | 626 | 0 | 0 |
| **Root** | Yamhill-2 | | | | |
| 6/15 | 0 | 0 | 0 | 1 | 0 |
| 7/13 | 0 | 12 | 1 | 0 | 0 |
| 8/10 | 0 | 0 | 0 | 0 | 0 |
| **Root** | Marion | | | | |
| 6/15 | 0 | 6 | 1 | 0 | 1 |
| 7/13 | 0 | 15 | 0 | 0 | 0 |
| 8/10 | | 0 | 7 | 0 | 1237 |
| **Root** | Yamhill-1 | | | | |
| 6/15 | 0 | 1 | 0 | 0 | 0 |
| 7/13 | 0 | 27 | 0 | 0 | 0 |
| 8/10 | 0 | 0 | 1 | 0 | 0 |

*If Criconemella were run through a centrifugation extraction method, numbers in column would be 15x.

Soil extractions are expressed in nematodes/quart of soil from a 5-day Baermann funnel.

Root extractions are expressed in nematodes/gram of roots extracted in a 7-day mist chamber.

This table is part of a reading and conference by Anne E. Connelly for Dr. Russ Ingham with assistance by: Kathy Merrifield, Paula Stonerod and Tom Forge.

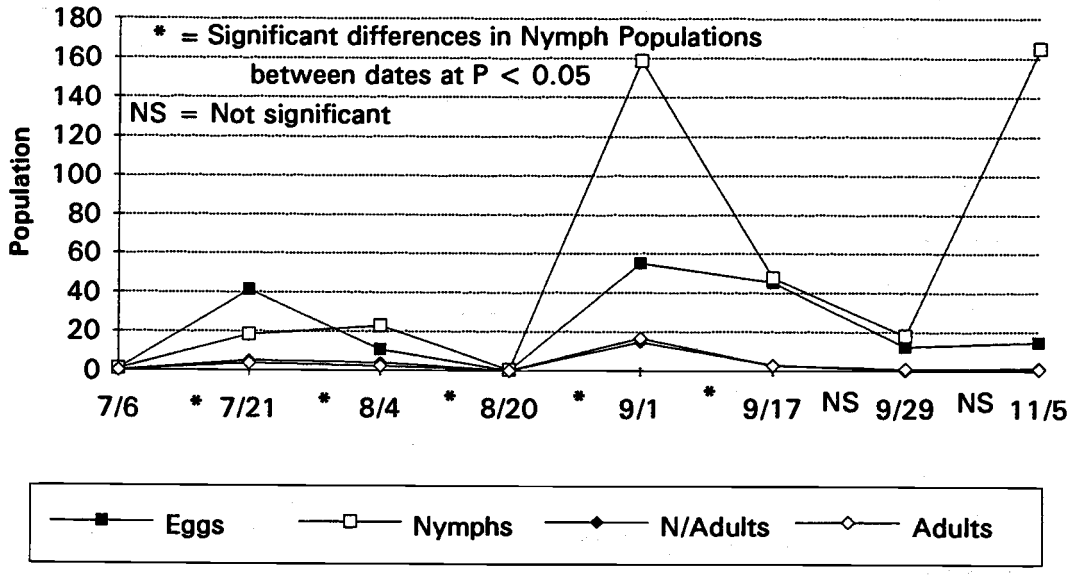



Figure 3.8 Marion grape phylloxera populations sampled in 1992.
Mean number of insects from 10 vines, pooled across two depths (15 to 30 and 30 to 45 cm), standardized to 30 grams of roots.

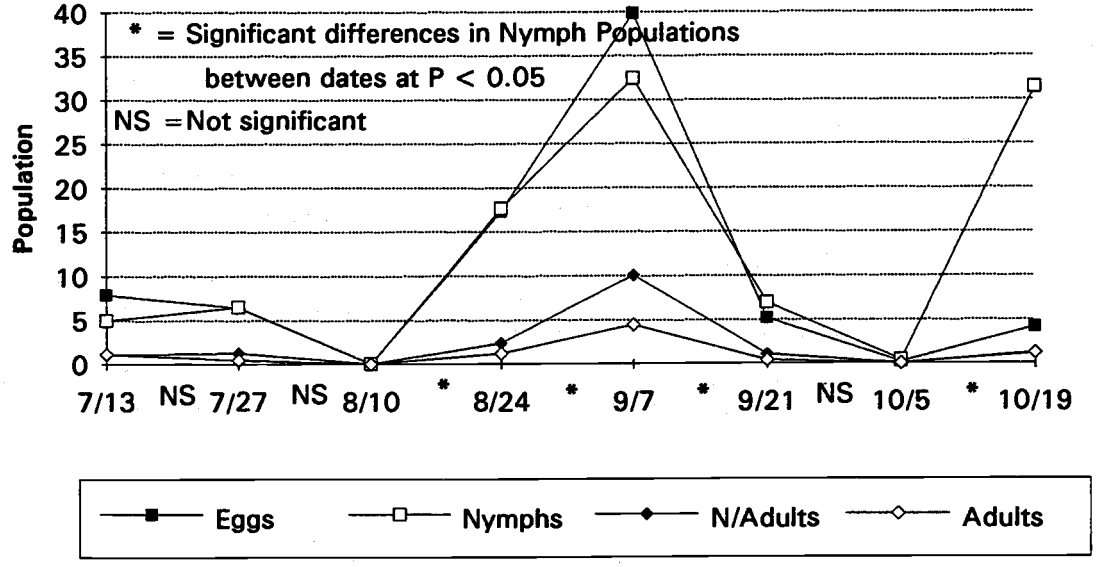



Figure 3.9 Marion grape phylloxera populations sampled in 1993.
Mean number of insects from 10 vines, pooled across two depths (15 to 30 and 30 to 45 cm), standardized to 30 grams of roots.

The drop in population levels in 1993 compared to 1992 may have been due to a reduction in root quality. Root growth at 15-30 cm appeared minimal and the percent moisture of roots was the lowest at Marion for half the sampling dates (Figure 3.10). Percent root moisture was also the lowest at Marion in nine out of twelve sampling dates at 30-45 cm (Figure 3.11). Individual GP population extraction levels and root moisture, were not significantly correlated. However, decline in moisture levels as the season progressed could have affected or been indicative of root substrate quality.

A relationship (e.g. competition for root substrate) between GP and nematodes may have also existed. Plant pathogenic nematode sampling done in 1993 at this site yielded over 53,000 *Meloidogyne sp.* per quart on August 10 and high numbers of *Pratylenchus sp.*, *Criconemella sp.*, and *Paratylenchus sp.* on September 21 (Table 3.6).

The effect of date on eggs and nymphs were highly significant for both years (P=0.0001 for both lifestages). The comparison of dates by least squares means yielded significant differences in nymph and egg population peaks and their preceding valleys for both years in most cases. In 1992, the 9/29 and 11/5 comparison for nymphal populations was not significant (NS) and in 1993, the 7/13 and 7/27 comparison for nymphal populations was not significant (NS) (Figures 3.8 and 3.9). Analysis by the least squares means substantiated the significant differences in peaks and valleys which allowed greater predictability in generation peaks. Two nymphal population peaks occurred for Marion in 1992 and 1993. In 1992, the first peak was on August 4 and the second on September 1 (Figure 3.8). The two nymphal population peaks in 1993 occurred on September 7 and October 19 (Figure 3.9).

