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Title: Verticillium Wilt, Nematodes, and Soil Fertility Interactions in Hop Yards

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Verticillium wilt of 'Willamette' hops (*Humulus lupulus*) was investigated to identify the causal organism, to determine the incidence of the disease, and to explore the possibility of interactions with soil fertility and/or nematodes. In the first year of a three year study, sampling of yards followed a "searching for extremes approach". Selection of yards was based on a preliminary survey of all (35) hop growers in the Willamette Valley. Participating growers (10) were asked to identify one "good" and one "not-so-good" yard. Each of the 20 specified yards was subdivided into 4 plots; two representing a "good" and two representing a "not-so-good" area.

In all 80 plots, data were collected to determine incidence of vascular colonization by *Verticillium* and stem necrosis in vines; soil and root parasitic nematode

populations; concentrations of nitrate-N, ammonium-N, P, K, Ca, Mg, and pH in the soil surface, and nitrate-N, ammonium-N , and K in the subsoil; concentrations of total-P, K, and Zn in the leaves; and concentrations of nitrate-N, phosphate-P, and K in the petioles.

The causal agents of the wilt were *Verticillium dahliae* in 13 yards and *V. albo-atrum* in one yard. Recovery of the pathogen within a yard ranged from 0 to 50% of sampled vines, while stem necrosis ranged from 0 to 68%. The frequency of infection was not significantly different among plots or yards, which suggests that the disease is present in all hop growing districts in Oregon.

Soil nematode populations ranged from 0 to 3000 juveniles/100 g of dry soil. *Heterodera humili* (hop-cyst nematode) was the predominant parasitic nematode, while *Pratylenchus* (root-lesion nematode) and *Paratylenchus* (pin nematode) were recovered only occasionally. Densities of nematodes extracted from roots ranged from 0 to 2000 juveniles/g of moist root material and were primarily *H. humili*. A significant association between nematode populations and Verticillium incidence was not detected.

Soil nutrient concentrations exhibited a high degree of variability among yards. The nitrate-N content, measured to a depth of 36" (90 cm) for individual hop yards, ranged between 65 (73) and 417 lb/A (468 kg/ha) with a mean value of 270 lb/A (302 kg/ha). Concentrations of ammonium-N were determined to be approximately one-fourth of the nitrate

concentrations. Phosphorus and potassium concentrations ranged from 55 to 155 ppm and 118 to 799 ppm, respectively, in the surface soil. For the same depth, soil pH ranged from 5.15 to 6.78.

Petiole concentrations of nitrate-N and potassium ranged from 0.16 to 1.3% and from 1.26 to 6.84%, respectively. While it is believed that the duration of the sampling period may have been responsible for the wide range in nitrate-N values, petiole potassium concentrations are thought to reflect the potassium content in the soil. The concentrations of K in petioles increased steadily with increasing soil test values up to 350 ppm K.

Soil and tissue nutrient concentrations found within and among hop yards did not correlate significantly with the incidence of Verticillium wilt. However, petiole nitrate-N concentrations were significantly higher ($p < 0.05$) in plots infected with *Verticillium* (0.73%) as compared to non-infected plots (0.56%).

Verticillium Wilt, Nematodes, and Soil Fertility Interactions in Hop Yards

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INTRODUCTION

The United States leads the world in the manufacture of beer. In 1989, 231 million liters were brewed which represented approximately 23% of the total world market. Since the production of beer requires the use of hops as a flavoring component, in excess of 40 million lbs (17 million kg) were consumed in the process.

Although hops are produced in most countries between the 35 and 45 parallel (northern and southern hemisphere), close to half the total production in 1989 came from West Germany (26%) and the United States (22%). Yet, hop cultivation between these two countries differs significantly in terms of farm size, climate, varieties, and diseases. While the average hop operation in Germany is approximately 12 acres (5 ha), an American (Oregon) farm encompasses 250 acres (100 ha). Precipitation during the growing season in Germany is 22" (560 mm), whereas in the Pacific Northwest rainfall is only 2.5 to 8" (64 to 203 mm) and, thus, hop fields require irrigation.

In general, replacement of varieties in the Pacific Northwest has occurred as a response to market preferences, whereas the decision to change varieties in Germany (and also in England) has been strongly influenced by disease

susceptibility. Up to the late 1950's, each growing region in Germany grew predominantly only one variety. However, these region-typical cultivars were all susceptible to Verticillium wilt to some degree. The most notable example was the 'Hallertauer mittelfruh'. Before the emergence of Verticillium wilt in the 1950's, this aroma hop represented the dominant variety grown in the Hallertauer region. However, by 1979, Verticillium wilt susceptibility had reduced its acreage to only 15%. Today, the 'Hallertauer mfr' has become a variety of only minor importance, being cultivated on only 8% of the total hop acreage.

It may be argued that breeding for Verticillium wilt resistance has also produced a new class of varieties that may have influenced the hop production and the subsequent hopping rate in beer more than any other factor. Based on the American wild hops (*Humulus lupulus neomexicanus*), the new Verticillium wilt resistant cultivars contained higher resin contents, the alpha acids, which are responsible for the bitter flavor in beer. As a result, breweries could formulate their beer with lower quantities of hops and still retain the desired bitterness.

Yet, some experts believe that a high quality beer can only be achieved by adding aroma hops to the brew, and for this reason, aroma varieties are still in demand. As the Oregon climate is well suited for the cultivation of aroma hops, most growers in the Willamette Valley have planted substantial quantities of this type. By far the most

important of all aroma varieties is the 'Willamette', which is grown on close to half of the total acreage in Oregon. However, possessing up to 70% of the germplasm of the 'Fuggle', the 'Willamette' has been recognized as moderately susceptible. Although symptoms of Verticillium wilt had been observed in 'Willamette' throughout the last five to six years, growers became particularly concerned in 1988 when severe wilting and yield losses were reported for some 'Willamette' yards.

The purpose of this study was to investigate the importance of Verticillium wilt in Oregon hops, especially in 'Willamette', and to explore possible interactions with soil fertility as have been observed in England and Germany, and also with nematodes as have been demonstrated on other crops.

REVIEW OF LITERATURE

Verticillium

Verticillium wilt of hops is caused by the fungi *Verticillium dahliae* and *Verticillium albo-atrum*. Although recognized worldwide as two separate species in only the last 20 years (Schnathorst, 1973), these two species exhibit very important differences in their morphology and disease severity.

V. albo-atrum was first reported by Reinke and Berthold in 1879 as the cause of a vascular wilt in potatoes (Pegg, 1984). The most notable sign was the production of dark mycelia (dauermycelium) or sclerotia (Shufelt, 1987). In 1913, 34 years later, Klebhan isolated a similar wilt causing fungus from Dahlia. Since this fungus produced small, black pseudosclerotia (microsclerotia), he thought it different from the previously isolated fungus and called it *V. dahliae* (Zehsazian, 1968).

That these morphological differences translated into distinct diseases became apparent when both types were isolated from hops (Keyworth, 1942). Thus, the literature describing hop wilt has, for the most part, accurately differentiated between the two species. However, in the few cases where the improper nomenclature was used, the particular fungus will be reported with its true name to avoid confusion.

England

The first report of Verticillium wilt of hops came from Kent, England, where, in 1924, Harris isolated *Verticillium albo-atrum* from a diseased 'Fuggle' yard at Penshurst (Harris, 1927). In describing this new wilt disease, Harris noted that yellowing and eventual wilting of leaves, the most striking symptoms, appeared on the bottom of the vines, and could commonly be seen after cone formation. Less apparent, but still invariably associated with this disease, was the fact that infected vines could easily be pulled off their base, sometimes even before visible symptoms occurred. Examinations of entire hills revealed that one or several vines could be infected and that heavily infected crowns were usually discolored and in a rotting condition. However, these crowns appeared not to be killed immediately by this particular fungus (Talboys, 1987).

Harris continued his observations over several years at this yard and found that infected plants succumbed to the disease in one year, but remained without symptoms the next. In searching for the cause of this fluctuation, he remarked that not all infected plants exhibited symptoms in a year of low Verticillium wilt occurrence. Thus, disease severity, he concluded, was more a function of predisposing environmental factors than of new infections (Harris and Furneaux, 1938).

Harris identified this predisposing factor as soil moisture. Continued observation of one particular field

resulted the formulation of the theory that the outbreaks are associated with an annually variable high water table during the growing season (Harris and Furneaux, 1938). But, as Verticillium wilt became more wide-spread, a total of 20 yards in 1938, their theory became insufficient to adequately describe newly reported cases of disease incidence. Outbreaks that occurred in the mid to late 1930's did not fluctuate, but reappeared each year and became progressively worse (Talboys, 1987).

It was Keyworth (1939) who recognized that farmers were now plagued with an additional strain of the pathogen. Aside from the differences in its recurrence, the progressive wilt (Keyworth) also produced symptoms distinct from the fluctuating wilt (Harris). While the fluctuating type was usually associated with mid and late season yellowing and eventual wilting, swollen stems (lending the vine a bark-like appearance), and a brown vascular tissue; the progressive strain displayed wilting early in the season and produced leaves with definite black streaks. Stems usually died before swelling or even browning. Crowns infected with the progressive type produced vines that never recovered and always died within one or two seasons (Keyworth, 1942; Isaac and Keyworth, 1948).

In mapping the outbreaks, it became clear that both types of diseases originated from two separate locations as two different strains, even though no morphological dissimilarities were ever discovered (Keyworth, 1942).

Furthermore, it was realized that transmission could be achieved both by mechanical soil cultivation and by human movement of plant material. Since diseased dead leaves carried verticillate conidiophores, hop pickers spread the inoculum throughout the yard and also carried it to other farms as well (Talboys, 1987).

Although the introduction of wilt resistant varieties ('OR55', 'Progress', 'Alliance') had saved the industry from disaster, it had also produced a false sense of security. In 1972, two more strains of *V. albo-atrum* emerged which were more pathogenic than the already existing progressive wilt. The "V2" and "V3" strains could now effectively produce severe and moderate symptoms, respectively, in the previously considered resistant variety 'Target' (Pegg, 1984).

Continued pathogenicity tests have not uncovered additional, more pathogenic strains. However, they have shown a constant broadening of virulence in the already existing fluctuating and progressive types. Thus, today the distinction between all strains is based more on arbitrary thresholds than on statistical significance (Talboys, 1987).

Another Verticillium species, *V. dahliae*, has been detected (Keyworth, 1942), but has not been considered to be of great significance. All trials with *V. dahliae* isolates from hops or other hosts have only resulted in fluctuating wilt outbreaks (Chambers, 1987).

Germany and Tasmania

The first incidence of Verticillium wilt in Germany was reported in 1952 and occurred in the 'Hallertauer mittelfruh' variety (Kremheller, 1980). Subsequent outbreaks varied from year to year, being severe, for instance, in 1955 but not in 1956 and 1957 (BLBP, 1957). Pathogenicity tests indicated that this strain of *V. albo-atrum* was similar to the English fluctuating type, although one isolate was found that approximated the virulence of the progressive strain, which by that time was the predominant cause of Verticillium wilt in England.

Yet, as was later determined, this mild form of *V. albo-atrum* found in Germany caused more disease in the resistant 'Bramling' (English variety) than in the very susceptible 'Fuggle' variety (BLBP, 1965). Conversely, the English strain could attack the 'Northern Brewer', which was considered to be resistant in Germany (Rintelen, 1974). This indicated that both countries had to cope with similar diseases, but which were caused by dissimilar strains. In 1969, another difference in strains was reported: a second strain of the fluctuating *V. albo-atrum* type was isolated from the 'Hallertauer mfr' which, in addition to physiological distinctions, also proved to be morphologically discrete. With only weak branching, pure cultures could easily be mistaken for *Fusarium*. Although this new strain occurred in mixed populations in several

varieties, it was isolated as a pure population from the 'Hallertauer mfr' (BLBP, 1968).

A severe outbreak of wilt occurred in 1975 in both susceptible and resistant varieties, which indicated a possible emergence of new, more virulent races that could break the resistance of wilt resistant varieties (Kremheller, 1976). Subsequent pathogenicity tests confirmed the presence of several strains of *V. albo-atrum* with varying degrees of virulence.

By the early 1970's it was recognized that the hop wilt was also caused by *V. dahliae*, though in much lower frequencies. Experiments demonstrated that this species, in contrast to *V. albo-atrum*, occurred more in coarse textured soils that were rich in organic matter (Kremheller, 1974). In the same trials, it was also concluded that *V. dahliae* exhibited no preference in infecting any particular variety.

As was subsequently shown, however, symptom expression did depend on the variety. In resistance trials visible wilting was observed in every infected 'Hallertauer mfr' test plants. Isolation of the causal organism yielded 80% *V. albo-atrum* and 20% *V. dahliae*. This indicated that both species produced similar symptoms in the Verticillium susceptible 'Hallertauer mfr'. Yet, in the resistant variety 'Northern Brewer', only three of nine infected plants expressed some symptoms. Stem isolations revealed that these three vines were exclusively infected with *V. albo-atrum*. However, of the remaining six plants not

showing symptoms one plant was also infected with *V. dahliae* (Kremheller, 1981). Thus, in resistant varieties, *V. dahliae* apparently lacks the strength to produce symptoms, even though it may be capable of infection. This ability of infection was reflected in the amount of *V. dahliae* diseased plants received at the Bavarian hop research institute at Hull. For the year of 1989 it was estimated that 15% of all the samples were infected with *V. dahliae* (Kremheller, personal communication).

In Tasmania, Verticillium wilt was reported as early as 1946, at which time the causal agent was identified as *V. albo-atrum*. However, 10 years later, Cartledge (1956) was able to isolate only *V. dahliae*. In describing the disease, he noted that symptoms usually did not appear until vines had reached the later stages of maturity. Wilting was observed on the bottom of vines and occurred in one, two or in a few cases, in all six vines per hill. Once leaves began to yellow, the whole leaf died in a period of 2 to 3 days and could easily be pulled off.

United States

In 1956, a conspicuous yellowing of leaves and premature dying of vines in a 'Fuggle' yard located near Independence, Oregon, led Horner (1965) to conduct tissue isolations. He consistently recovered *V. dahliae* from brown, necrotic stems. Continuous observation of this and other yards that were surveyed in 1960 revealed the disease

to be present in several yards but not in epidemic proportions. Only 5 years later, however, another survey indicated that wilt was more widespread. *V. dahliae* was confirmed in six of eight well distributed farms and all plants in one 30 acre (12 ha) field were apparently infected (Horner, 1965).

Pathogenicity tests later indicated that several strains of *V. dahliae* existed. Based on propagule counts it was determined that strain 138 (isolated from a 'Fuggle') represented a strain which was specific to potato and possibly to strawberry. Another strain (150, also isolated from a 'Fuggle') was identified as pathogenic to mint, while strain 148 (isolated from a 'Bullion', producing the severest symptoms) was not specialized on any crop tested. (Horner, 1967; 1968).

The fact that *V. dahliae* was demonstrated not to be a pathogen specialized on hops may explain the observation that crowns have never been observed to die as a result of infection. Furthermore, by 1967 the widely accepted hypothesis of disease induced yield declines still lacked conclusive proof. Horner suggested that reduced yields may also be due to some other environmental factors (Horner, 1967).

Nevertheless, Verticillium wilt in hops needed to be monitored, especially since *V. albo-atrum* had emerged in 1962. Isolated from a 'Fuggle' yard near Salem, it reappeared in the same yard in 1963 (Horner, 1964; 1965b).

Another outbreak of this pathogen occurred in 1969 and in the 1970's in a 'Cascade' yard (Haunold, personal communication). (This yard was taken out of production but *V. albo-atrum* reappeared in 'Willamette' in 1989, as will be described later).

Interactions with Verticillium

Although Harris' soil moisture theory did not sufficiently explain outbreaks of the progressive wilt, its relevance to the fluctuating type has, nonetheless, never been disproved (Keyworth, 1942). However, another closely related environmental factor, soil temperature, has been conclusively shown to correlate with the fluctuating wilt. At soil temperatures between 50 and 65 ^{o}F (10 and 18.3 ^{o}C), Sewell and Wilson (1973) demonstrated an average wilt increase of 5% for each 1 degree (1.8 ^{o}C) drop in soil temperature. This inverse linear relationship was observed for the fluctuating type infecting both susceptible and tolerant varieties. The virulent (progressive) strain, on the other hand, caused severe symptoms in all varieties, regardless of temperature.

This linear relationship between wilt and soil temperature transformed into an interaction when temperatures fell below 50 ^{o}F (10 ^{o}C). At these temperatures, even the fluctuating type induces severe wilt in both susceptible and resistant varieties (Sewell and Wilson, 1973). The researchers therefore concluded that the

unusually low spring temperatures (March - July) were the cause for the widespread and severe outbreak in 1972.

In the United States, interactions of Heptachlor and also of Chlorodane (insecticides) with Verticillium wilt have forced farmers to take affected fields out of hop production (Haunold; Probasco, personal communication). Such outbreaks occurred particularly in the 'Cluster' variety, but 'Willamette' and 'Fuggle' have also been affected (Skotland and Johnson, 1985).

Therefore, Verticillium wilt needs to be further examined, not only as a self perpetuating disease, but as a disease complex that includes both environmental and management factors. Since the previously discussed environmental factors are beyond the control of farmers, even more emphasis must be placed on those factors that a grower can influence by his management decisions. Research on hops and other crops has identified two areas: the management of plant pathogenic nematodes and the management of soil fertility.

Nematodes

One of the first known wilt-nematode interactions was observed by Atkinson (1892). He noted that severe wilting in cotton only occurred when both the fungus (*Fusarium* spp.) and the nematode (*Meloidogyne* spp.) were present together. Although the root-knot nematode by itself caused severe galling on cotton roots, no wilt symptoms were seen. Early

explanations of such an interaction centered on the nematode's ability to provide a pathway for the fungus (BLBP, 1957). However, Faulkner et al. (1970), working on peppermint, demonstrated in a split-root experiment that infections by *Pratylenchus minyus* must have physiological consequences. Both severity and incidence of wilt due to *V. dahliae* were dramatically increased when *P. minyus* was present, even though the two pathogens parasitized separate root systems from the same plant. Studies on cotton discovered another detrimental effect of a nematode-wilt interaction. Inoculation of a wilt resistant cotton variety with *V. dahliae* and with *Rotylenchus reniformis*, respectively, did not lower yields significantly. Yet, when both pathogens were added together, wilt resistance was broken and a significant yield loss was obtained (Tchatchoua and Sikora, 1983).

In order to study nematode-wilt disease complexes in hops, a review of the dominant hop pathogenic nematodes is essential. In Oregon and probably in most hop growing areas around the world, *Heterodera humuli* (the hop cyst nematode) represents the most prevalent nematode in hop yards (Jensen et al., 1962; Sen, 1968; von Mende, 1985; Simon, 1957). *Pratylenchus* (the lesion nematode), on the other hand, is less frequently found (Simon, 1957). Yet, its widely recognized interactions with *Verticillium* spp. on other plants merits examination of the role of *Pratylenchus* in Verticillium wilt of hops.