Date was significant for mean number of n/adults and adults at Marion (P=0.0080 and 0.0026 respectively). These two life stages had a similar pattern of development and population peaks were seen in early September for both years (Figures 3.8 to 3.9).

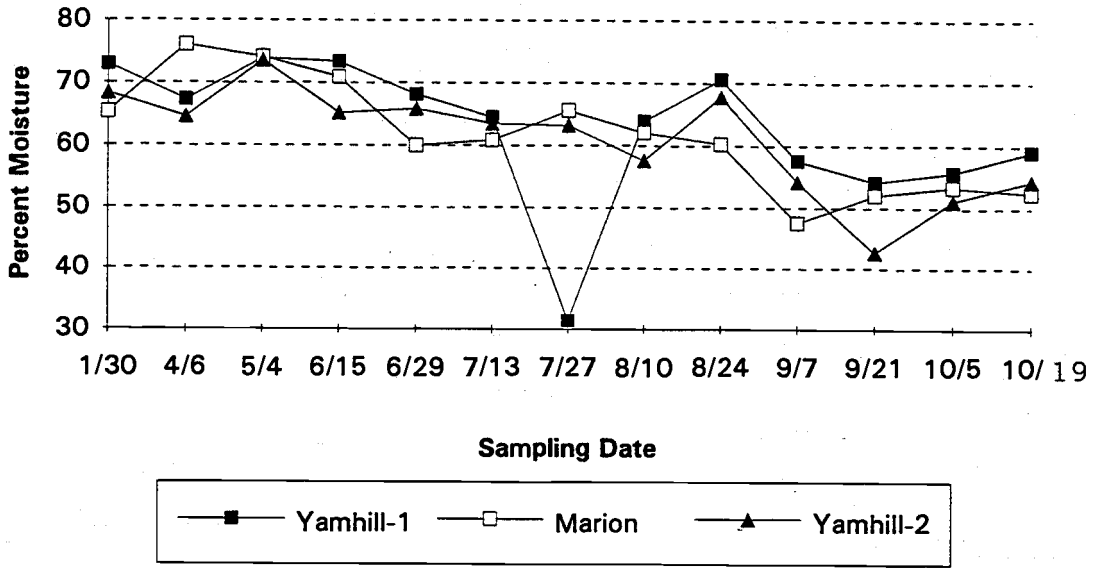



Figure 3.10 Mean percent root moisture from roots sampled for grape phylloxera at 15-30 cm soil depth, 1993. Percent moisture = [(root fresh weight - dry weight)/fresh weight]x100.

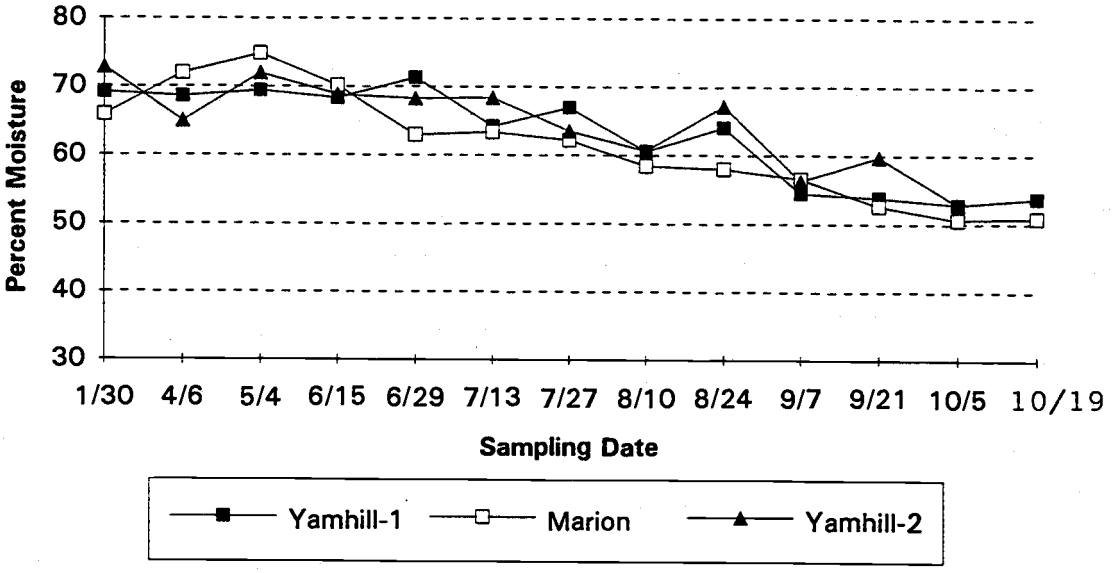



Figure 3.11 Mean percent root moisture from roots sampled for grape phylloxera at 30-45 cm soil depth, 1993. Percent moisture = [(root fresh weight - dry weight)/fresh weight]x100.

The effect of sampling side on nymph population levels was significant at Marion. Nymph populations on side one were significantly higher than side two and three in 1992 and 1993 (Table 3.5). The Marion site had many satellite weak areas surrounding the main lens, but the presence of GP was never verified in these areas. Figure 3.1 illustrates the low vigor contour ratings surrounding the Marion infestation.

There are many factors which contribute to reduced vine vigor within a grape phylloxera infestation and this site was a good example of that. Low soil temperatures along with inferior water and air drainage in the winter and nematode competition may have lead to low GP populations in 1993. Yet a comparative visual field observation, using above-ground vigor ratings (0 to 5 described in materials and methods), rated this site the worst of all three (Table 3. 8). The damage done by sampling may have confounded results, but the average rating for all vines counted within the infestated area was the lowest at the Marion site in 1992 and 1993 (Table 3.8). In addition, the manner in which a vineyard becomes infested can affect the symptomatic response. A single point source introduction may be more contained than an infestation resulting from infected plant material. This site was thought to be infested through planting material. These comments serve as conjecture, further research is needed to substantiate them. All these factors contribute to the difficulty in quantifying what effect GP populations have on vine decline.

The effect of date on egg and nymph populations was not significant for the Yamhill-2 site. Date was significant for n/adults and adult populations (P=0.014 and 0.0033 respectively). Populations of n/adults in 1992 were significantly different between the dates 8/4 and 8/20 (P=0.0039), and 8/20 and 9/1 (P=0.0036) (Figure 3.12). Date also affected adult populations for those date combinations in 1992 (P=0.0016 and 0.0027 respectively). In 1993, n/adult populations were significantly different between 9/21 and 10/5, but date had no effect on adult populations.

| Site | Year | Rating* | SD | Total n | % 0 (n) | % 1 (n) | % 2 (n) | % 3 (n) | % 4 (n) | % 5 (n) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| Yamhill-1 | 1990 | 3.5 | 1.3 | 47 | 0.0 (0) | 0.1 (6) | 0.1 (6) | 0.1 (6) | 0.4 (18) | 0.2 (11) |
| | 1991 | 3.6 | 1.1 | 104 | 0.0 (2) | 0.0 (4) | 0.1 (12) | 0.1 (11) | 0.6 (66) | 0.1 (9) |
| | 1992 | 3.5 | 1.0 | 159 | 0.0 (2) | 0.0 (3) | 0.1 (18) | 0.3 (45) | 0.5 (77) | 0.1 (14) |
| | 1993 | 3.6 | 1.1 | 145 | 0.0 (2) | 0.0 (5) | 0.1 (14) | 0.3 (37) | 0.4 (60) | 0.2 (29) |
| | | | | | | | | | | |
| Marion | 1992 | 2.5 | 0.9 | 333 | 0.0 (2) | 0.2 (55) | 0.3 (86) | 0.5 (161) | 0.1 (29) | 0.0 (0) |
| | 1993 | 2.7 | 1.1 | 333 | 0.0 (11) | 0.1 (24) | 0.3 (98) | 0.4 (121) | 0.2 (72) | 0.0 (7) |
| | | | | | | | | | | |
| Yamhill-2 | 1992 | 2.6 | 1.0 | 177 | 0.0 (3) | 0.1 (19) | 0.2 (37) | 0.3 (61) | 0.3 (47) | 0.0 (0) |
| | 1993 | 2.6 | 1.1 | 177 | 0.0 (5) | 0.1 (11) | 0.3 (56) | 0.3 (46) | 0.2 (40) | 0.1 (9) |

* Rating is the average of all vine ratings, the six rating categories were 0=dead vine, 1=nearly dead, 2=low vigor, 3=yellow leaves, 4=pale green leaves, 5=healthy vine.

SD = standard deviation

Total n = the number of vines rated

(n) = the number of vines in each category

Table 3.8 Percent vine decline ratings (0 to 5) for three grape phylloxera infestations from 1990 to 1993.

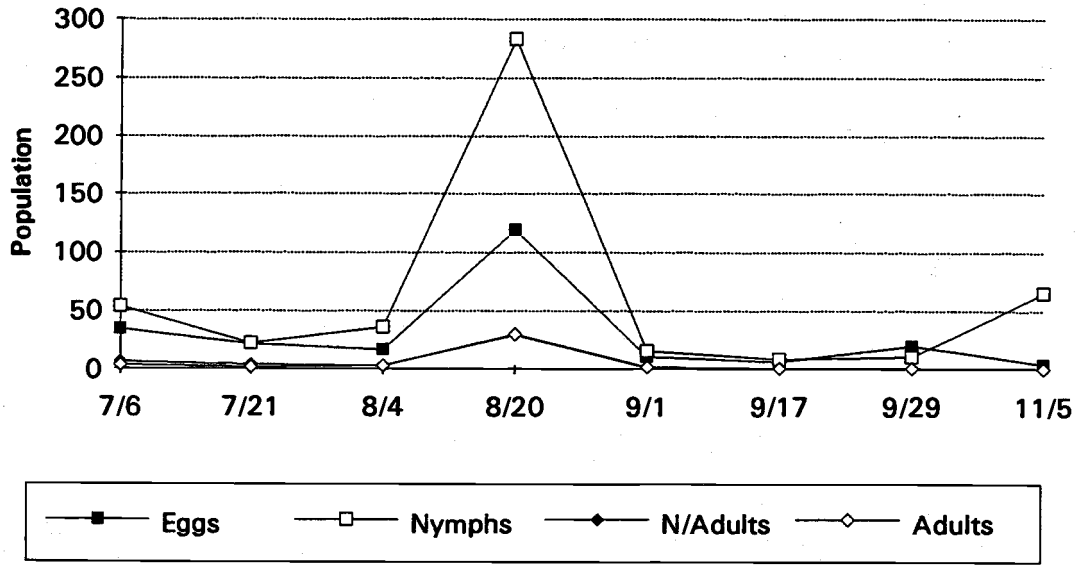



Figure 3.12 Yamhill-2 grape phylloxera populations sampled in 1992.
Mean number of insects from 10 vines, pooled across two depths (15 to 30 and 30 to 45 cm), standardized to 30 grams of roots.

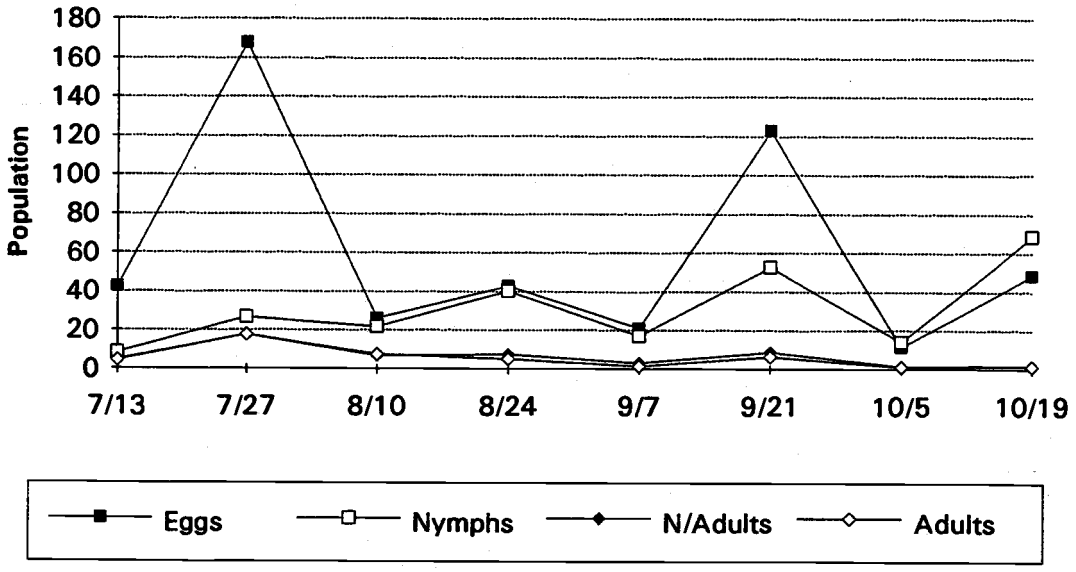



Figure 3.13 Yamhill-2 grape phylloxera populations sampled in 1993.
Mean number of insects from 10 vines, pooled across two depths (15 to 30 and 30 to 45 cm), standardized to 30 grams of roots.

Soil temperatures at Yamhill-2 were intermediate compared to those at the other two sites in both years (Figure 3.4 and 3.5). The percent root moisture was the lowest at Yamhill-2 for half of the sampling dates at 15-30 cm (Figure 3.10). The soil was highly compacted due to cultivation and digging became difficult as the summer progressed. Yamhill-2 was intermediate in vine decline ratings compared to the other two sites (Table 3.8). It is not known how this site was infested. This site also suffered from a poor powdery mildew control program in 1992 which may have increased the vine stress. Nematode populations at Yamhill-2 did not reach levels found at the other two sites (Table 3.6)

The side sampling at Yamhill-2 was similar to the other two sites with side one and three sampled within rows and side two across rows (Figure 3.1). One difference, however, was that side three was on the downward slope. Side three was significantly higher in nymphal populations than the other two sides (P=0.0001). This increase could have been due to the movement of GP in the topographical drainage (Table 3.5).

## Conclusions

Peak populations of eggs and nymphs were difficult to distinguish from valleys (low population levels) at the Yamhill-1 and Yamhill-2 sites, but the Yamhill-1 site showed a significant increase in nymphal populations over the summer in both years. N/adults and adults showed an effect by date at the Yamhill-1 and Yamhill-2 sites and could be used to distinguish moderate population changes. The Marion site showed a significant effect of date on egg and nymph numbers. Two distinct population peaks occurred at the Marion site in 1992 and 1993. It is not known whether these peaks were GP generations or population fluctuations due to some extrinsic factor. The simultaneous

rise and fall of the four life stages may have been due to changes in the soil environmental or plant physiological changes.