Heterodera humuli

First reported in Europe in 1894 (Sen, 1968) and described by Filipjev, 1934, *H. humuli* was identified in Oregon by Jensen et al. (1962) and in Washington by Cobb (1962). Although it was believed to be responsible for the "nettlehead" disease in hops, Duffield (1925) refuted that theory and implicated a virus. In Switzerland, surveys of hop yards found higher densities of this pathogen in older, less vigorous fields. Nevertheless, the researchers were uncertain if the lack of vigor could be attributed to the nematode's presence (Anonymous, 1988). In England, investigations were initiated in commercial hop yards, where patches of stunted vines with yellow leaves appeared. However, the study produced only inconclusive results and, hence, could not associate the hop cyst nematode with the observed field symptoms (von Mende, 1985). Thus, *H. humuli* has not been conclusively documented to cause direct damage to hop plantations.

Laboratory experiments examining threshold levels of the cyst nematode on hops confirmed these field results. Sen (1968) working with seedlings estimated significant yield reductions at 50 to 100 eggs/g of soil. However, von Mende (1985) found that these levels to actually stimulated plant growth, probably due to increased compensating root development. Plants were significantly decreased in height at 400 eggs/g, and at 1600 eggs/g of soil reached only half the size of control plants. Yet, it was also stated that

such high levels of the cyst nematode are probably never reached in commercial field situations. Since *H. humuli* has to share the soil environment with other microorganisms, the chance of parasitism on the nematode and especially on the cysts is great, keeping populations in check (Hirling, 1978). Furthermore, as the hop is a perennial plant, it develops an elaborate root system that apparently provides the plant a sufficient resistance against nematode attack (von Mende, 1985).

H. humuli, as a typical representative of the cyst nematodes, undergoes four molts. While still inside the egg, the first stage juvenile molts and eventually hatches as a second stage juvenile. This process is strongly accelerated in the presence of hop root diffusates (De Grisse and Gillard, 1963). It is the hatched second stage juvenile that continues to infect the adventitious roots which are produced during the growing season (Simon, 1957). Once inside the root tissue, and after inducing the formation of several "giant" nutrient providing cells (syncytia), the nematode undergoes its second molt, which, depending on the temperature, occurs after 45 days at 60 ^{o}F (15.5 ^{o}C) or after 16 days at 80 ^{o}F (26.6 ^{o}C) (Sen, 1968). Upon completion of the third stage, sexual differentiation can be observed. Sexual development becomes more pronounced after the third molt, as is the formation of its typical flask or lemon shape. This causes the developing nematode to protrude through the epidermis of the root. After the

fourth molt, males revert to a worm shape while females become even more swollen. As soon as the males have matured to adults and have left the root, the females will be fertilized (von Mende, 1985). Completion of the life cycle is indicated by the change in color of the female "cyst" cuticle, being white at first and finally brown when the female dies (Sen, 1968).

The duration of the life-cycle has been estimated to be 148 to 177 days by Sen (1968) and less than 50 days by von Mende (1985). However, both studies agree that *H. humuli* will produce at least two generations per growing season, if not three under favorable moisture and temperature conditions (von Mende, 1985).

Interactions between *Heterodera* spp. and *Verticillium* have rarely been reported. In potato, root infection with *H. rostochiensis* promoted wilting 15 days sooner than in plants inoculated only with *V. dahliae* (Corbett and Hide, 1971). The role of this cyst nematode was determined to be strictly physical. As the females swelled to take on their cyst shape, they ruptured the root cortex and, thus, provided access for fungal penetration. In tomato, however, Miller (1975) suggested that increased severity of wilt caused by the pathogen complex of *H. tabacum* and *V. albo-atrum* may have been due to physiological alterations in the host. Since the nematode induces the formation of giant cells via hormones, it may lower the host's resistance against parasitism by *Verticillium*.

Nematode-*Verticilllium* interactions in hops were suggested soon after the appearance of the Verticillium wilt in Germany. Simon (1957), although unable to correlate numbers of root nematodes with incidence of Verticillium wilt, did observe a general trend of higher *Heterodera* densities in diseased yards. Furthermore, von Mende (1985) suggested that as the nematode breaks through the endodermis, host resistance might be broken as well. This hypothesis is based on Talboys' observations that resistance to Verticillium wilt is due, at least in part, to the root's ability to "suberinize" (suberize) its endodermis (Beckman and Talboys, 1981).

In a laboratory experiment, von Mende (1985) demonstrated a true interaction (synergism) between the mild *V. albo-atrum* (V1) and *H. humuli*. The previously noted stimulation in hop seedling growth at 40 nematode eggs/g soil seemed to be reversed when plants were inoculated with both pathogens. Also, infection with V1 alone did not reduce plant height significantly. However, it was emphasized that such results are not readily transferable to field situations, since the timing of infection by either pathogen may influence the outcome considerably. In addition, this synergism could only be found in a susceptible variety, implying that the nematode was unable to break host resistance.

Pratylenchus

Pratylenchus penetrans follows a disease-cycle quite different from *H. humuli*. Eggs released in soil develop into first stage juveniles that hatch and subsequently undergo their first molt. At this time the juveniles are infective and may penetrate a suitable root or may continue to mature into sexually differentiated adults in soil. Once juveniles (second to fourth stage) have penetrated into a root, they persistently advance through the cortex and, in doing so, leave ruptured cell tissues in their path. It is this tissue that becomes predisposed to invasion by fungi and bacteria (Agrios, 1988).

Although Simon (1957) extracted higher *Pratylenchus* populations from wilt diseased hop yards, no study was ever undertaken to investigate this nematode's direct destructive potential or its possible role in a Verticillium wilt complex on hops. However, on crops such as potato and mint, conclusive evidence of a synergism between the two pathogens has been reported (Rowe et al., 1985; Riedel et al., 1985).

Rowe et al. (1985) concluded that both pathogens needed to be present to cause severe wilt or "potato early dying". At 10 microsclerotia/10 g soil, and nematode population levels of 100 to 150 propagules/100 cc of soil, a strong interaction between *V. dahliae* and *P. penetrans* occurred resulting in significant yield losses. This was especially true when test plants were subjected to heat stress. The same researchers in another study also concluded that the

disease complex was highly specific, exhibiting a differential interaction of *Pratylenchus* species. While a synergism was observed with *P. penetrans*, no such findings were made with *P. crenatus* (Riedel et al., 1985).

Soil Fertility

The effect of soil nutrients on the incidence and/or the symptom severity of Verticillium wilt may be the result of single or multiple elemental reactions. Furthermore, their influence may depend on the quantities that are present in the soil or that are applied in the annual fertilization program. Two nutrients, nitrogen and potassium, have been implicated to interact with or, at least, influence wilt outbreaks in hops and in other crops.

Many researchers have investigated hop nutrient consumption and have ensuingly identified nitrogen to be removed in the highest quantities. As can be seen from Table 1, the ratio of nutrient removal approximates 3-1-3 ($N - P_2O_5 - K_2O$).

Nitrogen

Precise nitrogen fertilization recommendations have not been fully established in all hop growing areas around the world and, thus, leave some farmers without solid guidelines. Due to the high cash value of hops and the comparatively low cost of fertilizers, growers have opted for more rather than less nitrogen. In England, some

Table 1. Nutrient removal in cones and vines of hops as reported by various author.

Nutrient	Roberts et al., 1985[1]		Burgess, 1964[2]	
	lb/A	kg/ha	lb/A	kg/ha
N	242	271	80 - 153	90 - 171
P_2O_5	65	73	25 - 64	28 - 72
K_2O	228	255	70 - 167	78 - 187

[1] removal based on a 10 bale/A 'Late Cluster' hops
[2] review of several authors

farmers still applied up to 350 lb/A (390 kg/ha) of N in the late 1960's (Sewell and Wilson, 1967). During the same time, growers in Germany commonly applied 220 to 280 kg N/ha (200 to 250 lb N/A) (Kamm, 1970). Even today, 200 lb/A (220 kg/ha) N are not uncommon in the United States.

In Germany, a long term fertilizer study has established 270 kg N/ha (240 lb N/A) as the rate which produces the highest cone yields. This value represents the sum of the mineral soil N reserve before the growing season (N min) measured to a depth of 90 cm and the farmer applied N as organic or inorganic fertilizer. Depending on the soil type, this means a fertilizer application of 89 to 174 kg N/ha (80 to 160 lb N/A) as determined over the 10 year period (Gmelch and Rossbauer, 1989; 1990). These recommendations are somewhat comparable to the Washington State fertilizer guide, which advises growers to add 30 to 140 lb/A (33 to 157 kg/ha) of N, if soil test values (N index, based on samples taken to a depth of three feet, 90 cm) are 40 to 10 ppm, respectively (Roberts et al., 1985). In Czechoslovakia, fertilizer trials involving the use of N min values have resulted in the highest yields when only 65 kg N/ha (60 lb N/A) were applied. However, recommendations for the Oswald clone variety ranged between 80 to 120 kg N/ha (70 to 115 lb N/A) (Mat'a'tko and Kopalova, 1989).

Even though these recommendations show that a significant fraction of the hop's N requirement can be satisfied by the soil and that higher than necessary

quantities may not be readily taken up by the crop, the immediate concern may not lie in the waste of this nutrient but in its interaction potential. Burgess (1964) remarked that a more than adequate supply of nitrogen will promote excessive leaf growth, altering the microclimate of the vine, and thus making it more susceptible to downy mildew. Perhaps, a more important interaction can be found between nitrogen and *Verticillium*.

In a pot experiment, Keyworth and Hewitt (1948) investigated the effect of excesses and deficiencies of several nutrient solutions on hop wilt. Although they found a reduction in disease severity in N, P, and K deficient plants, only nitrogen starved vines reproduced the same results in the subsequent year. Since these findings pointed toward a possible control strategy against wilt, large scale field trials were undertaken. Sewell and Wilson (1969) concluded that rates of 60 and 120 lb/A (67 and 135 kg/ha) gave 60% and 25% wilt control, respectively, compared to the commercially used rate of 180 lb/A (200 kg/ha). The researchers suggested that the lower symptom expression may be due to a diminished vascular colonization by the fungus (Sewell and Wilson, 1967).

It was also noted in this study that lower nitrogen applications did not affect yields adversely over the five year period. On the contrary, occasional yield depression and lower resin contents in the cones were observed at higher rates. Yet, even more important, the researchers

indicated that luxury applications of nitrogen seemed to promote symptom expression of Verticillium wilt.

In an experiment relating both soil temperature and nitrogen application to wilt Verticillium wilt severity, it was found that for each 10 units of N (11 kg/ha), wilt increased by 1.7%. Since detrimental soil temperature effects had already been established (Sewell and Wilson, 1973), the investigators were able to equate 25 units of N (28 kg/ha) with a temperature drop of 1 ^{o}F (1.8 ^{o}C), or 4.3% wilt. Thus, in a year where soil temperatures were favorable for severe wilt outbreaks, a low nitrogen program could lessen wilt symptoms and therefore also minimize yield losses. Conversely, high N applications would cause severe wilt even in years where temperatures were not conducive to wilt (Sewell and Wilson, 1974).

Still, low nitrogen policies were not implemented on all farms and, ultimately, excessive N applications may have lead to the emergence of two new, more virulent strains (V2 and V3). Although Talboys (1987) noted that these "supervirulent" strains probably have existed before, selection pressures that included a wilt resistant variety and high N must have provided a favorable environment for their emergence. In fact, *V. albo-atrum* appears to be a rather easily mutating fungus that can quickly adapt to a new environments.

In Germany, wilt appeared soon after farmers started to use large applications of inorganic (N, P, K) fertilizers.

Although this observation precludes a cause and effect relationship, evidence for increased wilt severity with high N supply has been obtained. Hydroculture experiments conducted in Germany tested the degree of wilt in the susceptible variety "Hallertauer mfr" as it was subjected to various nutrient solutions. The outcome of this experiment confirmed results by Keyworth and Hewitt (1948) in that the highest nitrogen supply produced the greatest amount of disease (Kremheller, 1975). Long term field trials with the this variety indicated that the actual incidence may not be influenced by nitrogen, as latent infection appeared to emerge regardless of soil extractable N. However, these trials did show that high nitrogen concentrations promoted greater symptom expression (Kohlmann, 1977).

These data obtained in Germany corroborated findings from England, where researchers were also able to isolate *V. albo-atrum* from plants receiving deficient amounts of N. This indicated that also in England susceptibility to pathogen entry was not a function of nitrogen (Keyworth and Hewitt, 1948). The implications of this finding are of particular importance, since, as previously noted, *Verticillium* strains from both countries produce different levels of disease in the same variety. Therefore, both the lack of increased incidence of infection and the enhancement of symptom expression as a response to excessive N rates may be universal characteristics that transcend specific environmental adaptations.

Taken a step further, continued experimentation with high nitrogen applications produced visible symptoms in the wilt resistant varieties 'Northern Brewer' and 'Perle' (Kremheller, 1985). Although these observations were probably not due to "supervirulent strains", they demonstrated the potential danger of a wilt-nitrogen interaction in even these varieties.

Although the lowest severities in some field trials with the "Hallertauer mfr" were obtained at rates of 90 kg/ha, 80 lb N/A (N min + inorganic fertilization) (Kremheller, 1982), these rates are clearly below the recommendations for optimum yields of 270 kg N/ha (250 lb N/A). For this reason, the effect of various nitrogen forms on wilt severity were explored.

In pot experiments with artificially inoculated soil, Maier (1976, 1977) found less disease in one year old "Hallertauer mfr" vines that had been fertilized with ammonium-N compared to nitrate-N amended plants. He suggested that the lower incidence may be due to accelerated suberization of the roots, i.e. a mechanical prevention of fungal penetration under the ammonium regime. Field trials also produced similar results (Maier, 1977). It is interesting to note that nitrification inhibitors did not further increase this "mechanical resistance".

In addition to the quantity and the form of nitrogen applications, a survey contrasting wilt infested with wilt free operations also indicated adverse effects of fertilizer

placement. Kamm (1970) observed more wilt when late fertilizer applications were positioned close to the base of the vine. Unfortunately, he did not offer an explanation for this observation.

Potassium

The management of potassium in hops is much less explored than that of nitrogen. A survey of German hop yards in the late 1960's estimated applications of potassium well in excess of 300 kg/ha (267 lb/A) (Kamm, 1970). Although these applications seem very high, fertilizer recommendations in Germany for potassium are still not based on soil test values (Gmelch and Rossbauer, 1990). For adequate hop production in East Germany, 125 kg K/ha (110 lb K/A) of K are recommended (Borde et al., 1989). In Washington, fertilizer guidelines have been established on soil test values. Soil K concentrations (measured in the top 12", 30 cm) below 30 ppm K require the application of 240 lb/A (265 kg/ha) while at or above 120 ppm K no applications are necessary to achieve maximum yields. Recent investigations of K fertilizer recommendations in Australia have resulted in an 84% decrease over previously recommended guidelines to only 50 kg K/ha (45 lb/A). At this new application rate yields were found to be significantly higher (Leggett, 1989).

The beneficial effects of potassium fertilization in reducing Verticillium wilt severity were recognized as early

as the 1920's on cotton (Rast, 1922). Applications of kainit (containing 12.5% K_2O) at a rate of 500 lb/A (560 kg/ha) enabled the treated cotton plot to "escape wilt infection", while the non-treated plot was devastated by the fungus. On hops, a similar observation was made. In a hydroculture experiment that explored the change in disease susceptibility of both *V. albo-atrum* and *V. dahliae* to altered nutrient levels, all three macro-nutrients (N, P, K) were varied from deficient to excessive concentrations. The lowest disease incidence was obtained in plants that grew in a solution with an excess of K (Kremheller, 1975).

Experiments with potted hops have demonstrated that the benefits from potassium are not necessarily derived from its concentrations in soil alone, but that its effectiveness depends on concentrations of nitrogen as well. It also appears that the ratio of N:K varies according to the degree of wilt susceptibility. While the tolerant 'Northern Brewer' exhibited the highest degree of wilt at a ratio of 1 N : 0.95 K_2O, the very susceptible 'Hallertauer mfr' showed the greatest amount of disease at a ratio of 1 N : 2 K_2O. In both cases there was a steady decrease in Verticillium wilt at higher potassium rates (Rintelen, 1974). Unfortunately, this report does not differentiate between incidence of infection and symptom expression, so it can only be inferred that potassium aided in the prevention of infection.

MATERIALS AND METHODS

Design of Survey

The 1989 survey of Oregon hop yards represented the first stage in an ongoing three year project. As such, it relied exclusively on field observations which were gathered in a non-random "searching for extremes" approach.

Preceding the actual collection of field data and in accordance with the "searching for extremes" approach, a questionnaire was mailed to all hop farming operations in Oregon. Growers were requested to select fields of contrasting quality. Although not specified, the selection criteria for choosing one yard as "good" and the other as "not-so-good" was based, in most cases, on past yield performance (personal communication with growers).

The questionnaire itself was partitioned into five sections which were duplicated, i.e. identical for both yards. In the first section, inquiries were made about yield declines, Verticillium wilt, and cropping history among others. The following section requested information about the 1989 fertility program, in terms of times and rates of fertilizer applications. Similar data was requested in the third section, the "pesticide program". To compare different management philosophies, the fourth section asked for information on the treatment of soil, the use of irrigation, and the management of plants. The last

section solicited information on previous records and requested permission for sampling.

In order to reduce varietal variability, the questionnaire restricted selection of yards to the variety 'Willamette'. There were two reasons for choosing this variety: First, during the 1989 season, the acreage planted to 'Willamette' amounted to 3842 acres (1555 ha), which represented close to 50% of the total Oregon hop acreage of 7,781 acres (3149 ha). Second, containing at least three fourths, if not nine-tenths of the 'Fuggle' germplasm (Haunold, personal communication), 'Willamette' have been described as moderately susceptible to Verticillium wilt (Shufelt, 1987). In its release documents, the 'Willamette' variety was not recommended for cultivation in the state of Washington due to its susceptibility to this disease. Therefore, symptom expression should occur first in this variety, providing suitable data even in years unfavorable for Verticillium wilt expression.

The locations of the 10 participants included all hop growing regions in the Willamette Valley. One farm was situated near Independence, two in Keizer, two near Mt. Angel, two near Gervais, and three around St. Paul. The predominant soil series for the 10 farms were Cloquato silt loam and Woodburn silt loam (Table 2).

Sampling of all 20 'Willamette' yards also followed the "searching for extremes" approach. Each "good" and "not-so-good" yard was further subdivided into two "good" and two

Table 2. Description of soil series found on hop farms surveyed.