Grape phylloxera populations from 1992 to 1993, decreased for Marion, increased for Yamhill-1, and remained at the same level for Yamhill-2. The site and cultural practices may have sustained GP levels without experiencing excessive vine decline at Yamhill-1. Yamhill-1 experienced warmer temperatures and superior air and water drainage. In addition, vines received drip irrigation during establishment years and a between row sod remained in place after the GP infestation had been verified. Root moisture at Yamhill-1 was the highest overall in 1993 (Figures 3.10 and 3.11). The average above-ground rating for the entire site was highest (healthiest) for this infestation than Marion or Yamhill-2 (Table 3.8). There were no significant differences in temperatures or GP populations, during the summer season, between the 15-30 cm and the 30-45 cm soil depth.

Granett and Timper (1987) calculated the mean generation time of 37 and 36 days (at 21 and 24°C, respectively). The Marion site often had temperatures between the 16 and 21°C treatments studied by Granett and Timper (1987). The approximated 45 days between peaks at Marion could have been the result of cooler temperatures and poor root quality.

The variance of population samples precluded any predictions of generation time at Yamhill-1 and Yamhill-2. Degree day accumulation may provide the information necessary to distinguish peaks that would be related to generation time and further research of the root and soil environments could help to establish the effect of extrinsic factors on population fluctuations.

The emergence of life stages was identified using a combination of soil/root extractions and sticky trunk wraps. Extractions were successful in finding the first generation eggs in April/May and the first hibernants in July. The sticky trunk wraps recovered above-ground crawlers and alates in July/August. Generation number

prediction was more difficult due to the variance in samples and the interaction between sites, years, and dates. Sides of infestation can vary in population levels based on proximity to satellite infestations, and topographic air and water drainage. The clumped distribution of grape phylloxera in the field necessitates a large number of samples on frequent sampling dates. Soil temperatures and vine phenology will influence the overall response of GP regeneration, but site, cultural practices (eg. irrigation and cultivation), and vine age can have a profound effect on the number of insects.

# CHAPTER 4. Laboratory Low Temperature Threshold Experiment

## Introduction

The extent to which vineyards decline due to grape phylloxera feeding is dependent in part on the root type (Davidson and Nougaret, 1921; De Benedictis and Granett, 1993; Granett et al., 1983; Granett et al., 1987; Granett et al., 1992), soil type (Davidson and Nougaret, 1921; De Klerk, 1974; King et al., 1982b; Stevenson, 1968), and climatic variables (Belcari and Antonelli, 1989; Davidson and Nougaret, 1921; De Klerk, 1974; Granett and Timper, 1987; Raspi et al., 1987). These factors influence the rate of development of the insect.

Grape phylloxera was found in a commercial vineyard in Oregon in August of 1990 (Connelly and Strik, 1992). Since that time, nine more infestations have been found throughout the state (Strik et al., 1995). Over 95 % of Oregon's 6000 acres are planted to root-susceptible *Vitis vinifera* vines. Once infested, susceptible vineyards reach low economic thresholds that warrant replanting. Understanding this insect in our cool climate viticultural region will help forecast this costly replant procedure.

## Demography

Parameters used to calculate the rate of increase of an insect population have adopted human demographic procedures established by Lotka (Birch, 1948). The true, inherent, or intrinsic rate of natural increase has been calculated on a number of species. The intrinsic rate (*r)* is defined as the instantaneous rate of increase accounting for only birth and death. The 'finite' rate of increase is the anti log of *r*. Calculating *r* can be done

by maintaining a specified number of insects under controlled conditions, where age-specific rates of fecundity and mortality can be determined (Birch, 1948). To approximate *r*, life tables must calculate the survival ($l_x$) of adults and immatures, and the age-specific fecundity rates ($m_x$) established on each time interval ($x$). A net reproductive rate ($R_0$) can be calculated by $\Sigma l_x m_x$. Gross reproductive rate (GRR) is calculated by $\Sigma m_x$. Generation time must be estimated by calculating $T = \Sigma x l_x m_x / l_x m_x$. With these values established, $r$ can be derived from the equation $r = \log_e R_0 / T$, which is essentially the rate of increase per individual, per interval (Birch, 1948). Life tables can then be established at different temperatures where $r$, as well as developmental rates or survivability and fecundity, will vary. Having determined $r$, the question becomes to what extent do field conditions affect rate of increase, where extrinsic factors such as environment, food availability, and interactions with other species are present.

**Grape Phylloxera Temperature and Demographic Research**

Regional differences in the severity of phylloxera have been attributed to temperature (DeKlerk, 1974; Granett and Timper, 1987). Temperature may influence fecundity, cohort survivorship, and generation time (Birch, 1948, Granett and Timper, 1987). In addition, insects may adapt to regional climates and threshold temperatures previously determined may no longer be valid under new conditions.

In a study conducted by Granett and Timper (1987), egg-hatching tests were performed on a Napa County colony at 10 temperatures (7, 10, 13, 16, 21, 24, 28, 32, 35, and 39°C). Life tables were constructed for laboratory-reared GP on excised root pieces at five different temperatures (16, 21, 24, 28, and 32°C). Results indicated egg survival was significantly less at 16 and 36°C than between 21 and 28°C, and nymphal survival was significantly less at 16°C. Developmental rates at 16°C indicated that the duration of the

immature stages was greater than 2 months, generation time was greater than 2 months, and doubling time was approximately 1 month. Granett and Timper (1987) substantiated previous data by Davidson and Nougaret (1921) that showed GP went into hibernation at 19°C and became active again when temperatures rose to 15°C, a lower temperature than hibernation commencement.

Davidson and Nougaret (1921) in laboratory and field studies in California, estimated the duration of the hibernant at 5 months. The first generation length was 4 to 7 weeks long once egg deposition had begun. The average length of egg deposition was 45 days with a daily fecundity mean of 2.5. Incubation period was 5 days in July and 30 days in December (Davidson and Nougaret, 1921). The time for the insect to reach maturity was calculated at 15 days for midsummer and 34 days for April and November.

In laboratory research conducted by Raspi et al. (1987) a thermal constant for foliar grape phylloxera was calculated from the thermal summation above 8.7°C (developmental zero for eggs). Peak population periods were used to determine generation numbers in the field, but late-season generation peaks ran together making determination difficult. The laboratory thermal constant was correlated with mean field temperatures and six generations were calculated.

Belcari and Antonelli (1989) studied the influence of temperature on duration and rate of development of foliar GP reared at eight constant temperatures (13, 15, 18, 20, 25, 30, 32, and 34°C). Mean durations for the first instar nymph ranged from 45 days at 15°C to 15 days at 30°C. Developmental zero was calculated at 6.4°C for first instar nymphs (Belcari and Antonelli, 1989). The three stages following the first instar nymph had a developmental zero of 9.9°C.

The objectives of this grape phylloxera low temperature threshold study were to: 1) determine the development times of three Oregon GP populations at five temperatures (7, 10, 13, 16, and 21°C); 2) compare laboratory findings with field population numbers and soil temperatures (Chapter 3) found in the three vineyards from which the GP

populations were sampled; 3) calculate temperature thresholds for development of the GP life stages present at each of the five temperatures.

## Materials and Methods

Grape phylloxera populations were collected from three infested vineyards in the Willamette Valley, Oregon during the summer of 1993. Roots were collected from holes dug 15-to 45-cm deep and within 20 cm of vine trunks.

Samples were transported back to the laboratory in plastic bags and run through a sieve centrifugation extraction method (Griesbach, personal communication). Roots were sprayed at high pressure with tap water for approximately 15 seconds. Insects and debris dislodged from roots were collected on two sieves (W.S. Tyler Inc.,U.S.A.) with 500 and 106 micrometer mesh size. GP collected on the 106 micrometer screen were rinsed with distilled water into a 100 ml centrifuge tube and left to settle for one hour. The supernatant was poured off and a sucrose solution (450 g /liter) was added to the pellet containing settled insects. Samples were stirred, centrifuged at 2800 rpm for 3 minutes, and the supernatant poured off onto a 45 micrometer mesh screen suspended in water. GP collected on this screen were rinsed into a 5 cm petri dish for counting. Extracted GP were then poured off onto a 15 cm filter paper-lined petri dish. Low speed sucrose centrifugation settles out large particles and fragments thereby cleaning samples for accurate counting.

Eggs, 1 to 14 days old, were transferred with a fine sable brush from petri dishes to clean, excised 'Pinot Noir' root pieces 4 cm long and 3 mm in diameter (Granett et al., 1983; 1986). These root pieces were collected from a non-infested vineyard, washed in tap water, then rinsed with distilled water. Root ends were wrapped in moistened cotton and placed over two sheets of filter paper. Once transferred, root pieces with eggs were

placed in plastic petri dishes 10 cm in diameter. Petri dish lids had a 2 cm hole covered with a fine cloth to allow ventilation, but prevent GP from escaping. Petri dishes were then wrapped with parafilm (National Can Inc.) and placed in sealed containers. The colonies of GP were held in incubators at 21° C for a minimum of two months with weekly colony maintenance prior to conducting the low temperature tolerance experiment. Colony maintenance included transferring eggs to new root pieces, replacing soiled cotton and filter paper, and maintaining petri dish moisture level. Petri dish contents were kept moist, but saturation of cotton and root surface was avoided.

Five temperatures were selected for the study: 7, 10, 13, 16, and 21° C. This was thought to represent a range of temperatures where conditions would result in death, hibernation, and near-optimum growth.

From each of the three vineyard colonies, five replicates of ten eggs each were transferred to five fresh 'Pinot Noir' root pieces. Five incubators were run simultaneously at each temperature (±1°C). Each incubator had a representative 10 parental eggs on each of five root pieces, in two ventilated petri dishes placed in sealed plastic containers (eg. food storage-type containers), for each of the three vineyard populations. Insects remained in total darkness except during counting. Soil-borne GP remains in darkness except when emerging above-ground in late-summer, therefore controlling for light or photoperiod was not necessary.

The experiment ran for 56 days and counts of insect stages (eggs, nymphs, n/adults-late instar nymphs, and adults) were performed every seven days with a 70x dissecting scope. The insect life stages were divided into the four categories on the basis of identification (morphology). Eggs were easily distinguished, nymphs represented the smaller early-instar nymphs (first 1-2 molts), and n/adults represented the larger late-instar nymphs. Adults were distinguished from late-instar nymphs by their larger dorsal tubercular areas (Davidson and Nougaret, 1921). Relative measurements of size were made in the absence of a lens micrometer. A partial life table was constructed for the

colonies reared at 21°C. Day numbers (e.g. 7, 14, 21, 28, 35, 42, 48, and 56 days) refer to colony counting periods after experiment initiation (day 0). Differences between vineyard populations and temperatures were analyzed with a two way factorial ANOVA, repeated measures analysis of variance, and regression (SAS, 1988). Vineyard site populations were pooled after no significant effect of temperature was seen between individual vineyard populations.

## Results and Discussion

The average age of parental eggs at the start of the experiment was six days old. This was calculated from the weighted mean egg hatch seen between 0 and 14 days at 21°C. All colonies were reared at this temperature two months prior to day 0. "Days" refers to the day number, after experiment commencement, that insect counts were made. "Egg hatch" is defined by the decrease in egg numbers in the egg column and the appearance of nymphs is defined by the increase in numbers in the nymph column. The mean number of life stages for the three populations pooled, and their standard deviations at the five temperatures are presented in table 4.1. Temperature significantly affected grape phylloxera development.

Table 4.1 Mean number (plus SD = standard deviation) of grape phylloxera at four life stages for three vineyards (pooled) reared at 7, 10, 13, 16, and 21°C for 56 days.

| | Life stages* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Eggs | | Nymphs | | N/Adults | | Adults | | Eggs+ | |
| Day | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| **7°C** | | | | | | | | | | |
| 0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 49.0 | 1.0 | 0.3 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | 46.7 | 3.1 | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | 44.0 | 3.6 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | 38.3 | 7.1 | 3.0 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | 34.0 | 5.6 | 3.3 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 42 | 34.3 | 4.5 | 2.0 | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 49 | 34.3 | 4.0 | 0.3 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 56 | 32.0 | 4.0 | 1.3 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **10°C** | | | | | | | | | | |
| 0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 48.3 | 2.1 | 0.3 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | 42.0 | 4.4 | 5.7 | 2.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | 39.0 | 4.6 | 6.3 | 2.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | 30.7 | 3.2 | 12.7 | 2.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | 28.3 | 2.5 | 12.0 | 1.7 | 0.7 | 1.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 42 | 24.7 | 4.7 | 12.0 | 3.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 49 | 18.7 | 9.0 | 14.0 | 6.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 56 | 14.0 | 7.2 | 12.0 | 7.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **13°C** | | | | | | | | | | |
| 0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 43.0 | 4.4 | 3.0 | 3.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | 19.7 | 5.5 | 22.7 | 5.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | 6.0 | 6.0 | 29.3 | 3.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | 1.7 | 2.1 | 37.0 | 6.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | 2.3 | 4.0 | 36.7 | 6.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 42 | 0.3 | 0.6 | 36.7 | 5.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 49 | 1.0 | 1.7 | 34.7 | 4.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 56 | 0.7 | 1.2 | 34.0 | 4.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

* Life stages: Eggs= parental eggs, Nymphs= early instar nymphs, N/Adults= late instar nymphs, Adults= mature insects, and Eggs+= offspring generation

Table 4.1 (cont.) Mean number (plus SD = standard deviation) of grape phylloxera at four life stages for three vineyards (pooled) reared at 7, 10, 13, 16, and 21°C for 56 days.