Series	Soil Classification	Grower
Amity	Fine-silty, mixed, mesic Argiaquic Xeric Argialbolls	1, 3, 4
Chehalis	Fine-silty, mixed, mesic Cumulic Ultic Haploxerolls	2, 7
Cloquato	Coarse-silty, mixed, mesic Cumulic Ultic Haploxerolls	2, 7 - 10
Woodburn	Fine-silty, mixed, mesic Aqualtic Argixerolls	3, 5, 6

"not-so-good" plots, which resulted in a total of 80 plots. This quality assessment was based on the relative appearance of a plot within its yard and was determined by the researchers.

Each plot received an absolute point score in addition to this relative rating. Being independent of the quality of a particular yard, this absolute point score was based on four criteria: development of sidearms, amount of foliage, height of vines, and color of foliage. For each of these criteria, plots were given either one point for being good or no points for being bad. Thus, plots could receive a maximum of four points (representing a very good plot) or a minimum of zero points (representing a very weak plot). Furthermore, due to its absolute nature, plots with the same point score appeared similar, regardless of yard or grower. However, most plots rated with two points or less were also categorized as "not-so-good" plots, and most plots of three or four points were usually classified as "good" plots.

The area of a plot included 16 hills arranged in a square of four hills per side. Corners were marked with standard yellow field flags and labeled with the initials of "OSU" to differentiate them from red flags commonly used by growers. Since individual hills within Oregon hop yards are spaced 7.5 feet (2.22 meters) apart, the total surface area of one plot amounted to 900 square feet (79 m^2)

The number of hop vines included per plot depended on the growers. Especially in younger yards, three vines were

trained on a string, but two vines were not uncommon. Since there are two strings to every hill, 64 to 96 vines were grown per plot. As four plots were identified per yard, 256 to 384 vines were available for sampling.

With the exception of whole stem cuttings, all plant and soil samples were taken in a 32-day period, between July 24 and August 24. In 8 of the 10 growers, both the "good" and the "not-so-good" yards were sampled within a period of 24 hours.

Verticillium Wilt

Field Sampling

Within one week after harvest, all 80 plots were sampled for *Verticillium*. Plant material was cut from the "pigtail", the unharvested 3 to 4 feet (90 - 120 cm) portion of the vines. Continuous on site inspections revealed that the "pigtail" remained alive for at least two weeks, which made it possible to re-sample individual hills.

From each of the 80 plots, four hills were chosen in a diagonal pattern. Then, at random, one vine was selected from each hill, producing 4 vines per hill or 16 per yard. Care was taken not to sample stems that had been damaged or that grew out from the side of a hill. An approximately 6" (15 cm) portion was cut 5" (12 cm) above the hill surface using a regular non-sterilized field knife. All four cuttings from one plot were placed in a standard plastic

vegetable bag, marked, and temporarily stored in an ice cooled Styrofoam cooler.

A special sampling procedure was employed in both "not-so-good" plots in the "not-so-good" yard from grower # 8. In this case, all six vines per hill and all 16 hills per plot were sampled. Vines from different hills were separately labeled but otherwise handled the same.

For long-term storage, all 504 (78*4 + 2*16*6) stem cuttings were placed in a cold room where they remained for up to two weeks before being analyzed.

Soil sampling for microsclerotia was carried out during the season. Four soil samples were taken at random from different hills to produce one composite sample per plot. At the two locations previously described from grower # 8, every hill was sampled to obtain 16 separate samples for each of the two plots.

These samples were taken with a bulb planter that was 6" (15 cm) long, and had a diameter of 4" (10 cm) at the top tapered to 3" (7.5 cm) at the bottom. By inserting the bulb planter at a 50 to 60^{o} degree angle into the side of a hill after loose soil had been scraped off, the effective depth of soil cores was approximately 4" (10 cm). It is important to note that these samples did not contain any soil that was below the regularly cultivated soil.

All samples taken from the hill also were used for soil and root nematode counts, and for nutrient analysis. They were kept cool during transportation. Samples for nutrient

analysis were processed immediately, while samples for nematodes and microsclerotia were kept in the cold room until analyzed, i.e. 2 to 3 weeks and 3 months, respectively.

Isolation of *Verticillium*

Both ends of the stem sample were cut off with an ethanol sterilized razor blade and discarded to obtain a 2 to 3" (5 to 8 cm) section of tissue. The "bark" or epidermal layer of the stem was peeled off using ethanol cleaned fingers. With flamed tongs the stem sections were submerged into a bleach bath (10% bleach in double distilled water) for 10 sec. The surface sterilized stems were then rinsed in a distilled water bath. Both baths were renewed after 16 or 32 samples, depending on need.

Three slices were cut from the stem sections with an ethanol sterilized razor blade at different locations. Flamed tongs were used to transfer the slices onto a disposable plastic petri dish.

In place of a culture medium, filter paper was used. Whatman 2 or 3 (medium coarseness, quantitative) filter paper was laid into 9 cm plastic disposable petri dishes. The filter paper was wetted with distilled water until all pores appeared to be saturated. Prepared plates not used immediately were stored in the original plastic sleeves, closed air tight, and kept away from direct sunlight.

Each prepared plate received six stem slices, with three slices (coming from the same stem) on either side. At this point, individual slices were examined for stem necrosis. A slice received a "+" when strong or light vascular browning was present and a "-" when tissue was completely white.

The first growth of verticillate mycelium could be detected after four days using a dissecting microscope. Good identification was possible after 7 to 10 days, at which point an infected slice was well covered with the *Verticillium*-typical "Christmas tree" structures. Although some contamination appeared, it did not interfere with the identification.

The most rapid growth of *Verticillium* occurred when the filter paper moisture was such that individual slices formed a gelatinous layer on their surfaces. In cases where moisture levels were too low, this did not occur and the growth of the fungus was retarded. However, this condition could be corrected by adding more distilled water.

Detection of microsclerotia was possible after 10 to 14 days. If infection was due to *Verticillium dahliae*, these resting structures could be seen underneath the stem slice. Sometimes microsclerotia were deeply embedded in the water softened filter paper, in which case they needed to be uncovered with a dissecting needle. In some cases, production (or detection) of microsclerotia occurred after

more than two weeks, at which time the *Verticillium* fungus was overgrown by contaminants.

When microsclerotia were not detected, conidia were transferred with a flamed needle onto standard PDA nutrient medium. If infection was due to *V. albo-atrum*, dauermycelium formed after approximately 10 days.

For all the described work, it appeared that a surface sterilized work area sufficed to minimize contamination. All samples on filter paper were stored in plastic bags that contained small amounts of free water to prevent drying. These plates and isolations on nutrient medium were kept for multiple re-checking, after which they were discarded.

Estimation of Microsclerotia

About 20 to 30 g of soil were subsampled from composite and single hill samples (grower # 8) and allowed to air dry for two to three days. Two 0.1 g subsamples from each plot were plated on selective medium (NPX) as described by Butterfield and DeVay (1977) using an Andersen Air sampler (Andersen Air Samplers and Consulting Service, Provo, UT). After approximately 2 weeks of incubation at room temperature, all soil particles were carefully removed from the plates' surface under mildly flowing tap water. Cleaned plates were then examined under a dissecting microscope and colonies of microsclerotia were counted.

Unsuccessful Methods

Collection of stem materials for the isolation of *Verticillium* was originally conducted during the season along with the other sample collection. However, cuttings that were obtained directly from strung vines at a 2 foot height, measuring 1 to 2 inches (3 to 5 cm) in length and half of the stem thickness (0.4 to 0.8 cm), had several disadvantages: Sampled vines usually died prematurely; cuttings demonstrated poor storageabilty by drying out too rapidly even when kept in a cold room; their size was too small, especially for unswollen younger vines with diameters of less than 1 cm; and they could be free of disease even though the source vine may have been infected. (This was due to the sometimes irregular, one-sided colonization of the vascular tissue).

Plating these cuttings on PDA resulted in severe contamination even after the "bark" was peeled off and the cutting was surface sterilized. The same problem occurred when petioles were used in place of stem sections.

For pure cultures of *V. albo-atrum* Czapek Dox medium was tried. However, the fungus grew only very slowly and never developed the typical dauermycelium.

Nematodes

Soil Nematodes

All soil samples from the hill were sieved through a 6 mm screen to separate fibrous hop roots and extracted for

nematodes with a modified Bearmann funnel. The sieved soil was mixed and 100 g of soil was placed on a Rapid-Flo milk filter (Filter Fabrics, Inc E. Jefferson Street, Goshin, IN) which was supported on a plastic window screen glued to a PVC ring of 4 cm diameter and 2 cm in height. This filter-screen complex was then placed on top of a plastic funnel (12.4 cm in diameter) filled with tap water to within 1 cm of the rim. Water was retained in the funnel by a metal clamp that was mounted on a 5 cm long latex tubing which extended from the bottom of the funnel. Soil samples remained there for 5 days, during which the water level was checked daily to assure a constant soil-water contact.

After the extraction period, about 150 ml of water was drained from the funnel into a small bottle and stored in a refrigerator until counting. Before samples were counted, extracted water was poured through a 500 micron sieve to concentrate the nematodes. Counting of all samples was carried out by the nematology staff in the Botany and Plant Pathology Department at OSU.

Root Nematodes

Roots, having been previously separated from soil, were washed clean with cold tap water over a series of fine screen. Any non-hop roots or older, woody roots were discarded at this time. All remaining fibrous roots were blotted with paper towels and weighed. Root tissue was then wrapped in Kimwipes and placed onto a regular window screen

which was inserted into a glass funnel. The glass funnels themselves were suspended from a rack that left sufficient space for glass tubes to collect water. The racks, supporting 10 glass funnels, were set into a mist chamber that ran on a 5 min cycle, emitting a fine spray of lukewarm water for 5 sec. After 7 days, racks were removed from the mist chamber. The collected water from the glass tubes was poured into small bottles and refrigerated until counted as described above.

Soil Nutrients

Field Sampling

Soil samples were collected from the hill (the uncultivated soil mount around the base of vines), the first foot (0 - 12"; 0 - 30 cm), the second foot (12 - 24"; 30 - 60 cm), and the third foot (24 - 36"; 60 - 90 cm). Depth samples were obtained using a 4-foot (1.2 m) Veihmeyer sampler (king tube) with a tube diameter of 1 inch (2.5 cm). Samples were bored at two locations, one between two hills and the other in the center of four hills. Although both locations were chosen at random, at least one row separated each site.

As the king tube was pushed into the ground, loose dry surface soil was cleared away from the shaft to prevent high-nutrient soil from falling into the hole and thus contaminating lower depth samples. Each one foot (30 cm) increment was placed into an appropriate plastic bucket

where clods were broken up and samples were mixed into composites. Samples were then transferred into OSU Soil Testing bags, labeled, and transported in an ice cooled cooler.

Laboratory Analysis

Upon arrival at the lab, samples were immediately processed to prevent nitrification. Preparation of soils followed the guidelines established by the OSU Soil Test Laboratory (Horneck et al., 1989). All soils for the hill and the first foot (0 - 30 cm) were analyzed for pH, P, K, Ca, Mg, NH_4, and NO_3. The second and third foot (30 - 60 cm and 60 - 90 cm) were analyzed for K, NH_4, and NO_3. The analysis was carried out by the OSU Soil Testing Laboratory according to published procedures (Horneck et al., 1989).

Tissue Nutrients

Field Sampling

Petiole and leaf samples were taken from every plot. All 16 hills per plot were sampled by randomly choosing one vine per hill. For each vine, both petioles and leaves came from the same node. Since 'Willamette' has an opposite arrangement of leaves on the lower part of the vine, two petioles and two leaves were taken at one node, resulting in composite samples containing a total of 32 petioles and 32 leaves for each plot.

In the first two weeks of sampling (the last week in July and the first week in August) petioles and leaves were clipped by hand from the second node of one sidearm at a six foot (1.80 m) height. As vines matured and leaves began to turn dark green at that location, indicating the onset of senescence, sampling was modified to collect only recently matured plant tissue. As such, even climbing suckers were used to obtain leaf and petiole material, provided that color and lobing of leaves matched the recently matured tissue which was collected in the first half of the sampling period. All plant tissue material from each plot was placed in a white paper bag and kept cool during transportation.

Laboratory analysis

Upon arrival at the laboratory, all samples were immediately dried at 60^{o}C for two days or until completely dry. All plant tissue was ground in Wiley mill with a 20 mesh screen and then stored in small coin envelopes at room temperature. Before being analyzed, samples were re-dried for at least 24 hours. The following analytical work was carried out by the Department of Soil Science plant analysis laboratory.

Petioles were analyzed for the concentration of nitrate-N, phosphate-P, and potassium using an acetic acid extraction. A total of 20 ml acetic acid (2% v/v) was added to 0.2 g plant material and diluted to a 1/200 concentration with 1500 ppm LiCl. This extract was analyzed for K in a

flame atomic absorption spectrometer. The same extract was analyzed colormetrically for nitrates in a continuous flow analyzer ("ALPKEM", RFA 300) by the cadmium reduction method. Concentrations of PO_4-P in the petioles were determined as described below for total P in leaves.

A nitric perchloric digest of leaves was analyzed for phosphorus, potassium, and zinc. This digest was analyzed for K and for Zn using a atomic absorption spectrometer. For P, this digest was run through the ALPKEM continuous flow analyzer with P concentrations determined using a molybdate colorimetric method.

Statistical Analysis

Due to the stratified survey design, ANOVAs were performed using a completely randomized three factor factorial split-split plot design (Table 3). In cases where the main effect "Plot" was not significant, data were reanalyzed using a completely randomized two factor ("Grower" and "Yard") factorial split plot design. For some variables, where both the "Plot" and "Yard" main effects or their interaction terms were not significant, a one factor factorial was used to analyze differences between the main factor, "Growers".

A mean separation test (LSD @ $p = 0.05$) followed the appropriate ANOVA. This procedure and the ANOVA was conducted using MSTAT release 4.1 computer program.

Table 3. Design of statistical analysis of survey data.

Source	Degrees of Freedom
GROWER	9
Error a	10
YARD	1
Grower x Yard	9
Error b	10
PLOT QUALITY	1
Grower x Plot Quality	9
Yard x Plot Quality	1
Grower x Yard x Plot Quality	9
Error c	20

Further statistical analyses were carried out in SAS release 6.3. Generation of a matrix correlation table was produced by the "Proc Corr" procedure. Multiple regression equations were obtained using "Proc Stepwise" with MaxR. Also, principal component analyses were performed using "Proc Factor" followed by a "Varimax" rotation. However, results from all principal component analyses were unsatisfactory and will not be presented.

RESULTS AND DISCUSSION

Verticillium

The Causal Agents

Verticillium wilt in 'Willamette' during the 1989 season was caused by both *V. dahliae* and *V. albo-atrum*. *V. dahliae* was positively identified in 13 yards, while *V. albo-atrum* was isolated from one yard (Figures 1 and 2). Only one species was present wherever the disease occurred.

Description of Symptoms

Although no formal records were kept on symptom expression in 'Willamette', the following general observations should, nevertheless, be noted: 1) Symptoms developed in early to mid-August. 2) Severity of symptoms appeared to be greater wih *V. albo-atrum* than with *V. dahliae* infected hops. 3) Infected vines were usually hidden by leaves from healthy plants from the same hill, often making wilted hop vines difficult to detect. 4) Only in rare cases was every vine from a hill infected and in even rarer cases did all infected vines express foliar wilt symptoms. 5) Symptoms first developed on leaves slightly below or slightly above the arching string. There, leaves showed necrotic margins which, at this stage, could be confused with natural senescence. As the disease progressed, these leaves turned completely yellow and/or

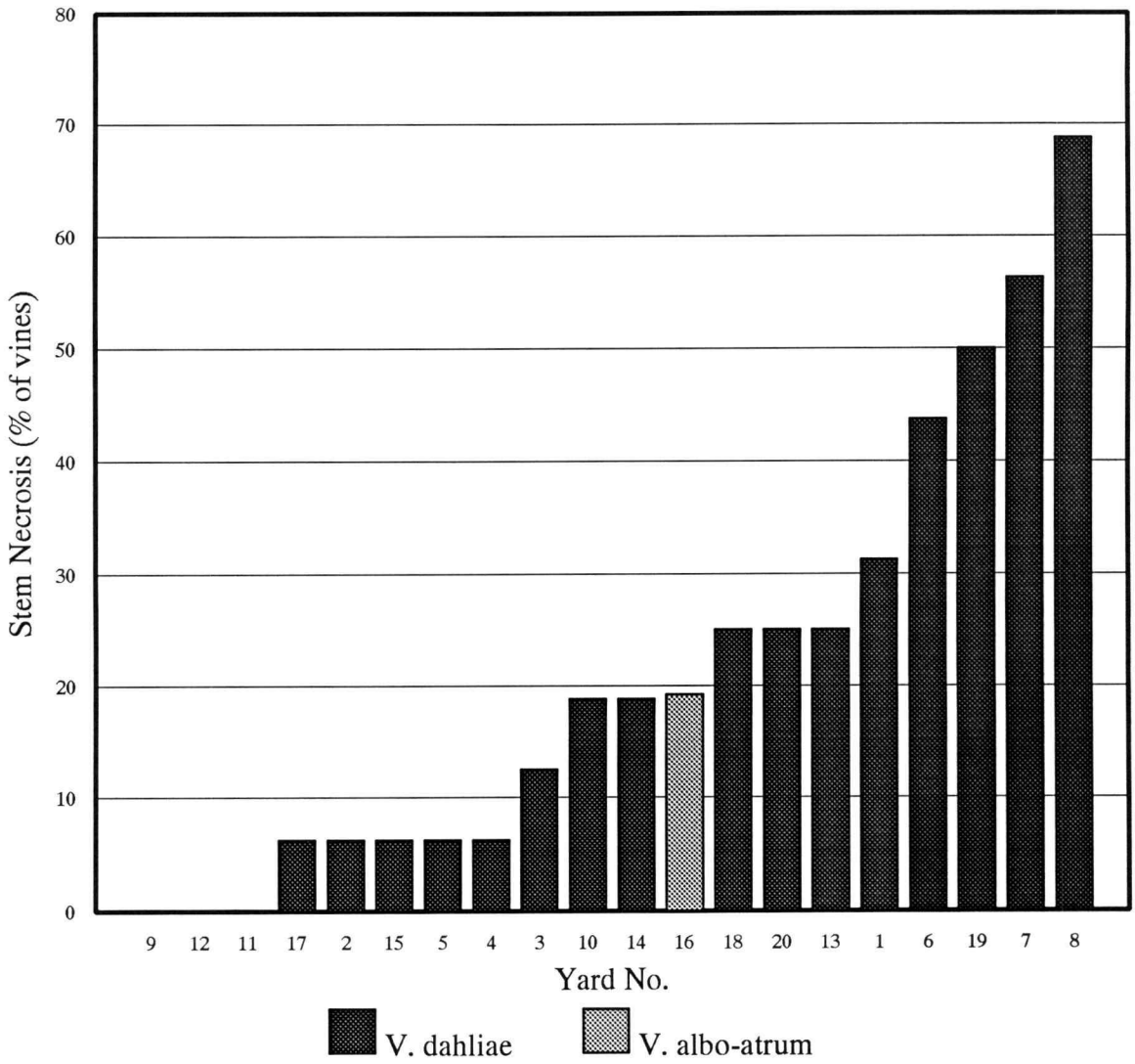

Figure 1. Frequency of vascular necrosis in vine samples from 20 hop yards.