| | Life stages* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Eggs | | Nymphs | | N/Adults | | Adults | | Eggs+ | |
| Day | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| **16°C** | | | | | | | | | | |
| 0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 44.7 | 6.1 | 4.3 | 5.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | 3.3 | 3.5 | 39.3 | 2.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | 0.0 | 0.0 | 38.0 | 18.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | 0.0 | 0.0 | 42.7 | 2.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | 0.0 | 0.0 | 44.3 | 3.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 42 | 0.0 | 0.0 | 43.0 | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 49 | 0.0 | 0.0 | 45.0 | 4.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 56 | 0.0 | 0.0 | 43.7 | 5.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **21°C** | | | | | | | | | | |
| 0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 30.3 | 7.8 | 14.7 | 9.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | 0.0 | 0.0 | 41.0 | 1.7 | 0.7 | 1.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | 0.0 | 0.0 | 36.0 | 1.7 | 4.0 | 6.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | 0.0 | 0.0 | 22.7 | 11.6 | 14.0 | 9.5 | 0.0 | 0.0 | 39.3 | 62.9 |
| 35 | 0.0 | 0.0 | 27.0 | 9.0 | 9.3 | 1.5 | 12.7 | 3.5 | 155.7 | 51.3 |
| 42 | 0.0 | 0.0 | 159.0 | 88.5 | 7.0 | 1.7 | 28.3 | 8.0 | 449.3 | 173.8 |
| 49 | 0.0 | 0.0 | 197.3 | 63.4 | 8.3 | 2.5 | 26.0 | 2.0 | 239.7 | 47.2 |
| 56 | 0.0 | 0.0 | 694.0 | 156.0 | 12.3 | 12.0 | 23.7 | 4.2 | 210.0 | 33.2 |

* Life stages: Eggs= parental eggs, Nymphs= early instar nymphs, N/Adults= late instar nymphs, Adults= mature insects, and Eggs+= offspring generation

**7 to 16°C**

Temperature had a significant effect on parental egg hatch. The percent egg hatch by day 14 was: 7% at 7°, 16% at 10°C, 61% at 13°C, and 93% at 16°C.(Figure 4.1).

No insects survived past the nymphal stage at temperatures between 7 and 16°C, except on day 35 at 10 °C (Table 4.1). These late instar nymphs did not survive past this date. Results from this experiment would indicate that under laboratory conditions, 16°C is the low temperature threshold for development past the early nymph stage.

**21 °C**

The first appearance of nymphs was between day 0 and day 7, and all parental eggs had hatched by day 14 for populations reared at 21°C(Table 4.1).

Adults were first observed on day 35, while offspring generation eggs (eggs+), were first observed on day 28 (Table 4.1). This could be an indication of paedogenesis (the ability to reproduce at an immature stage) (Chapman, 1982) or some GP were recorded as immature when actually an adult. The nymph and n/adult life stages were distinguished solely on the basis of body size. Thus n/adult category, devised to group late-instar nymphs, may have had some adults who had not reached average size. Paedogenesis does occur in Aphidoidea (Chapman, 1982) thus further research to test this hypothesis in grape phylloxera is warranted.

It was impossible to determine what proportion of the n/adults contributed to fecundity after day 28, consequently all succeeding generation eggs (egg+) were calculated as the proportion contributed by adults only.

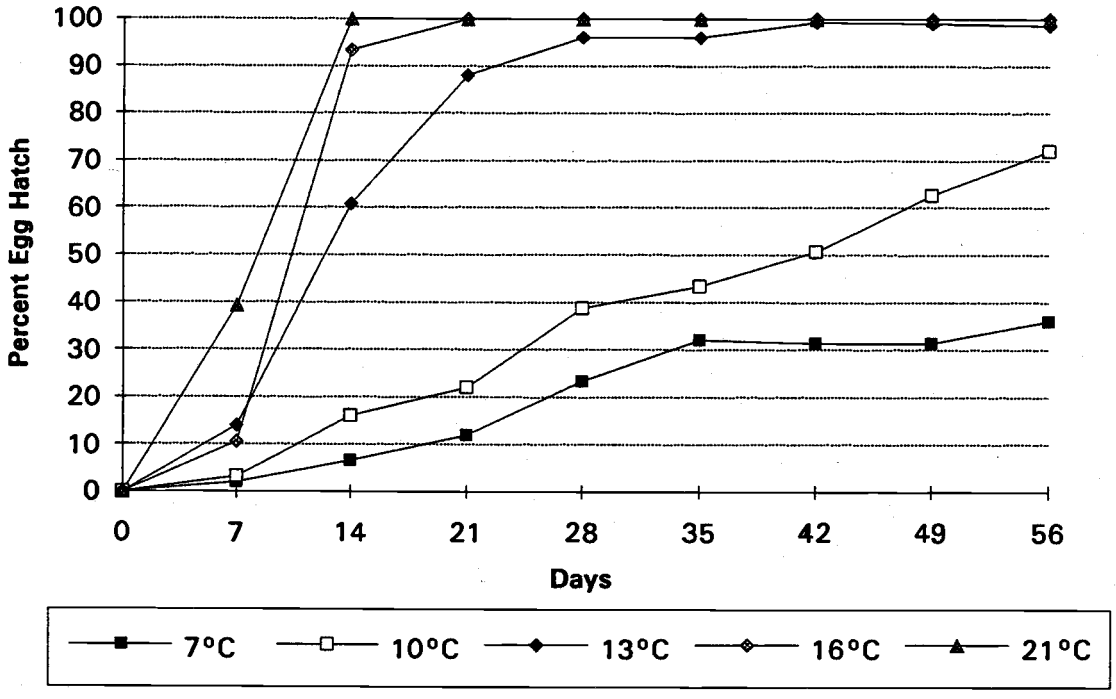



Figure 4.1 Percent egg hatch of grape phylloxera with laboratory rearing at five temperatures.

The offspring generation eggs (egg +) was not significant by vineyard population but was by day ( $P < 0.0001$). Eggs + increased by 60 to 68% between day 35 and day 42 (Table 4.2). When considering the number of parental eggs at day 0 (50 eggs), the mean of the three populations experienced a nine-fold increase in egg number by day 42.

Table 4.2 Partial life table of the mean of three GP populations reared at 21°C

| *x* | Egg | Nymph | N/adult | Adult | Egg+ | *lx* * | *mx*** | *lxmx* | *xlxmx* |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 50 | 0 | 0 | 0 | 0 | 1.00 | | | |
| 7 | 30 | 15 | 0 | 0 | 0 | 0.90 | | | |
| 14 | 0 | 41 | 1 | 0 | 0 | 0.84 | | | |
| 21 | 0 | 36 | 4 | 0 | 0 | 0.80 | | | |
| 28 | 0 | 23 | 14 | 0 | 39 | 0.74 | 0.40 | 0.30 | 8.29 |
| 35 | 0 | 27 | 9 | 13 | 157 | | 1.73 | | |
| 42 | 0 | 159 | 7 | 28 | 449 | | 2.29 | | |
| 49 | 0 | 197 | 8 | 26 | 240 | | 1.32 | | |
| 56 | 0 | 694 | 12 | 24 | 210 | | 1.25 | | |

x = day lifestages counted
Egg = parental egg
Nymph = early nymphal stage
N/Adult = late nymphal stage
Adult = adult from parental egg
Egg+ = offspring generation egg
lx* = cohort survival calculated through day 28
mx** = fecundity (eggs/cohort/day) calculated on day 28 for n/adults and day 35 to 56 for adults
lxmx = cohort survival x fecundity for n/adults
xlxmx = day interval x cohort survival x fecundity for n/adults

Nymphs increased in number after day 35, when the offspring generation eggs hatched (Figure 4.2). An increase in nymphs such as this could result in a high percentage of crawlers in the field as they move about to seek new food sources.

Generation time could not be established due to the assay methods used in this experiment. The removal of eggs would have been necessary to calculate adult cohort survivorship and generation time based on the methodology employed by Granett and Timper (1987).

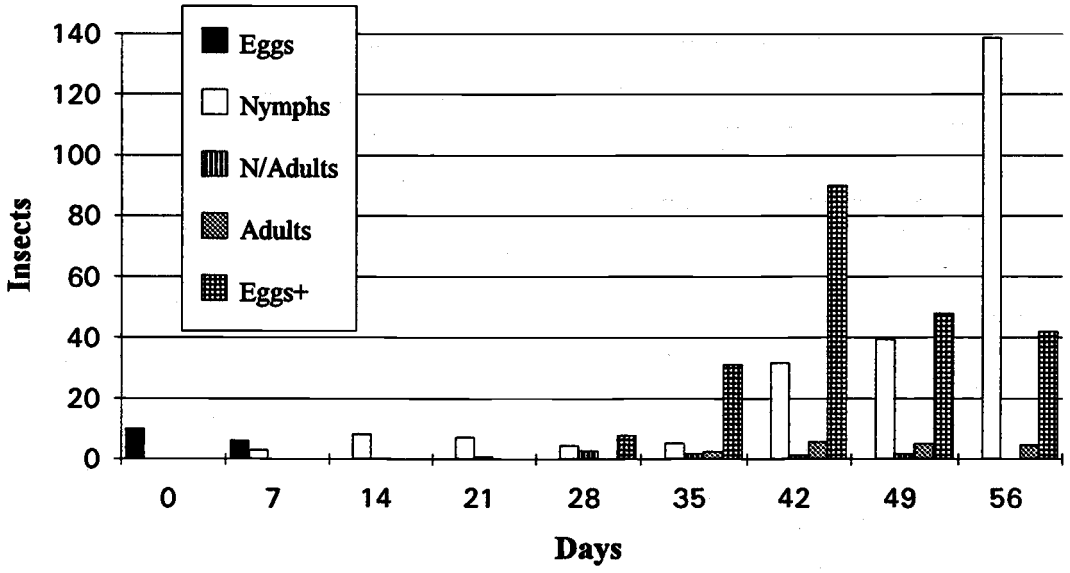



Figure 4.2 Mean number of grape phylloxera reared at 21°C over 56 days.

The partial life table did provide information on 'time to adult' for populations at 21°C. Time to adult was estimated to be between 28 to 35 days (with the six day parental egg age added) if adults were present in the 'N/Adult' category. If adults were not present in this category then the estimate is 35 to 42 days (with the six day parental egg age).

**Comparison with Field Results**

Grape phylloxera did not develop beyond the small nymphal stage in the laboratory by day 56 at temperatures of 7 to 16°C, but full development took place at 21°C. The shortest immature developmental time at 21°C was 28 days, while the longest development time was 42 days. Distinguishing certain n/adults from small adults was difficult and development time could have varied because of this.

Soil temperatures in the field at 15 cm during the summer months approximated the 21°C constant temperature. Results from the 21°C laboratory assay indicated the mean time to first adult was 35 days. Laboratory assays could help establish field generation times by substituting laboratory generation time under similar temperature regimens. Further analysis is necessary with this data to corroborate this.

Number of GP generations per year was previously predicted at three to four for three vineyard sites but the variance between sample dates prevented the statistical confirmation of generations per year (Chapter 3). The Marion site had two population peaks each in 1992 and 1993 and soil temperatures at 15 cm never rose above 20°C in 1993 (Figure 3.4). Mean soil temperatures at the Yamhill-1 site more closely approximated the 21°C constant temperature used in this laboratory study but sample variance prevented the prediction of populations in the field.

Over all, soil temperatures for the three sites at 15 cm ranged from 15 to 25°C in 1992 and 15 to 22°C during the growing season in 1993. Eggs first appeared in the field in-late April to early-May when soil temperatures were 10.5 to 12.0°C (Chapter 3), yet by day 56 in the laboratory study, generation eggs (eggs+) had not appeared at temperatures of 16°C. Either survival and development proceeds in the field at lower temperatures than in the laboratory or development to maturity takes longer than 56 days.

While constant temperature studies can be misleading (Roltsch et al., 1990), the subterranean form of grape phylloxera does experience slow changes in temperature due to the buffering capacity of soil. In this study (Chapter 3), mean soil temperatures at 15 cm rarely deviated more than 1°C in a 24 hour period. Constant temperature is thus a suitable laboratory methodology for calculating GP developmental times when comparing to summer field temperatures in Oregon at 15 cm. Insect sampling was done at the 15-to 45-cm depth in the field study due to higher number of roots at this depth. Future laboratory temperature assays should include temperatures between 16 and 21°C in order to model development under spring conditions.

**Temperature Threshold**

The mean proportion of nymph survival at five temperatures for all three populations were linearly related for temperatures between 7 and 21°C (Figure 4.3). All life stages are included in figure 4.3, eggs and nymphs for temperatures 7 to 16°C and eggs to adults for 21°C. The formula for the predicted values was calculated as Y = 0.635X - 3.181. Using this linear model, the temperature for zero development or no insect survival (all life stages) was calculated to be 5.0°C. Three percent of the nymphal population was still alive in the laboratory at 7°C on day 56. When the 21°C assay was added to the regression, nymph, n/adult, and adult survival was calculated at day 35. This was due to the offspring generation, present after this day, which confounded the survival results the parental generation. All other temperatures were represented by nymphal survival at day 56. Nymphs did not molt past the nymphal stage and survive at the four lowest temperatures.

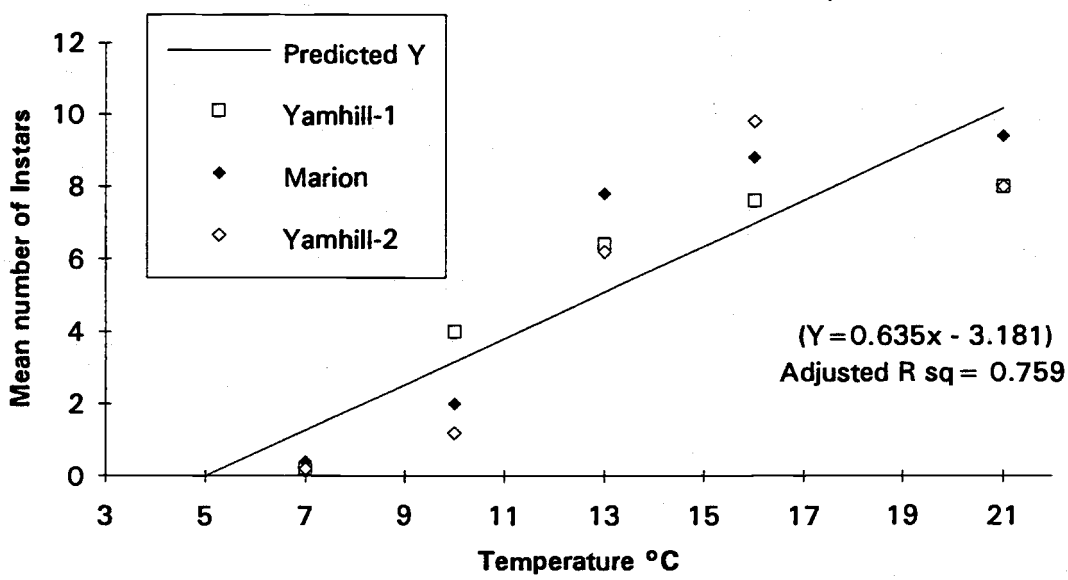



Figure 4.3 Low temperature threshold for development of phylloxera calculated from contstant temperatures of 7 to 21°C.