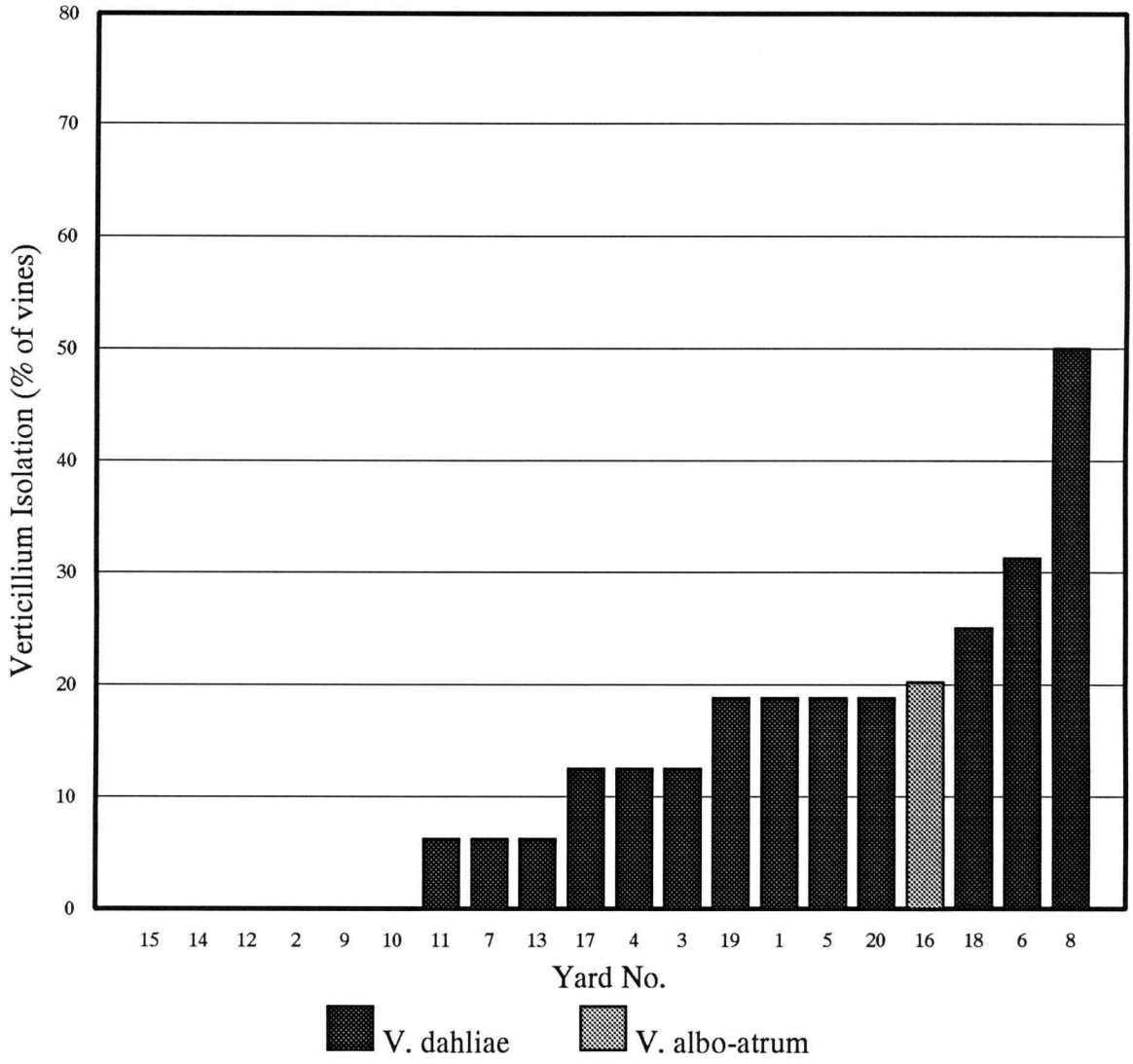

Figure 2. Frequency of isolated *Verticillium dahliae* and *V. albo-atrum* in vine samples from 20 hop yards.

partially necrotic. At this time the petioles still appeared to be healthy. (Tiger striping of the leaves, as has been described in England by Keyworth (1942), was not observed in the 1989 season). 6) Infected vines thickened (up to 1.5 cm) and produced a light brown "bark". 7) The best indication of an infection was the occurrence of vascular necrosis. In general, the darker the water conductive tissue, the greater the chance of recovering the fungus.

Precision of the "Filter Paper" Isolation Technique

The general recovery rate of the "filter paper" method, based on the isolation of both fungal species, was approximately 70%. As is indicated in Table 4, the isolation technique produced better results in vines infected with *V. albo-atrum* than with *V. dahliae*. While *V. albo-atrum* was isolated from 88% of stems exhibiting vascular necrosis, *V. dahliae* was positively identified from only 52% of stems showing some vascular browning.

There are several reasons that may explain this observed difference in recovery rates. 1) All *V. albo-atrum* isolates came from only two closely spaced, intensively sampled plots, whereas *V. dahliae* isolates were obtained from 13 regularly sampled yards. 2) Judgement of vascular necrosis for *V. albo-atrum* infected slices was simple because stems either did or did not exhibit the symptom.

Table 4. Frequency of recovery of *Verticillium dahliae* and *V. albo-atrum* from hop vines with or without stem necrosis.

		Verticillium albo-atrum isolated[1]		*Verticillium dahliae* isolated[1]	
		+	-	+	-
Stem necrosis[2]	+	63	8	34	31
	-	16	84	3	236

[1] a "+" indicates vascular colonization by either fungus
[2] a "+" indicates observed stem necrosis

This was not the case for all other yards where *V. dahliae* occurred. *V. albo-atrum*, being the more virulent pathogen, may produce a stronger necrosis in the tissue. Since, in general, a stronger vascular browning increased the chance of recovery of the pathogen, the filter paper method may be indirectly sensitive to virulence.

Table 4 further illustrates that not all non-necrotic stems are free of infection. Again, a difference between the two fungal species exists, but in this case, the trends are reversed. While 25% of the tissue containing *V. albo-atrum* did not produce vascular browning, only 9% of the vines remained symptomless when infected with *V. dahliae*.

An adequate explanation for this difference has not been found. However, the fact that a *Verticillium* isolation can be made before vascular necrosis appears is important. It shows that this technique is probably capable of isolating the fungus from plants that do not exhibit symptoms. Thus, the filter paper method might also be applied in screening resistant varieties which may harbor the fungus without expressing symptoms.

Statistical Analysis

An analysis of variance of the variable "isolated Verticillium" resulted in non-significant differences for all main effects and their interactions. Therefore, data were re-analyzed using "Grower" as the only effect. Again,

no statistical differences were obtained which suggests that Verticillium wilt may be present in all locations sampled.

Contrary to "isolated Verticillium", an analysis of variance of "stem necrosis" which included all three main effects produced significant differences ($p < 0.001$) among "Growers". (Other effects were not significantly different). A separation of means (LSD @ 0.05 = 0.237) resulted in three groups (Table 5). The first group included growers who had healthy (non-necrotic) stems but who were not significantly different from growers with 22% or less stem necrosis in their vine samples. While the second group constituted growers who had stem necrosis in 25 to 38% of their vines, the third group was made up only by vines from grower # 4, who exhibited stem necrosis in 63% of the samples.

Although the statistical analysis detected a significant difference among growers, it does not invalidate the conclusion that *Verticillium* may be found throughout the valley. Instead, stem necrosis should be regarded as a supplement to the isolation of the pathogen. As such, it may indicate the degree of wilt infection for particular growers.

The reason for this particular interpretation lies mostly in the efficiency of the isolation technique. Since the testing procedure is only 70% efficient, *Verticillium* may have been present where it was not isolated. For grower # 5, none of the 32 vines were demonstrated to contain the

Table 5. Incidence of vascular necrosis in 32 vine samples for 10 hop growers.

Grower	Stem Necrosis	
	-- % --	
6	0	d[1]
5	9	cd
2	9	cd
8	13	cd
9	16	bcd
1	19	abc
7	22	bcd
3	25	bc
10	38	b
4	63	a
LSD @ 0.05	23.7	

[1] values followed by the same letter are not significantly different at p = 0.05

fungus, but three vines did exhibit stem necrosis. In this case, the isolation procedure may have failed to recover the pathogen. If these necrotic stems were, in fact, infected with *Verticillium*, then grower # 5 also would not be free of disease. Hence, this interpretation would again suggest that Verticillium wilt can be found on every farm.

The preceding interpretation is based on the hypothesis that stem necrosis in hop vines is caused exclusively by *Verticillium* infection. Although both downy mildew and *Phytopthora* crown and root rot will produce some stem browning, this symptom has not been reported to occur at a 3 to 4 foot (90 to 120 cm) height. Thus the previously described stem necrosis seems only to be caused by an infection of *Verticillium*.

In order to bring data on *Verticillium* isolation and on stem necrosis into perspective, it must be remembered that 32 stems from two yards containing at least 80,000 vines represented an extremely small fraction. Thus, it was surprising that *Verticillium* was actually recovered from that many plants. For this reason, it appears very likely that a more intensive sampling scheme would have detected *Verticillium* in every yard.

The fact that the analysis of variance for both variables did not produce significant differences among the effects "Yard" and "Plot Quality" has important implications. This may imply that the differences in appearance are the result of factors other than infection by

Verticillium alone. Certainly a more precise statement could be made, if all vines in a plot had been sampled.

A similar conclusion can be drawn from the lack of significant differences among yards. If both the incidence of the pathogen and/or the stem necrosis could be treated as a representative level of the disease, then the presence of the pathogen alone does not determine the "Quality" of a yard. Since the quality of a yard was, in most cases, a reflection of yield, it may be speculated that *Verticillium* was not associated with a yield reduction in the 1989 season.

Microsclerotia Counts

Results from the incubation of soils were disappointing. The Andersen Air Sampler technique, in most cases, did not produce any colonies of microsclerotia in plots from which *V. dahliae* had previously been isolated. In cases where colonies were obtained, counts were very low, approximately 15 ms/g soil. Furthermore, in 10 out of 17 cases, microsclerotia were extracted from plots that did not have an incidence of Verticillium wilt as was earlier determined by the "filter paper" method.

Although these findings do not establish an inoculum level required for *V. dahliae* infection in hops, they do strengthen the hypothesis that Verticillium can be found throughout the valley and do indicate that only a low density of microsclerotia is needed to infect hops.

Nematodes

Distribution of Soil and Root Nematode Populations

Five genera of nematodes were recovered from the soil and *Heterodera* was the most predominant (Figure 3). Populations of *Heterodera* ranged from 2 to more than 2000 juveniles/100 g of oven dry soil and were found in all but one yard. Although the species was not identified, it is reasonable to believe that this nematode is the hop cyst nematode, i.e. *Heterodera humuli*.

Pathogenic nematodes recovered in lower numbers were *Pratylenchus* spp., the root-lesion nematode, and *Paratylenchus* spp, the pin nematode. Each was recovered from 15 and 9 yards, respectively, with populations averaging less than 100 juveniles/100 g soil for both. Both genera occurred in the one yard that was free of *Heterodera*. Thus, all 20 yards had at least one genus of plant pathogenic nematodes present.

Heterodera was the dominant nematode extracted from roots. In the 12 yards where it was recovered, counts ranged from 1 to greater than 1700 juveniles/g of fresh root material and averaged at 173 juveniles/g (Figure 4).

This was in contrast to both *Pratylenchus* and *Paratylenchus*. Although the root-lesion nematode was found in 14 yards, its populations never reached more than 300 juveniles/g fresh root and averaged 32 juveniles/g soil. The pin nematode was recovered in only 3 yards and averaged 16 juveniles/g soil.

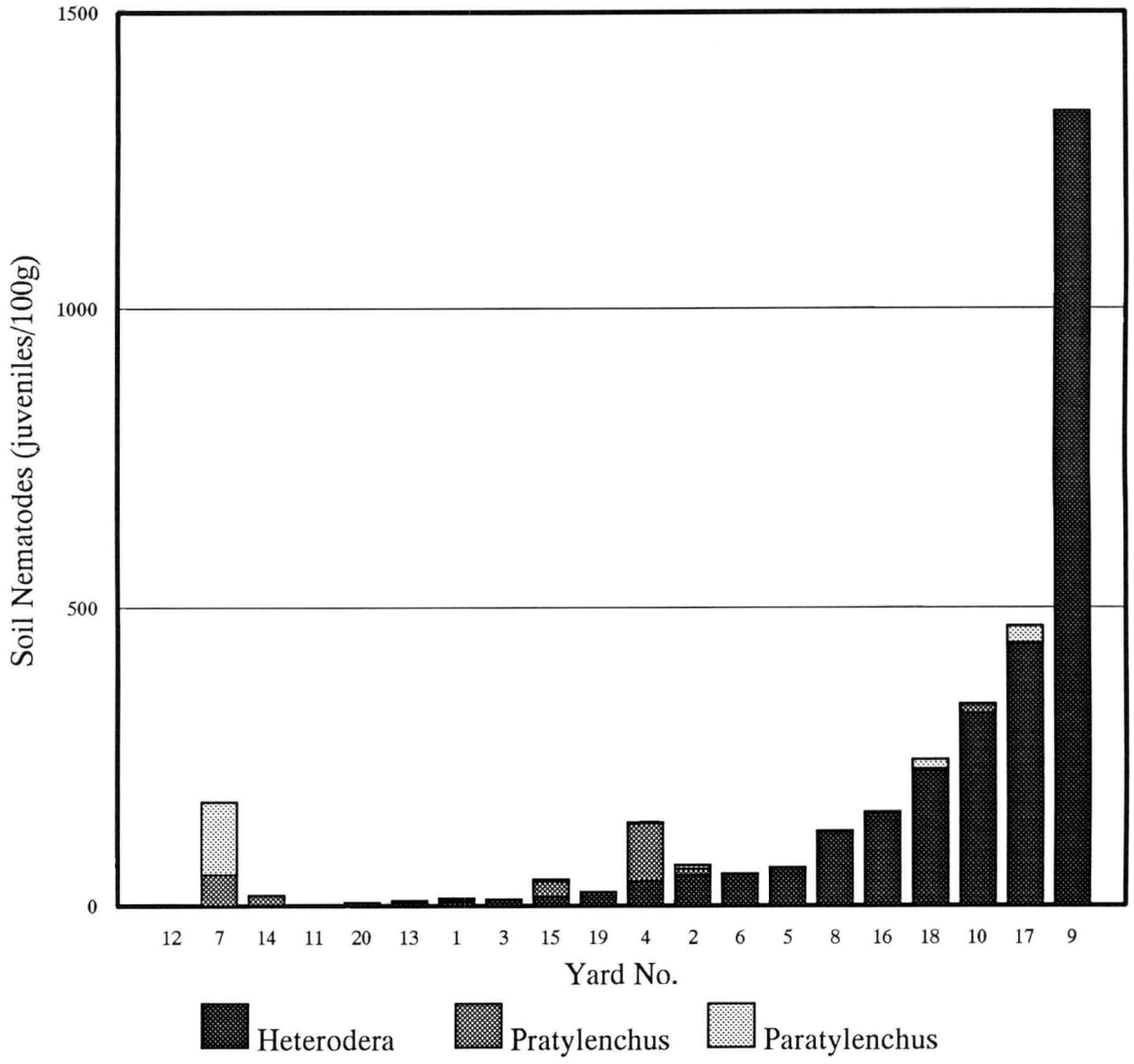

Figure 3. Populations of plant parasitic nematodes recovered from soil samples collected in hop yards.

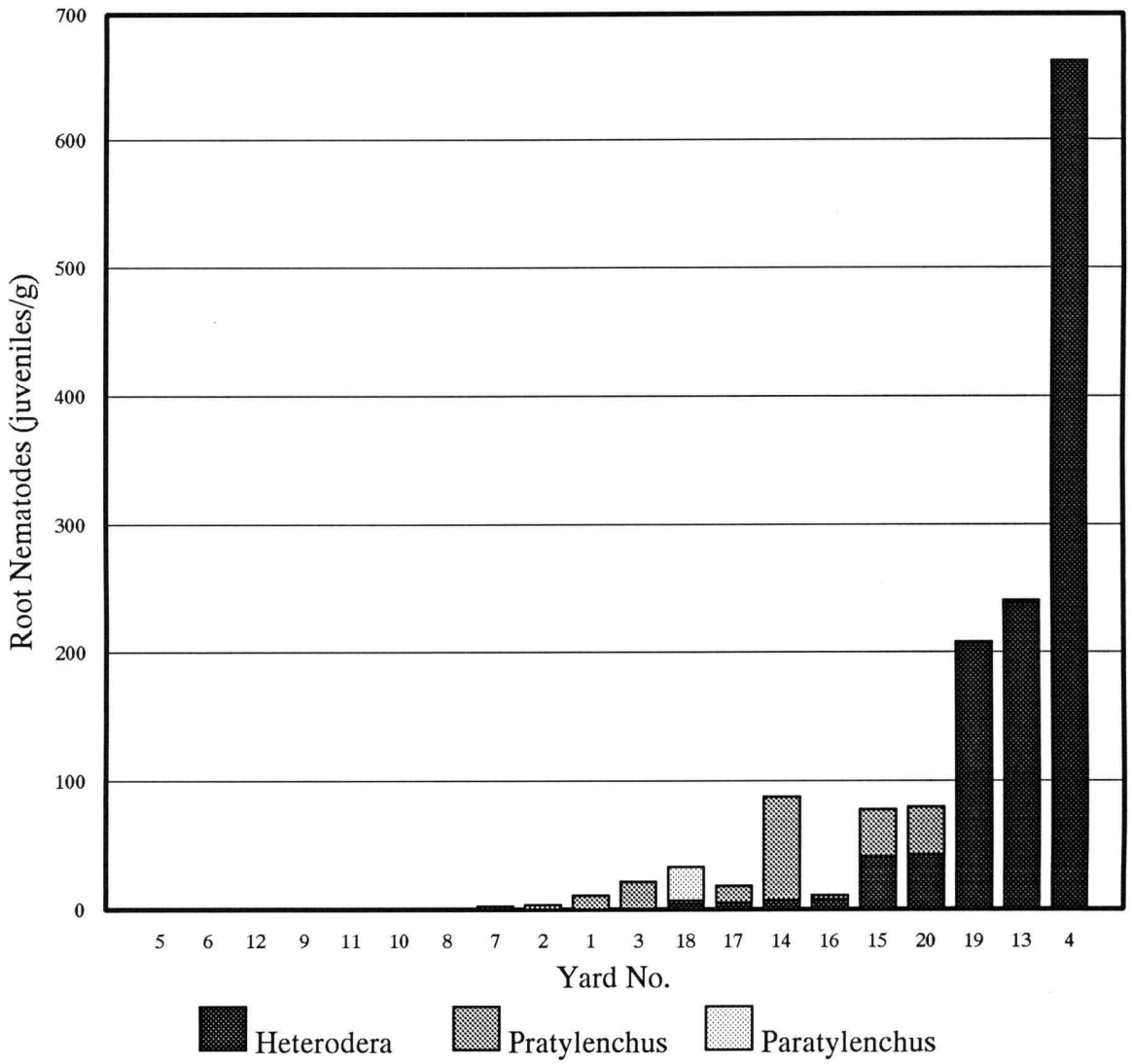

Figure 4. Populations of plant parasitic nematodes recovered from fresh fibrous roots collected in hop yards.

Statistical Analysis

In analyzing the influence of all three effects on "soil" *Heterodera*, a significant interaction ($p = 0.019$) between "Growers" and "Plot Quality" was detected. Although means for "Plot Quality" within "Growers" were significantly different in only one case (grower # 5, LSD @ 0.05 = 298), 6 out of 10 growers had higher nematode levels in the "not-so-good" plots compared to the "good" plots. This may suggest that higher *Heterodera* populations may be recovered from areas where hops appear stressed (Table 6).

This trend seemed to be enhanced when total soil nematode populations were investigated. For the same interaction ($p < 0.001$), 7 out of 10 growers exhibited higher total nematode counts in the "not-so-good" plots. However, more significant differences (LSD @ 0.05 = 288) were not obtained (Table 6).

An analysis of variance of nematodes extracted from the roots resulted in a "Grower" x "Yard" interaction for *Heterodera* ($p = 0.009$) (Table 7). An interpretation of this interaction, however, is impossible due to the low occurrence (less than 8 juveniles/g) in 80% of the yards. Yet, such low root populations still possess value in possibly indicating a concurrent completion of its life cycle with the hop harvest. In fact, all *Heterodera* that were found in the roots, came from samples taken from during the first part of the sampling period (a time correlation was significant, $p = 0.0275$; $r = -0.246$). This may

Table 6. Soil nematode populations as influened by "Grower" and "Plot Quality".

Grower	Plot	*Heterodera*		Total Nematodes	
		---- juveniles/100 g ----			
1	good	20	d[1]	27	d[1]
	not-so-good	39	cd	54	cd
2	good	32	cd	32	d
	not-so-good	19	d	118	bcd
3	good	59	bcd	58	cd
	not-so-good	59	bcd	59	cd
4	good	110	bcd	163	bcd
	not-so-good	13	d	135	bcd
5	good	269	bcd	283	bcd
	not-so-good	1384	a	1387	a
6	good	1	d	3	d
	not-so-good	1	d	1	d
7	good	1	d	4	d
	not-so-good	5	d	23	d
8	good	23	d	51	cd
	not-so-good	147	bcd	147	bcd
9	good	324	bc	334	bc
	not-so-good	342	b	378	b
10	good	22	d	21	d
	not-so-good	7	d	7	d
LSD @ 0.05		298		288	

[1] values followed by the same letter in one column are not significantly different at p = 0.05

Table 7. Root *Heterodera* populations as influenced by the "Grower" and "Yard".

Grower	Yard	*Heterodera*
		juveniles/g
1	good	0 b[1]
	not-so-good	0 b
2	good	1 b
	not-so-good	663 a
3	good	0 b
	not-so-good	0 b
4	good	0 b
	not-so-good	0 b
5	good	0 b
	not-so-good	0 b
6	good	0 b
	not-so-good	0 b
7	good	238 ab
	not-so-good	8 b
8	good	41 b
	not-so-good	8 b
9	good	5 b
	not-so-good	4 b
10	good	207 ab
	not-so-good	43 b
LSD @ 0.05		278

[1] values followed by the same letter are not significanlty different at p = 0.05

indicate that as the hop plant matures in mid-August and nutrient uptake becomes less, the nematode responds to these physiological changes by maturing into adults. Since adult *Heterodera* either exit the roots as males or become encysted females, they would not have been recovered by the previously described extraction procedure.

Results from an intensive sampling of two "not-so-good" plots (soil samples were collected from all 16 hills) revealed extreme variations in nematode populations among hills (Figures 5 and 6). While for the whole plot 8-2-2-1 the average nematode count amounted to 468 juveniles/100 g, individual hill populations ranged from 6 to 3240 juveniles/100 g in plot 8-2-2-2. This may imply that hill nematode densities for other plots may have been up to four or more times as great as the mean for any particular plot. Conversely, extremely high means for some plots may have been the result of sampling hills with unrepresentatively high nematode populations.

Although the plot size may have been too small to detect any pattern in distribution, it is not believed that a larger area would permit the detection of a pattern either. Still, future studies should take samples from larger areas and should be based on more subsamples.

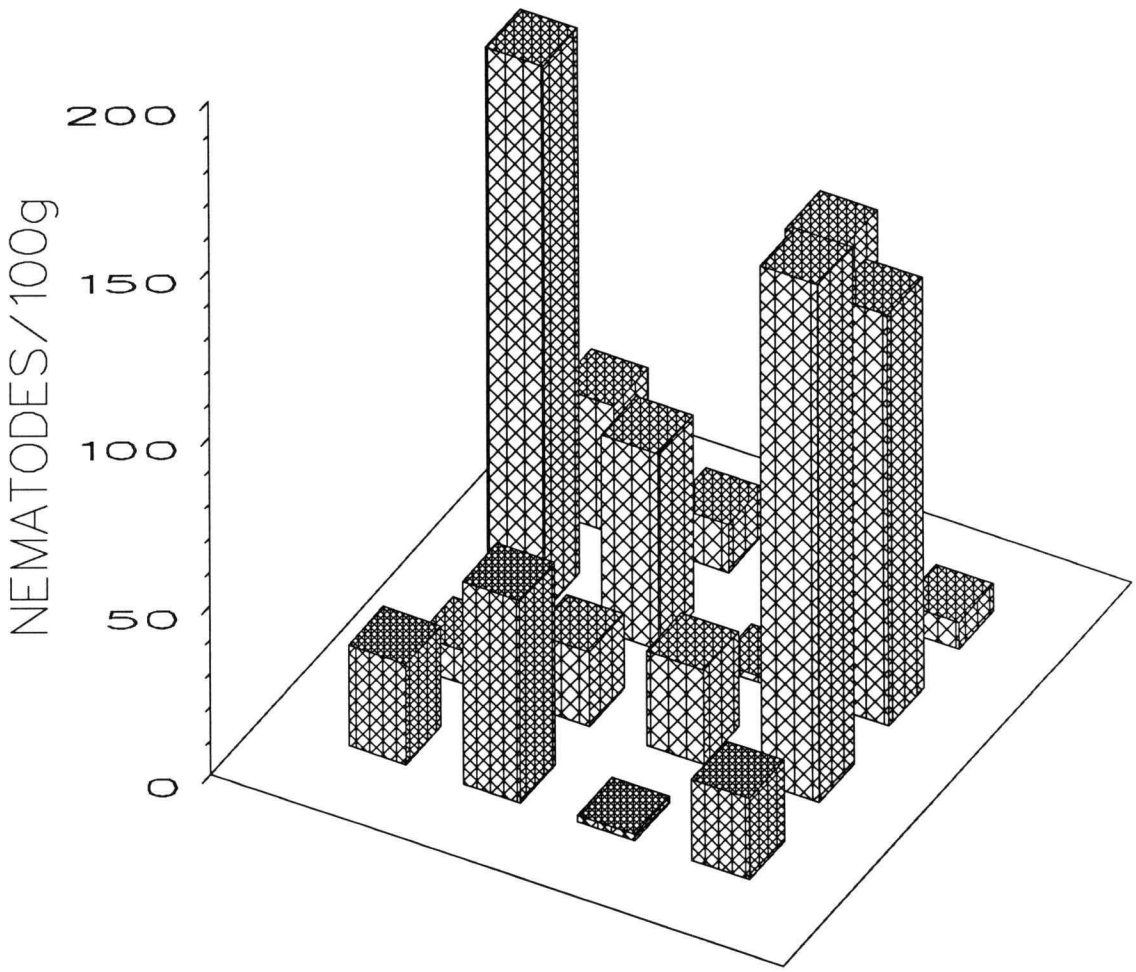

Figure 5. Distribution of plant parasitic nematode populations recovered from every hill in plot 8-2-2-1.

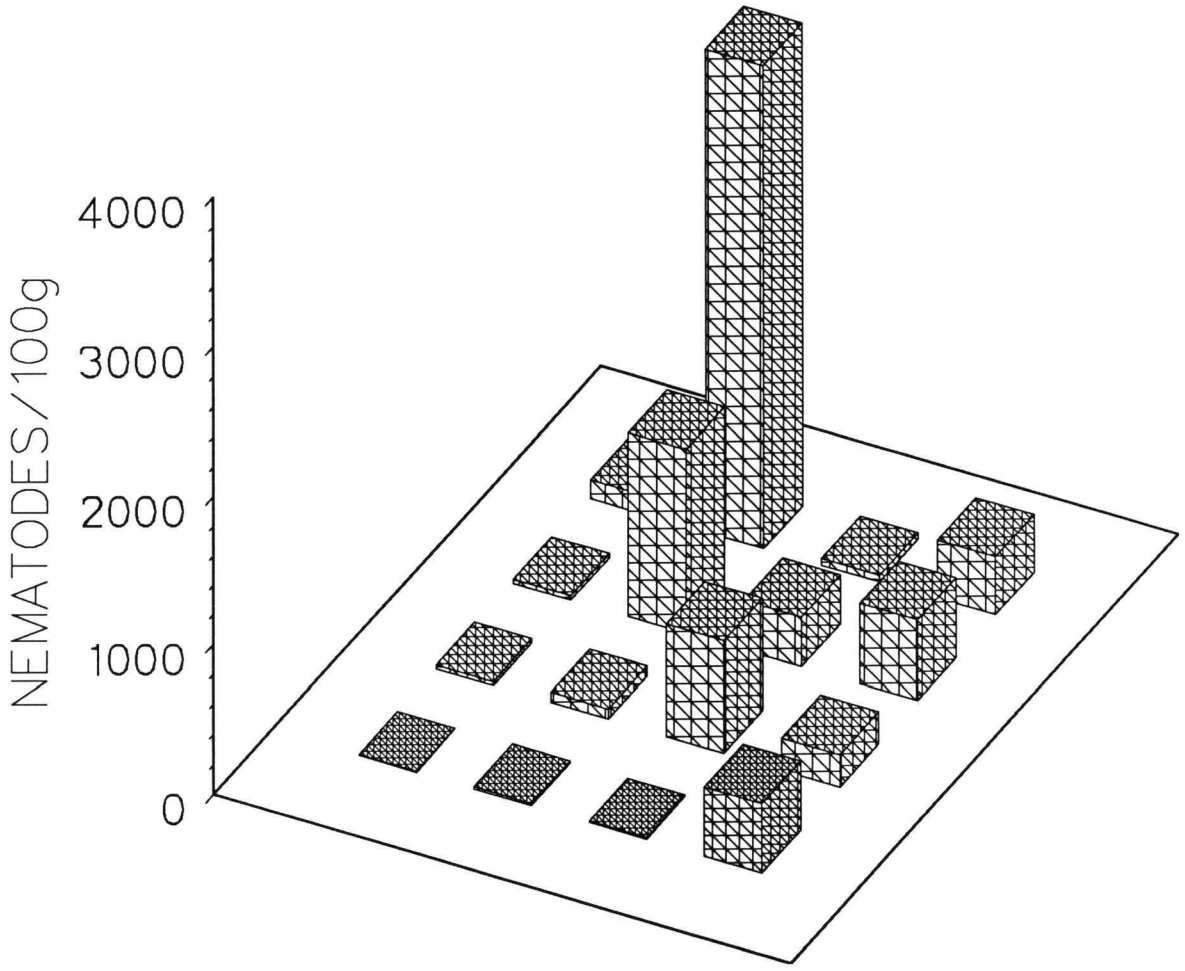

Figure 6. Distribution of plant parasitic nematodes populations recovered from every hill in plot 8-2-2-2.

Soil Nutrients

The analysis of soil nutrients included soils in the associations Woodburn-Amity-Willamette and Cloquato-Newberg-Chehalis. These associations are located on alluvial terraces and on floodplains between the elevations of 100 to 350 feet (33 to 100 m) and 100 to 650 feet (33 to 200 m), respectively, and receive 40 to 45 inches (1000 to 1100 mm) of rain annually.

The predominant soil series were the Cloquato and the Woodburn which were farmed, at least partially, by 80% of the growers, followed by the Chehalis and the Amity. The natural fertility of all these series is high, except for the Amity, where it is moderate.

Nitrogen (NO_3, NH_4)

As nitrogen represents the most abundantly consumed element in hops with 242 lb/A (270 kg/ha) removed for the production of cones and vines (Roberts et al., 1985), its adequate supply is essential. Growers in this survey applied approximately 100 to 200 lb N/A (110 to 220 kg/ha) in late March to early April either as a single or a split application.

Nitrate-nitrogen was most abundant in the hill and in the surface soil (0 - 12", 0 - 30 cm) between hills (Figure 7), with the mean of all plots having slightly higher concentrations for the hill (23.3 ppm) than for the surface soil (20.6 ppm). Hill nitrate-N concentrations for

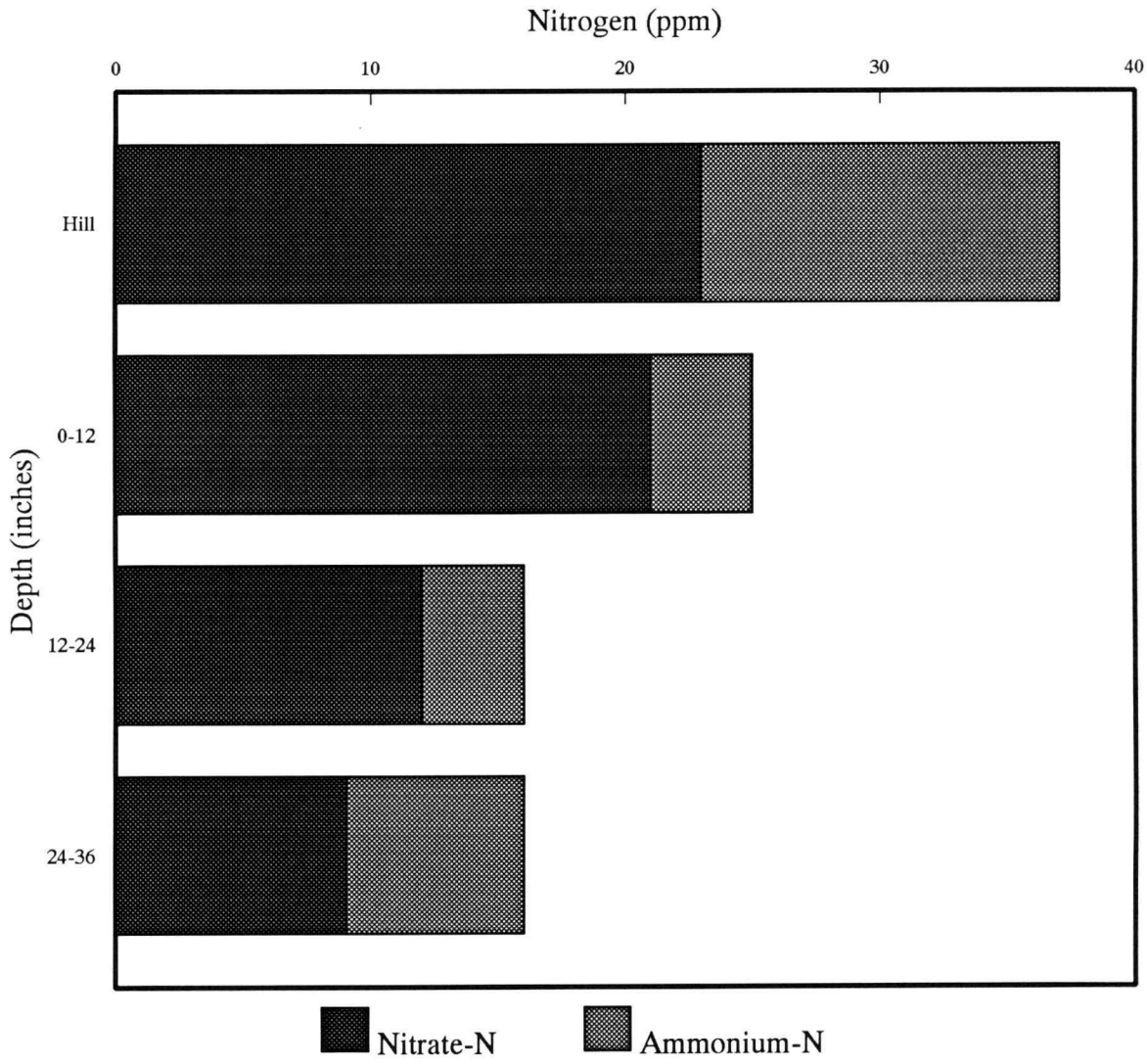

Figure 7. Distribution of nitrate-N and ammonium-N concentrations in soil averaged over 20 hop yards.

individual yards fell essentially into three range categories: a low range with 2.6 to 10.6 ppm; a medium range with 21.7 to 30.1 ppm; and a high range with 40.6 to 46.8 ppm. Nitrate-N content in the surface soil (0 - 12", 0 - 30 cm) exhibited greater variation and was not proportional to the hill. It ranged from 5.5 to 48.0 ppm (Figure 8).

Combined nitrate concentrations (Figure 8) in the subsoil (12 - 36", 30 - 90 cm) amounted to only a small fraction (33%) of the total nitrate-nitrogen (hill - 36", hill - 90 cm) (Figure 8). Mean nitrate-nitrogen concentrations were 12.4 ppm for the second foot (30 - 60 cm) and 9.3 ppm for the third foot (60 - 90 cm) and exhibited less variation than the samples from the hill or from the first foot. Notable exceptions observed in yards 13 and 16, where the highest concentrations were found at a depth of 12 - 24" (30 - 60 cm) with 33.2 ppm and 23.1 ppm, respectively. At a depth of 24 - 36" (60 - 90 cm), concentrations were also unrepresentatively high with 34.5 and 17.1 ppm for the same yards. Hence, for these two yards the subsoil nitrate-nitogen content accounted for 58 and 36%, respectively, of the total extractable nitrate-nitrogen. In addition, the high subsoil concentration for yard 19 should also be mentioned. However, in this yard extractable nitrate-nitrogen in the top soil (hill and 0 - 12", 0 - 30 cm) was also high.