Degree day calculations (accumulated degrees above threshold) were computed for the Yamhill-1 site in 1992 as degree days above the laboratory low temperature threshold of 5°C and below the maximum daily soil temperatures at 15 cm (Table 4.3). Table 4.3 also shows the number of degree days between dates and the accumulated degree days for both the low 5°C, and the standard 10°C base temperature (used for vine phenology).

Table 4.3 Accumulated and individual degree day calculations of the Yamhill-1 site from the maximum field soil temperatures at 15 cm using two threshold temperatures.

| Degree Days* | | | | |
|---|---|---|---|---|
| | 5°C Threshold** | | 10°C Threshold*** | |
| Date | Between | Accumulated | Between | Accumulated |
| 7/6 | | | | |
| 7/21 | 289.5 | | 220.5 | |
| 8/4 | 283.0 | 572.5 | 217.0 | 437.5 |
| 8/20 | 324.0 | 896.5 | 234.0 | 671.5 |
| 9/1 | 222.0 | 1118.5 | 167.0 | 838.5 |
| 9/17 | 254.5 | 1373.0 | 179.5 | 1018.0 |
| 9/29 | 199.0 | 1572.0 | 134.0 | 1152.0 |
| 11/5 | 402.0 | 1974.0 | 212.0 | 1364.0 |

*= Degree days, the maximum soil temperature minus the threshold temperature.
**= Low temperature laboratory threshold.
***= Standard vine phenology threshold.

Zero development for all stages was calculated in the first laboratory model at 5°C (Figure 4.3). The 10°C threshold more accurately describes development past the nymphal stage under field conditions than the 16 to 21°C temperature estimated in the laboratory (Table 4.1). Eggs were found in the field when soil temperatures were between 10.5 and 12.0°C.

The time to first adult was estimated to be 35 days at 21°C in the laboratory. When using the 10°C threshold model and the 35 days at 21°C, the calculation for estimating time to adult in degree days is: 35(21°C - 10°C)= 385 degree days. This

number can be compared to the accumulated degree days given in Table 4.3 and the field data in chapter 3. The population sample date of August 4 had a 10°C accumulation of 437.5 degree days (Table 4.3). This value is 52.5 degree days above the 385 degree day estimate of time to adult. The 52.5 degree days difference may be the interval required to complete the first generation. This estimation was presented only as an example of degree day modeling. Further studies are necessary to accurately represent GP generation times with degree days.

## Conclusions

Grape phylloxera development was quantified at the five temperatures (7, 10, 13, 16, and 21°C). The early instar nymph was the final stage of development at temperatures 7, 10, 13, and 16° by day 56. This indicates that temperatures greater than 16°C were necessary to complete development under laboratory conditions. The time to first adult was averaged at 35 days at 21°C.

When compared to field results, the low temperature populations did not produce eggs at temperatures 7 to 16°C. Offspring eggs were recovered from field extractions when mean soil temperatures were between 10.5 and 12.0°C. Laboratory assays may not represent field conditions for certain periods of development, but the laboratory determination of time to adult at 21°C could help to predict generation times.

The low temperature threshold varied depending on developmental stages and temperature parameters used in the calculation. Zero development was calculated at 5°C across life stages using data from all five temperatures. Further research is necessary to quantify thresholds and their applications in degree day modeling.

# CHAPTER 5

## General Conclusions

The results of this biological investigation will provide researchers and growers information regarding grape phylloxera regeneration under Oregon's cool climate conditions. It also illustrates the differences in GP development at three different vineyard sites.

The critical dates of GP activity such as emergence in spring and hibernation in fall were investigated. Adults from over-wintering hibernants and eggs from these adults were recovered in extractions in April/May when mean soil temperatures were between 8.5 and 12.0°C at the 15 cm depth. GP were trapped in above-ground sticky-trunk-wraps as crawlers and alates in July/August and hibernant-like nymphs were extracted in late July when soil temperatures ranged from 17.3 to 18.8°C at 15 cm. This early occurrence of darkened nymphs may have been the result of poor root substrate quality. The percent root moisture dropped 5% overall for the three sites from January 30 to July 27. The bulk of GP hibernation occurred during a protracted period between September to November with very few eggs found in extracts after October.

GP development and regeneration was determined by the combination of bi-monthly root extractions and sticky-trunk-wraps sampled on perimeter vines of three infested areas over two summers. Root extracted GP populations were used to identify population peaks which were used to estimate generation number. Generation number could not be established at two sites due to the extreme variance between samples. However, at Marion two generation peaks were observed for nymphal populations. Further research of modeling generation time with degree day accumulation could help

determine field generation time. The contribution of soil and root environments in GP development is still uncertain.

Vine sample location within site (e.g. side of lens) approached significance for population size and this appeared to be related to topographic drainage and proximity to satellite infestations. Depth of soil sample (15 to 30 cm versus 30 to 45 cm) did not significantly affect GP population size.

Populations varied between sites and sample dates but population levels appeared to be directly related to mean soil temperature. Low temperature thresholds for development for grape phylloxera vary depending on the life stage tested. The low temperature threshold for GP survival was calculated at 5°C from laboratory constant temperatures (Figure 4.3). Predicting the response of GP in the field based on laboratory studies may have some degree of error at lower temperatures tested. The laboratory temperature threshold calculation for zero development past nymph stage, by day 56, was between 16°C and 21°C (Table 4.1) yet eggs were recovered in the field when soil temperatures were between 8.5° and 12.0°C. A 10°C threshold temperature (used for grapevine development) for GP reproductive development may better reflect what takes place under spring field conditions in Oregon. Soil-borne grape phylloxera development must be regulated by both soil temperatures and sap flow (or vine development) and the two parameters cannot be separated.

Population numbers of grape phylloxera can vary between infested sites in Western Oregon. Site location and topography influences water drainage and soil temperature, which in turn, can influence GP dispersal, generation time, and above-ground vine decline symptoms. The site with the highest overall GP populations (Table 3.1 and 3.2) also had the least above-ground symptoms (Table 3.8). Grape phylloxera population size alone does not contribute to vine decline.

Vineyard cultural practices may also affect grape phylloxera populations. Drip irrigation during establishment years will increase vine root development and this may

compensate for root loss due to GP feeding and subsequent decay. Cultivation of GP weakened areas after insect development commences in June should be avoided as it may spread GP and lead to new satellite infestations. These observations serve only as speculations based on the limited number of sites sampled in this study. A controlled experiment to test these assumptions would provide further information regarding GP dispersal and vine-vigor resistance. The ultimate long term solution for growers is to plant with grafted vines on resistant rootstocks.

Vine age, soil type, viticultural practices, and the presence of other pathogens (e.g. nematodes) will influence the predictive analysis of GP infestations. Because of these confounding factors, it is difficult to predict the progression of an infestation in one vineyard based on the results in another. Grape phylloxera infested vineyards with good viticultural practices and without additional pathogens face only the natural progression of the insect. While this may be monumental, the above-ground rate of vine-decline can be reduced by proper vineyard care.

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