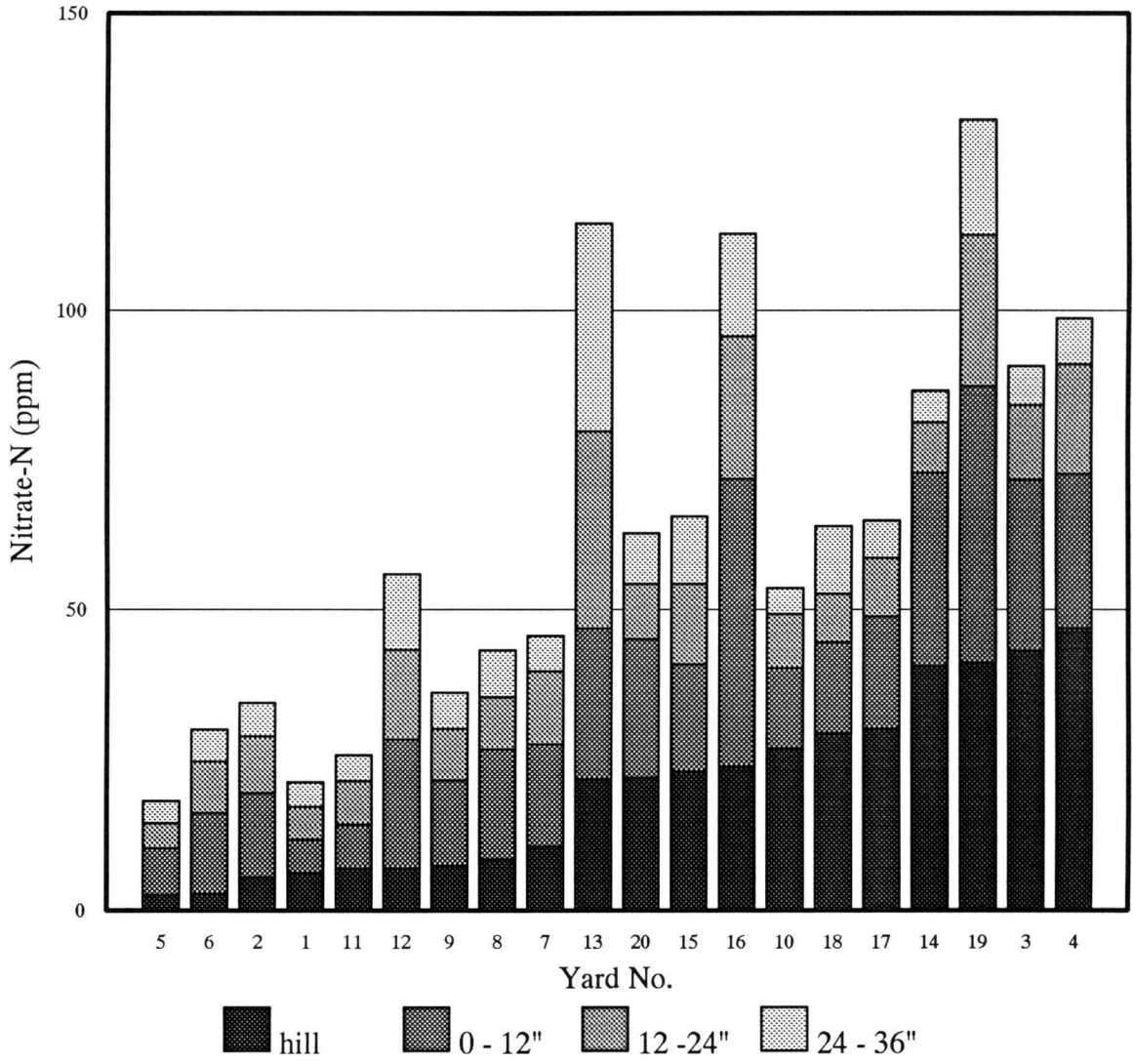

Figure 8. Soil nitrate-N concentrations at four depths in 20 hop yards.

Ammonium-nitrogen concentrations were generally lower than the nitrate concentrations at all depths (Figure 7). The highest quantities of ammonium-N were extracted from the hill with a mean concentration of 13.9 ppm for all plots. There was a decline in concentration from the hill to the surface soil (0 - 12", 0 - 30 cm) with the latter having a mean of 3.7 ppm. Further decreases with depth were not observed, in fact, mean ammonium concentrations increased at a depth of 24 - 36" (60 - 90 cm). This was primarily due to the exessively high concentrations (62 ppm) in yard # 1. (Figure 9).

Concentrations in the surface soil (0 - 12"; 0 - 30 cm) remained rather constant with a range of 2.0 to 8.8 ppm. For the subsoil, most of the variation was due to yard # 1, which exhibited values of 20.8 ppm and 61.9 ppm for 12 - 24" (30 - 60 cm) and 24 - 36" (60 - 90 cm), respectively. Hence, excluding this yard, concentrations averaged approximately 3 ppm for both of these depths.

Statistical Analysis

For the hill nitrate-nitrogen content, an analysis of variance found only "Growers" to differ significantly ($p < 0.001$), with values ranging from 2.75 to 44.9 ppm NO_3 (Table 8).

A complete explanation for such a wide range of values is difficult to find. Soil type, time and amount of nitrogen application, time of sample collection and

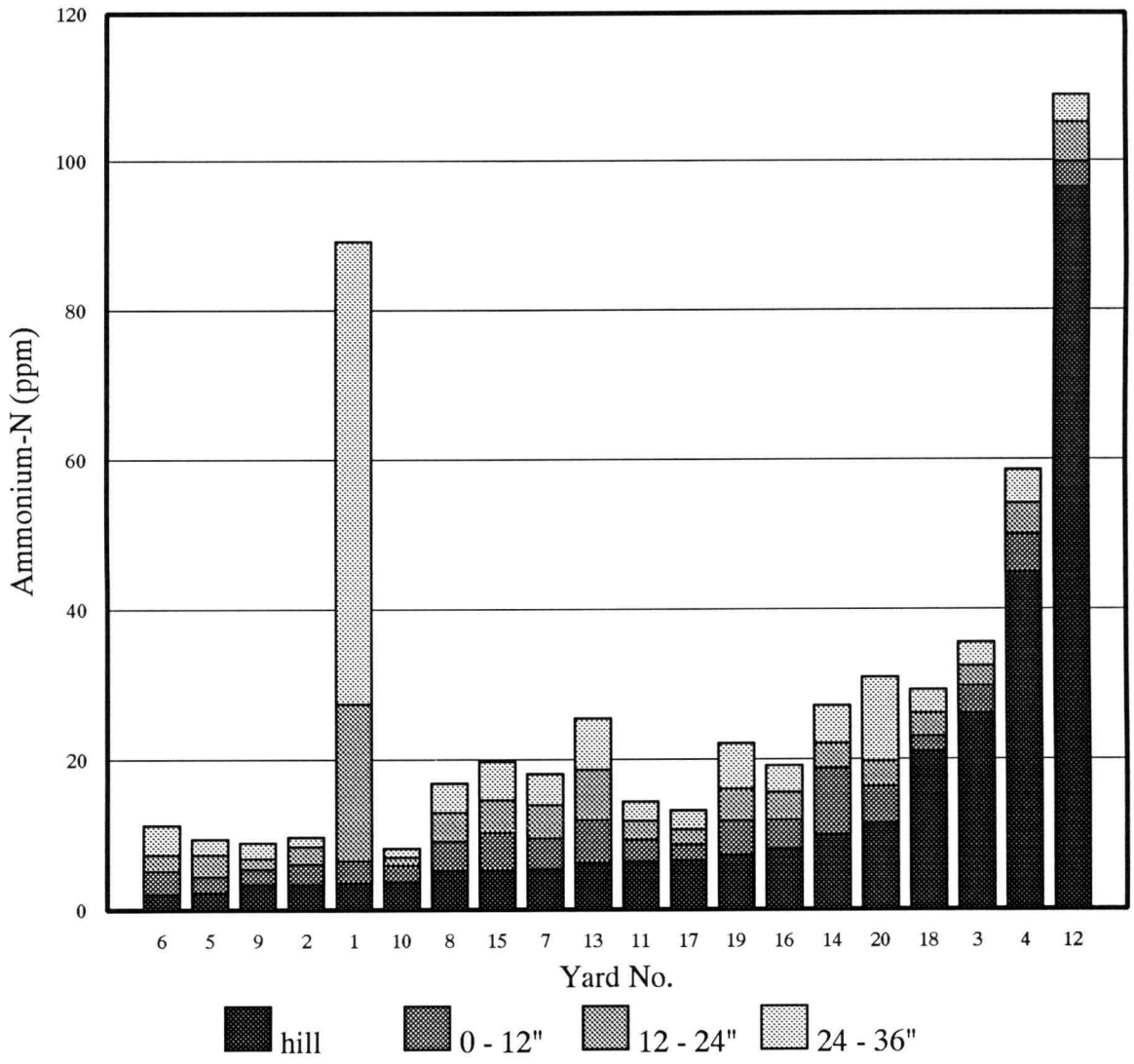

Figure 9. Soil ammonium-N concentrations at four depths in 20 hop yards.

irrigation management can influence nitrate concentrations greatly. Grower # 2, for instance, banded a third split application of 50 N lb/A (56 kg/ha) on the hill in the beginning of July, bringing his total N application up to 200 lb/A (220 kg/ha). As a result the hill nitrate concentrations were the higest with 44.9 ppm or translating into roughly 60 lb/A (67 kg/ha) at sampling time. On the other extreme, grower # 3 had the lowest hill nitrate concentration with only 2.75 ppm. According to the questionnaire, he applied only 86 lb N /A (96 kg/ha). In addition, he was the last grower to be sampled, which may have allowed more time for plant uptake of N.

For the surface and subsurface soil, significant differences ($p < 0.001$ for 0 - 12", 0 - 30 cm; $p = 0.049$ for 12 - 24" 30 - 60 cm; $p = 0.014$ for 24 - 36", 60 - 90 cm) were detected among yards of particular growers (Table 9). For the surface soil (0 - 12", 0 - 30 cm) the mean nitrate-N concentration was significantly higher (LSD @ 0.05 = 13.11) in the "not-so-good" yard of grower # 8. This unusually high value (48.03 ppm) may be explained be a late aerial application of calcium nitrate. No explanation can be given for the very high concentrations (46.3 ppm) that also were found in the "good" yard of grower # 10. Although significant differences were obtained at depths of 12 - 24" (30 - 60 cm) and 24 - 36" (60 - 90 cm), these differences were the result of the unrepresentatively high values from yards 13 and 9 (Growers # 7 and # 5), as discussed above.

Table 8. Nitrate-N concentrations in the hill as influenced by "Grower".

Grower	hill	
	NO_3-N (ppm)	
3	2.75	f[1]
1	5.94	f
4	9.54	ef
5	17.17	de
8	23.45	cd
9	29.77	bc
7	31.13	bc
10	31.56	bc
6	36.47	ab
2	44.90	a
LSD @ 0.05	11.07	

[1] values followed by the same letter are not significantly at p = 0.05

Table 9. Nitrate-N concentrations at three depths as influenced by "Grower" and "Yard".

Grower	Yard	0 - 12" 0 - 30 cm		12 - 24" 30 - 60 cm		24 - 36" 60 - 90 cm	
		NO_3-N (ppm)					
1	good	5.50	f[1]	5.38	d[1]	4.10	cd[1]
	not-so-good	13.85	def	9.45	cd	5.55	cd
2	good	28.80	bc	12.45	bcd	6.50	bcd
	not-so-good	26.00	bcd	18.38	abcd	7.60	bcd
3	good	7.70	ef	4.05	d	3.72	d
	not-so-good	13.15	def	8.65	cd	5.32	cd
4	good	16.98	cdef	12.10	bcd	5.95	cd
	not-so-good	18.20	cdef	8.70	cd	7.80	bcd
5	good	14.13	def	8.63	cd	5.97	cd
	not-so-good	13.30	def	9.00	cd	4.28	cd
6	good	7.28	ef	7.18	d	4.32	cd
	not-so-good	21.42	bcd	14.88	bcd	12.55	bcd
7	good	25.08	bcd	33.17	a	34.50	a
	not-so-good	32.40	b	8.45	d	5.28	cd
8	good	17.85	cdef	13.35	bcd	11.25	bcd
	not-so-good	48.03	a	23.77	abc	17.08	bc
9	good	18.67	cde	9.75	cd	6.28	bcd
	not-so-good	15.13	def	8.00	d	11.27	bcd
10	good	46.30	a	25.08	ab	19.23	b
	not-so-good	23.10	bcd	9.15	cd	8.43	bcd
LSD @ 0.05		13.11		15.19		13.12	

[1] values followed by the same letter in one column are not significantly different at p = 0.05

An analysis of variance detected a significant interaction ($p = 0.003$) between "Growers" and "Yards" for ammonium-nitrogen concentrations in the hill (Table 10). Although a mean separation (LSD @ 0.05 = 29.37) found only the "not-so-good" yard from grower # 6 to be significantly higher than the "good", the rather high value found in yards from grower # 2 should be pointed out. An ammonium-N concentration 44.92 ppm ("not-so-good" yard) represented the second highest hill value for all yards. It is interesting to note that this yard also tested the lowest in hill pH and, thus, these unusually high concentrations may have been a reflection of a reduced nitrification rate, or conversely, the presence of ammonium ions may have depressed pH. A scatter plot of hill pH and hill ammonium-N (log transformed) for all locations indicated an inverse relationship (Figure 10). As the pH decreased, the amount of ammonium-N increased.

For the surface soil (0 - 12", 0 - 30 cm), an analysis of variance only detected significant differences in NH_4-N ($p = 0.001$) among "Growers" (Table 11). Although most growers differed significantly from one another (LSD @ 0.05 = 1.65) no satisfactory explanation can be given.

Table 10. Ammomium-N concentrations in the hill as influenced by "Grower" and "Yard".

Grower	Yard	hill	
		NH_4-N (ppm)	
1	good	3.60	c[1]
	not-so-good	3.42	c
2	good	26.00	bc
	not-so-good	44.92	c
3	good	2.33	c
	not-so-good	2.13	c
4	good	5.50	c
	not-so-good	5.25	c
5	good	3.42	c
	not-so-good	3.78	c
6	good	6.50	c
	not-so-good	96.5	a
7	good	6.28	c
	not-so-good	10.07	c
8	good	5.32	c
	not-so-good	8.15	c
9	good	6.57	c
	not-so-good	21.02	bc
10	good	7.38	c
	not-so-good	11.48	c
LSD @ 0.05		29.37	

[1] values followed by the same letter are not significantly different at p = 0.05

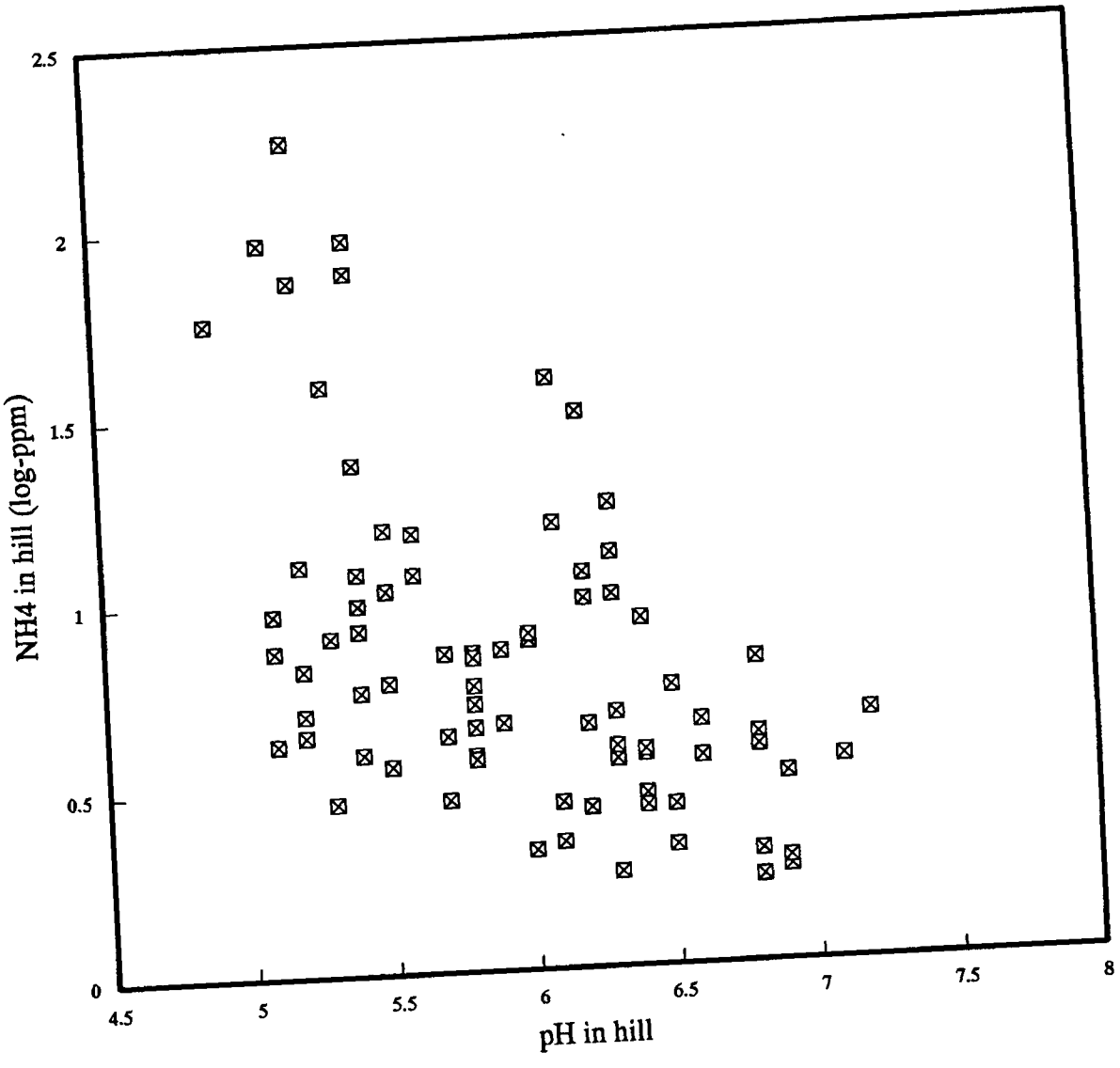

Figure 10. Scatterplot of pH and ammomium-N concentrations in the hill for all plots.

Table 11. Ammonium-N concentrations in the surface as influenced by "Grower"

Grower	0 - 12" 0 - 30 cm
	NH_4-N (ppm)
9	2.08 e[1]
5	2.10 e
3	2.59 de
1	2.85 cde
6	3.10 bcde
4	3.99 bcd
8	4.40 bc
2	4.44 bc
10	4.70 b
7	7.20 a
LSD @ 0.05	1.65

[1] values followed by the same letter are not significantly different at p = 0.05

Potassium

The nutrient that is consumed in the second highest quantities is potassium. During the season, an acre of cone bearing vines will take up 190 lb K/A (213 kg/ha) from the soil (Roberts et al., 1985). To provide this needed nutrient, growers broadcast an average of 100 lb K/A (112 kg/ha). It is interesting to note that two farms (9 and 10) followed a different fertility program, applying none and 228 lb K_2O/A (255 kg/ha), respectively.

Potassium was found in the highest quantities in the hill and decreased exponentially with depth (Figures 11 and 12). For the hill, yards could be grouped into range categories. The lowest range was represented by two yards (#'s 11 and 12), both belonging to grower # 6. Extractable soil K concentrations were only 154 and 118 ppm, respectively. The next category, comprising the most yards, ranged from 290 to 412 ppm. Noteworthy is the fact that those 11 yards belonged to 5 growers, suggesting that growers may have a greater ability to influence K availability than soil mineralogy. Another category with higher than average ranges (557 to 616 ppm) included 6 yards, managed by 4 growers. The highest value was found in yard 13, which had an average K concentration in the hill of 799 ppm.

The K concentrations for the surface soil were approximately 40% less than in the hill, with a mean of 276 ppm for all plots. On a yard basis, fields 11 and 12 again

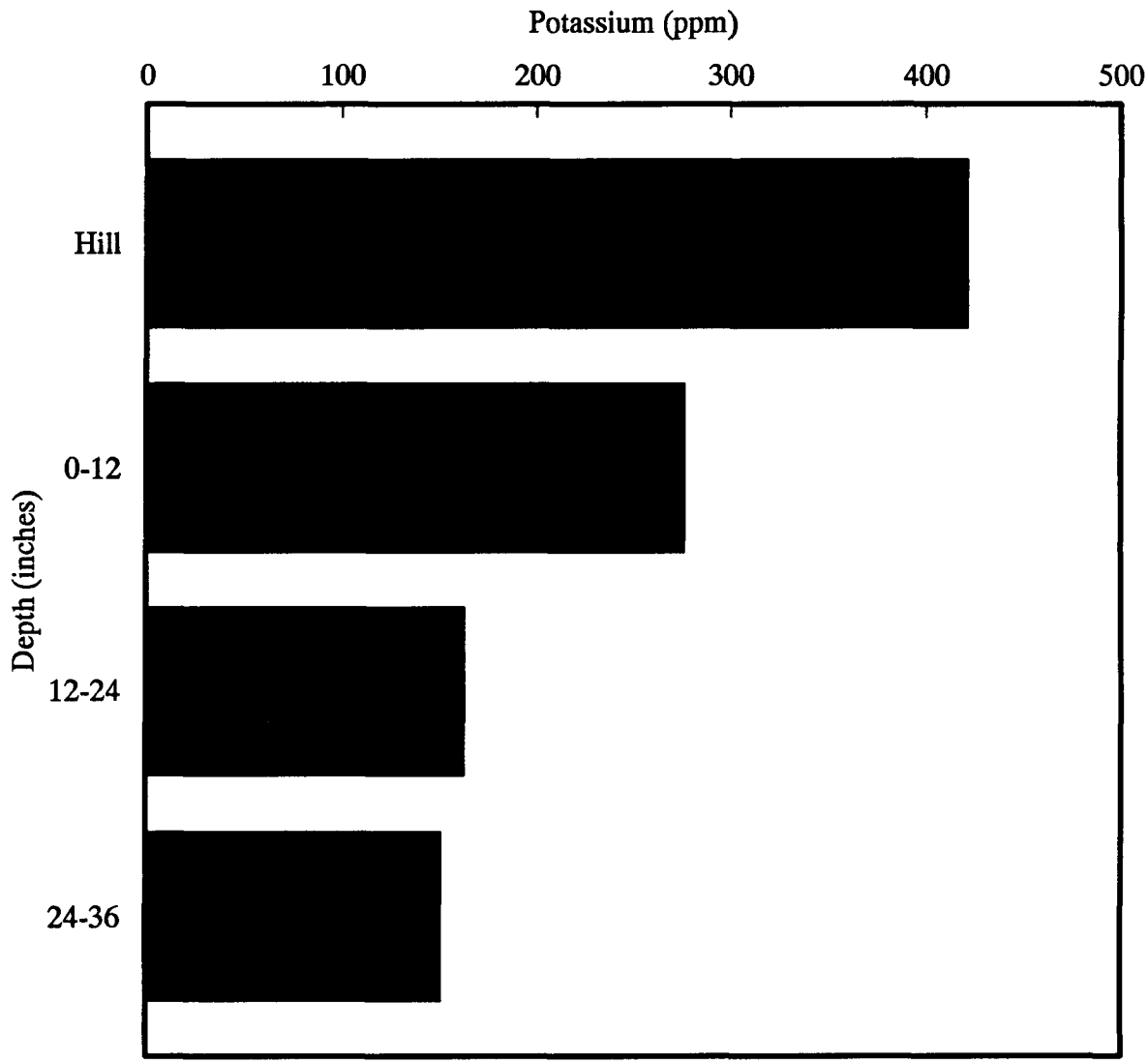

Figure 11. Distribution of potassium concentrations in soil averaged over 20 hop yards.

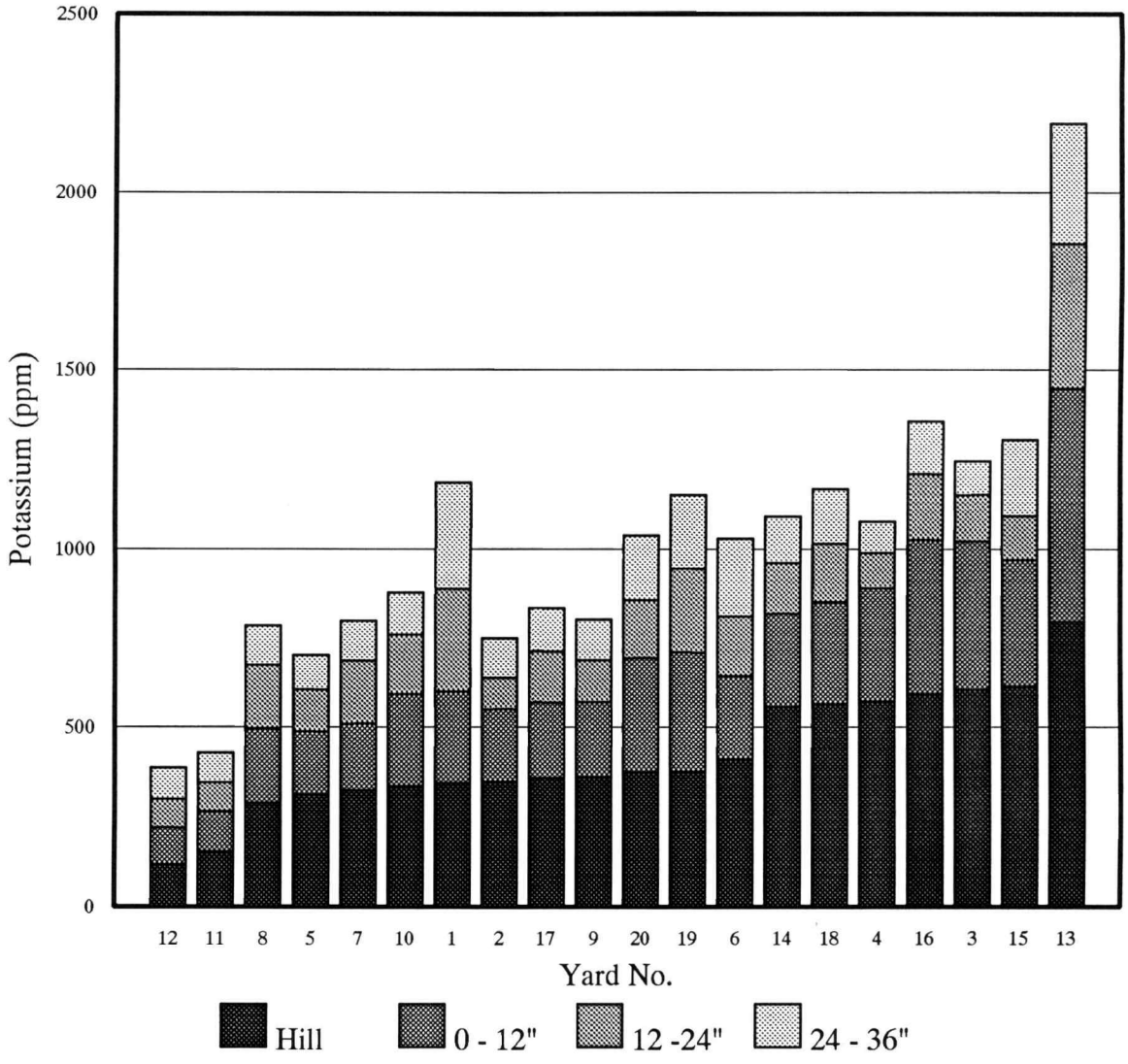

Figure 12. Soil potassium concentrations at four depths in 20 hop yards.

had the lowest concentrations with 111 and 101 ppm, respectively. A general mid-range of approximately 170 to 320 ppm was observed for 15 yards, while the highest concentrations ranged from 418 ppm to 649 ppm. Yards with the highest hill concentrations were also those with the highest K in the surface soil.

Concentrations of K found in the subsoil amounted to 30% of the total profile. The mean for the depth of 12 -24" (30 - 60 cm) was 163 ppm and for the depth of 24 - 36" (60 - 90 cm) was 150 ppm. Variation among yards was relatively low, however, those yards that had high surface K concentrations also had elevated levels in the subsoil.

Statistical Analysis

In examining potassium in the hill, the effect of "Plot Quality" proved to be significant ($p = 0.037$). With an average of 391 ppm, the "not-so-good" plots tested 60 ppm lower than the "good" plots. This may suggest that the determination of plot quality was influenced by a fertility effect.

"Plot Quality" was also a factor in the surface soil (0 -12"), although here it appeared to depend on the particular grower ($p = 0.014$). For two growers (7 and 10) where differences were significant (LSD @ 0.05 = 124), the "good" plots contained higher potassium levels than their counterparts (Table 12).

Table 12. Potassium concentrations in the surface soil as influenced by "Grower" and "Plot Quality".

Grower	Plot Quality	0 - 12" 0 - 30 cm
		K (ppm)
1	good	180 efg[1]
	not-so-good	279 cde
2	good	363 bcd
	not-so-good	376 bc
3	good	165 efg
	not-so-good	244 de
4	good	185 efg
	not-so-good	208 efg
5	good	229 ef
	not-so-good	238 e
6	good	109 fg
	not-so-good	103 g
7	good	530 a
	not-so-good	383 bc
8	good	421 ab
	not-so-good	366 bcd
9	good	281 cde
	not-so-good	219 efg
10	good	456 ab
	not-so-good	198 efg
LSD @ 0.05		124

[1] values followed by the same letter are not significantly different at p = 0.05

Table 13. Potassium concentrations in the hill and the surface soil as influenced by "Grower" and "Yard".

Grower	Yard	hill	0 - 12" 0 - 30 cm
		K (ppm)	
1	good	344 c[1]	256 defg[1]
	not-so-good	348 c	202 fgh
2	good	606 b	418 b
	not-so-good	571 b	321 cd
3	good	321 c	177 hij
	not-so-good	412 c	232 efgh
4	good	324 c	185 ghi
	not-so-good	290 c	207 fgh
5	good	360 c	211 fgh
	not-so-good	337 c	256 defg
6	good	154 d	111 ij
	not-so-good	118 d	101 j
7	good	790 a	649 a
	not-so-good	557 b	264 def
8	good	616 b	355 bc
	not-so-good	596 b	432 b
9	good	358 c	210 fgh
	not-so-good	565 b	290 cde
10	good	376 c	334 cd
	not-so-good	375 c	320 cd
LSD @ 0.05		133	78

[1] values followed by the same letter are not significantly different at p = 0.05

In addition, an analysis of variance showed a significant interaction between "Growers" and their "Yards" for both the hill ($p < 0.001$) and the surface soil (0 - 12") ($p < 0.001$) (Table 13). Two yards from growers # 7 and # 9 were significantly different from one another (LSD @ 0.05 = 133 ppm) for the hill, while these and one additional yard from grower # 2 exhibited a significant difference (LSD @ 0.05 = 78 ppm) in the surface soil (0 - 12"). A trend of "good" versus "not-so-good" was not apparent in either depth.

Phosphorus

Uptake of phosphorus by hops, based on a 10 bale/A yield (2054 kg/ha), is estimated to be 65 lb P_2O_5/A (72 kg/ha). Growers regularly apply an average of 100 lb P_2O_5/A (112 kg/ha) to adequately supply this nutrient.

The analysis of phosphorus in the hill revealed high to very high concentrations for most yards with values ranging from 55 to 155 ppm with an averaging 106 ppm (Figure 13). Although individual yards could not be grouped into distinct range categories, the majority of yards exhibited concentrations from 85 to 125 ppm.

Phosphorus concentrations in the surface soil (0 - 12", 0 - 30 cm) were considerably less than those in the hill, averaging 76 or 30 ppm lower. The overall variability among

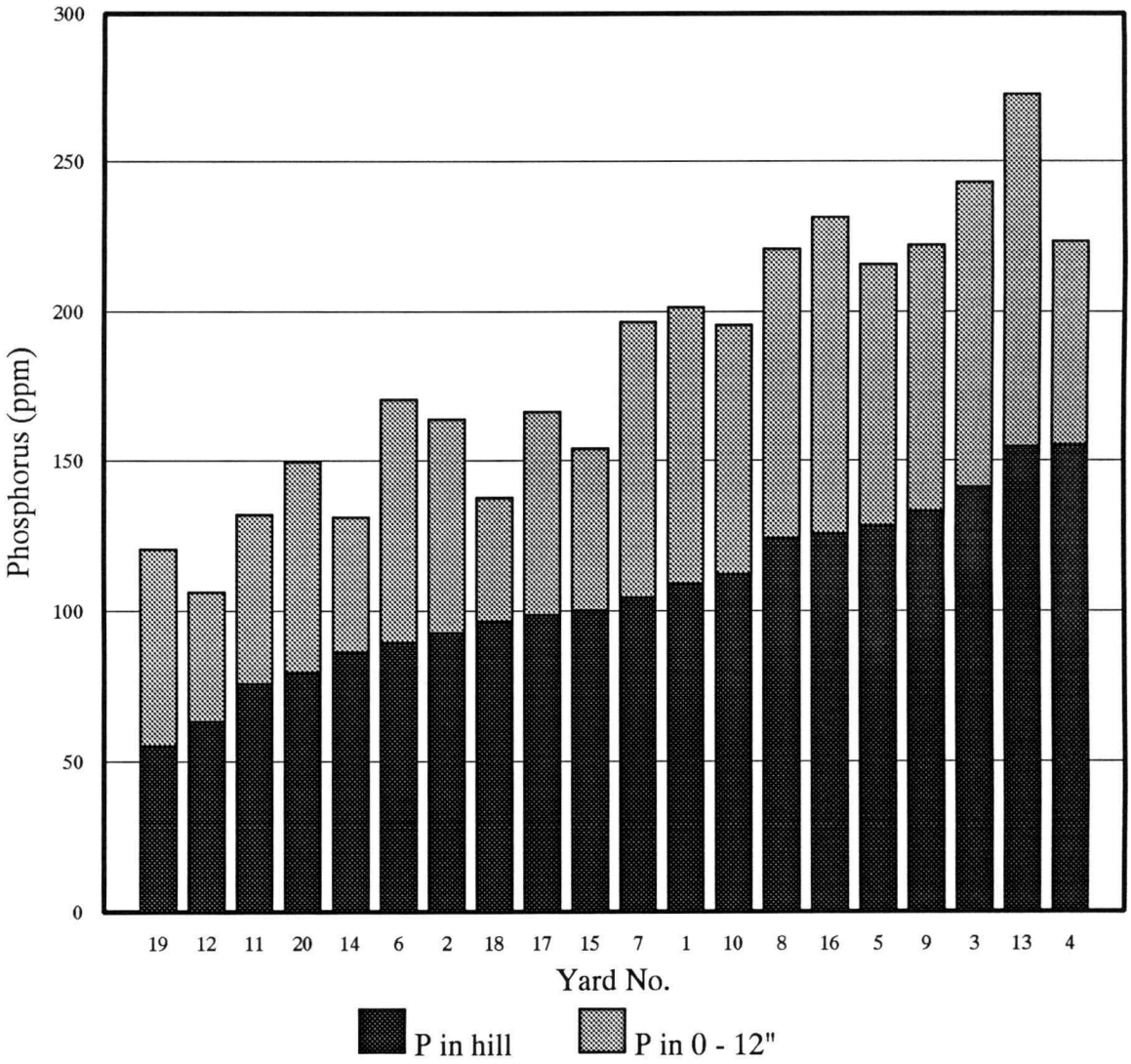

Figure 13. Soil phosphorus concentrations at two depths in 20 hop yards.

yards in P concentration in the 0 - 12" (0 - 30 cm) depth was similar to or even greater than in the hill, with values ranging from 43 to 118 ppm.

Statistical Analysis

An analysis of variance detected a significant interaction among "Growers" and "Yards" for both the hill ($p = 0.017$) and the surface soil ($p = 0.001$). For the hill, a mean separation (LSD @ 0.05 = 33 ppm) revealed both "not-so-good" yards from growers # 3 and # 7 to be significantly lower than the "good" yards (Table 14). However, no circumstances could be found that would adequately explain these differences. Mean concentrations for the surface soil exhibited significant differences (LSD @ 0.05 = 29) in growers # 2, # 7, and # 8. The fact that the "not-so-good" yard from grower # 8 contained significantly higher P concentrations may be attributed to the comparatively low values of the "good" yard.

Yet, it must be pointed out that phosphorus concentrations in all yards, whether or not they were significantly lower than their counterparts, probably represented more than adequate levels for optimum hop growth. Thus, it also appears reasonable that the soil phosphorus levels for both the hill and the surface soil (0 - 12") did not correlate significantly with the yield and appearance based classification of "good" and "not-so-good" for yards and plots, respectively.

Table 14. Phosphorus concentrations in the hill and the surface soil as influenced by "Grower" and "Yard".

Grower	Yard	hill	0 - 12" 0 - 30 cm
		P (ppm)	
1	good	109 bcdef[1]	92 abcd[1]
	not-so-good	93 efgh	71 cdefg
2	good	141 ab	102 ab
	not-so-good	155 a	68 cdegfh
3	good	129 abcd	87 bcd
	not-so-good	90 fgh	81 bcdef
4	good	105 cdefg	92 abcd
	not-so-good	124 abcde	97 abc
5	good	133 abc	89 abcd
	not-so-good	112 bcdef	84 bcde
6	good	76 ghi	56 efgh
	not-so-good	64 hi	43 gh
7	good	155 a	118 a
	not-so-good	87 fghi	45 gh
8	good	100 cdegf	54 fgh
	not-so-good	126 abcde	106 ab
9	good	99 defg	68 cdefgh
	not-so-good	97 defg	41 h
10	good	55 i	65 defgh
	not-so-good	80 fghi	70 cdefgh
LSD @ 0.05		33	29

[1] values followed by the same letter in one column are not significantly different at p = 0.05

Soil Acidity (pH)

Analytical determination of soil pH revealed surprisingly large variations. The pH for the hill ranged from 5.13 to 6.78 and for the surface soil (0 - 12", 0 - 30 cm) from 5.07 to 6.9. Although most experts believe that the pH should be kept close to pH 6, approximately half of the yards were considerably lower than this recommended level, both in the hill and in the surface soil (Figures 14 and 15).

Statistical Analysis

A analysis of variance for soil pH in the hill detected a significant interaction between "Growers" and their respective "Yards" ($p = 0.006$). Although such an interaction implies that the distinction between "good" and "not-so-good" yard has no direct relevancy on the pH level, it should be pointed out that where a significant difference (LSD @ 0.05 = 0.442) did occur, the pH in the "not-so-good" yard was consistently lower than the "good" yard (growers # 2, # 5, and # 10). Two of these "not-so-good" yards were strongly acidic (pH 5.28 and 5.15) and differed from the "good" yards by more than half a pH unit (Table 15). For the surface soil, the "not-so-good" yards also had the lower pH. Here the difference (LSD @ 0.05 = 0.319) was observed on five farms (#'s 2, 6, 8, 9, and 10), three of which had pHs below 6.

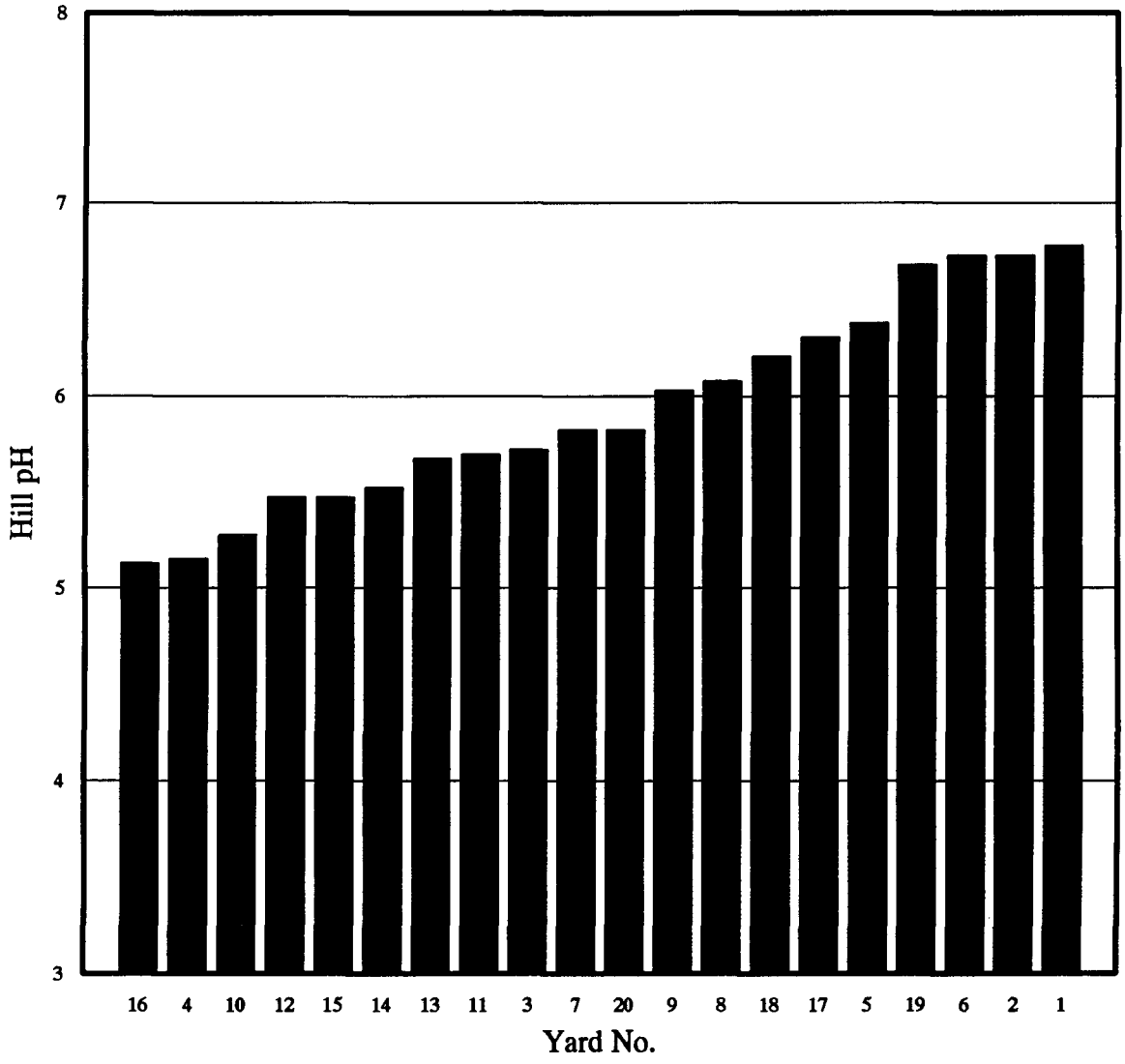

Figure 14. Soil pH in the hill for 20 hop yards.

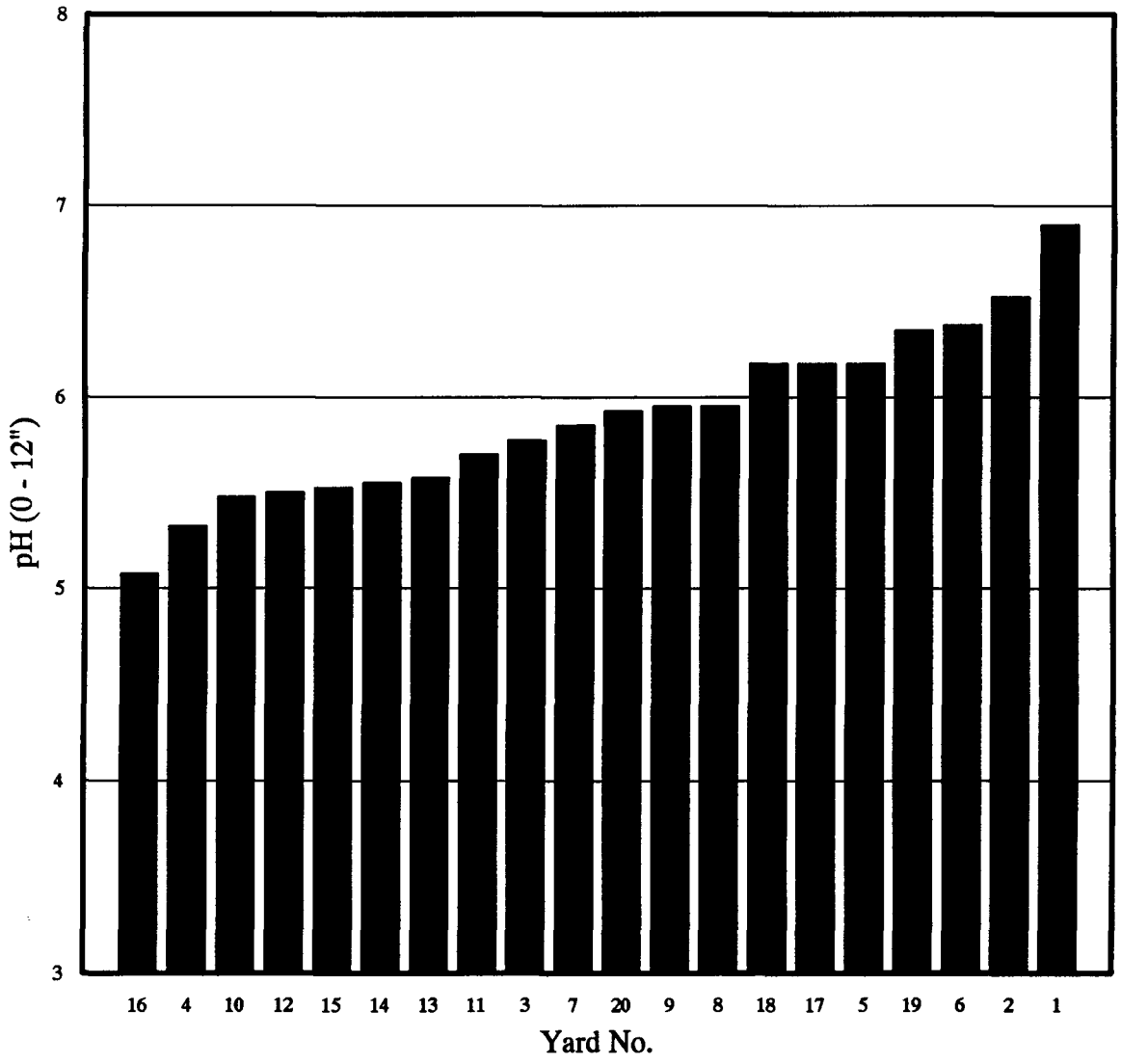

Figure 15. Soil pH in the surface soil for 20 hop yards.

Table 15. Soil pH in the hill and the surface soil as influenced by "Grower" and "Yard".

Grower	Yard	Hill	0 - 12" 0 - 30 cm
		---------- pH ----------	
1	good	6.78 a	6.35 bc[1]
	not-so-good	6.72 ab	6.38 bc
2	good	5.72 ef	5.95 de
	not-so-good	5.15 h	5.53 gh
3	good	6.38 abc	5.95 de
	not-so-good	6.72 ab	6.18 cd
4	good	5.82 def	5.78 efg
	not-so-good	6.07 cde	5.93 de
5	good	6.03 cde	5.55 fgh
	not-so-good	5.28 gh	5.32 hi
6	good	5.70 efg	5.85 ef
	not-so-good	5.47 fgh	5.50 gh
7	good	5.68 efg	5.47 gh
	not-so-good	5.53 fgh	5.70 efg
8	good	5.47 fgh	5.57 fgh
	not-so-good	5.13 h	5.07 i
9	good	6.30 bc	6.53 b
	not-so-good	6.20 cd	6.18 cd
10	good	6.68 ab	6.90 a
	not-so-good	5.82 def	6.18 cd
LSD @ 0.05		0.442	0.319

[1] values followed by the same letter in one column are not significantly different at p = 0.05

Whether these data suggest that "good" yards exhibit higher pH levels, or conversely, that "not-so-good" yards are associated with lower pH levels cannot be said with certainty. However, these results warrant further research into the effect of pH on yield or plant vigor.

Calcium and Magnesium

In controlling soil pH, the usual practice has been to apply 2 tons/A (2,240 kg/ha) of lime every four years. However, as lime represents an expenditure that produces no immediate returns, some growers in this survey have not limed since 1984 (grower # 4) or even since 1980 (grower # 2) (Table 16)

Concentrations of calcium in soil samples from the hill ranged from 6.03 to 22.85 meq/100g for individual yards, with most yards exhibiting values between 9 and 13 meq/100g. Concentrations of calcium for the surface soil (0 - 12", 0 - 30 cm) were similar, but showed an even wider range of 5.43 to 23.30 meq/100g.

Magnesium content in the hill and in the surface soil (0 - 12") was approximately one third to one fourth that of the calcium concentration. Concentrations for most yards ranged between 2 and 4meq/100g, although concentrations as high as 5.0 meq/100g ("not-so-good" yard from grower # 7 in the surface soil) and as low as 0.86 meq/100g ("good" yard for grower # 5 in the hill) have been observed.

Table 16. The year of the last application of lime and amount applied for selected yards.

Grower	Yard	Lime	Year applied
		tons/A	
1	good	3	1988
	not-so-good	3	1988
2	good	-	1980
	not-so-good	-	1980
3	good	1.5	1987
	not-so-good	1.5	1987
4	good	-	1984
	not-so-good	-	1984
5	good	2	1988
	not-so-good	2	1987
6	good	-	-
	not-so-good	2	1987
7	good	-	-
	not-so-good	-	-
8	good	-	-
	not-so-good	-	-
9	good	2	-
	not-so-good	2	-
10	good	2	1989
	not-so-good	-	-

Statistical Analysis

In analyzing the calcium concentrations in the hill, there was a significant interaction ($p = 0.001$) between the main effects of "Grower" and "Yard" (Table 17). Significant differences (LSD @ 0.05 = 3.06 meq/100g) among yards of growers # 1, # 2, # 7, and # 10 were found, and in all cases, except for grower # 7, the "good" yard had significantly higher Ca concentrations.

Concentrations of calcium in the surface soil (0 - 12", o - 30 cm) were also significantly different among yards within farms. In this case a mean separation (LSD @ 0.05 = 3.23) resulted in only two significant differences (growers # 7 and # 10). In both cases these differences were due to very high concentrations in the "good" yard (grower # 10) and in the "not-so-good" yard (grower # 7).

Magnesium concentrations in the hill also differed among yards from growers ($p < 0.001$) (Table 18). Where means were significantly different (LSD @ 0.05 = 0.68), the "not-so-good" yard had consistently higher values. This was also true for the surface soil where significant differences ($p < 0.001$) were higher (LSD @ 0.05 = 0.75) in the "not-so-good" yards for growers # 2, # 7, and # 9.

Table 17. Calcium concentrations in the hill and the surface soil as influenced by "Grower" and "Yard".

Grower	Yard	hill	0 - 12" 0 - 30 cm
		-------- Ca (meq/100 g) --------	
1	good	14.88 bc	10.95 def[1]
	not-so-good	11.75 def	9.90 egh
2	good	14.38 bcd	14.60 bc
	not-so-good	10.38 efg	12.60 cde
3	good	10.13 fg	8.90 fg
	not-so-good	10.10 fg	8.63 fgh
4	good	10.18 fg	10.85 def
	not-so-good	9.50 fg	9.05 fg
5	good	8.75 fgh	6.80 gh
	not-so-good	6.03 h	5.43 h
6	good	7.93 gh	8.90 fg
	not-so-good	8.68 gh	8.38 fgh
7	good	13.27 cde	13.30 cd
	not-so-good	16.90 b	17.42 b
8	good	11.80 def	12.57 cde
	not-so-good	9.75 fg	10.82 def
9	good	13.25 cde	12.88 cde
	not-so-good	15.27 bc	14.38 bc
10	good	22.85 a	23.30 a
	not-so-good	13.82 cd	14.63 bc
LSD @ 0.05		3.06	3.23

[1] values followed by the same letter in one column are not significantly different at p = 0.05

Table 18. Magnesium concentrations in the hill and the surface soil as influenced by "Grower" and "Yard".

Grower	Yard	Hill	0 - 12" 0 - 30 cm
		------- Mg (meq/100 g) -------	
1	good	1.70 efg	1.77 fg[1]
	not-so-good	1.14 ghi	1.45 fgh
2	good	3.35 d	3.58 de
	not-so-good	4.22 abc	4.75 abc
3	good	0.80 i	1.35 fgh
	not-so-good	1.33 ghi	1.04 gh
4	good	2.38 e	1.85 f
	not-so-good	2.05 ef	1.77 fg
5	good	0.86 i	0.87 h
	not-so-good	1.02 hi	1.12 fgh
6	good	1.27 ghi	1.35 fgh
	not-so-good	1.58 fgh	1.83 f
7	good	3.30 d	3.47 de
	not-so-good	4.65 ab	5.00 ab
8	good	4.30 abc	4.53 bc
	not-so-good	4.13 bc	4.10 cd
9	good	3.38 d	3.25 e
	not-so-good	4.90 a	5.30 a
10	good	3.75 cd	3.60 de
	not-so-good	3.35 d	3.30 e
LSD @ 0.05		0.68	0.75

[1] values followed by the same letter in one column are not significantly different at p = 0.05

Heterogeneity of Soil Nutrients within Plots

In the cultivation of hops in the United States, the formation of a hill around the base of a set of vines is a common practice. Probably the most important function of such a hill is to promote rhizome growth, i.e. develop roots that can be used to re-plant missing hills or to establish new fields. For this reason, care is taken not to disturb the area around the base of a set of vines, which measures approximately 4 sq feet (0.36 m^2).

The hill is formed by pushing soil between rows onto the base of vines. This task is performed with either of two machines: A regular disc cultivator from which both outer discs were removed and replaced by enlarged disks. When dragged through the rows, these outer disc will push soil from the row onto the base of vines. A triangular blade which will scrape off soil from the entire row onto the base. This machine will produce a larger and steeper hill.

In terms of soil fertility, there may be great differences among soil in the hill and soil between hills. Since this practice occurs after fertilizers have been broadcast, a blade will deposit significantly more recently fertilized soil onto the hill. Consequently, all of the surface soil between the rows will lack some of the recently fertilized soil and, thus, may exaggerate the difference.

To analyze the "hilling effect" statistically, nutrient concentrations for individual plot observations from the

hill were correlated with the corresponding surface soil between the rows (0 to 12"). In the case of nitrate-nitrogen, a significant correlation was obtained ($p = 0.005$), however, the R^2 was only 0.14. For ammonium-N the same correlation was not significant. Hence, biological and/or physical processes have established marked differences in the nitrogen availability between the untilled (hill) and the tilled soil (between hills). This implies the need for soil nitrogen testing to differentiate between the hill and the soil between the rows.

A significant ($p < 0.0001$) regression of the tilled soil (0 - 12") on the hill was obtained with potassium. In this case, an R^2 of 0.69 indicated a good linear relationship (Figure 16). The regression coefficient for this equation was 0.61, which suggests that the hill contained up to 40% more potassium. Therefore, it is also essential for potassium soil tests to distinguish between the hill and the surface soil. Fertilizer recommendations based on samples of extractable K from the tilled soil may be too high, if most of the uptake occurs in the hill. (Circumstantial evidence for such an uptake will be presented later.)

Phosphorus content in the hill correlated significantly ($p = 0.0001$) with concentrations found in the tilled soil (0 - 12", 0 - 30). A regression, using the hill as the independent variable produced an R^2 of 0.54, indicating that a straight line relationship would only explain half of the

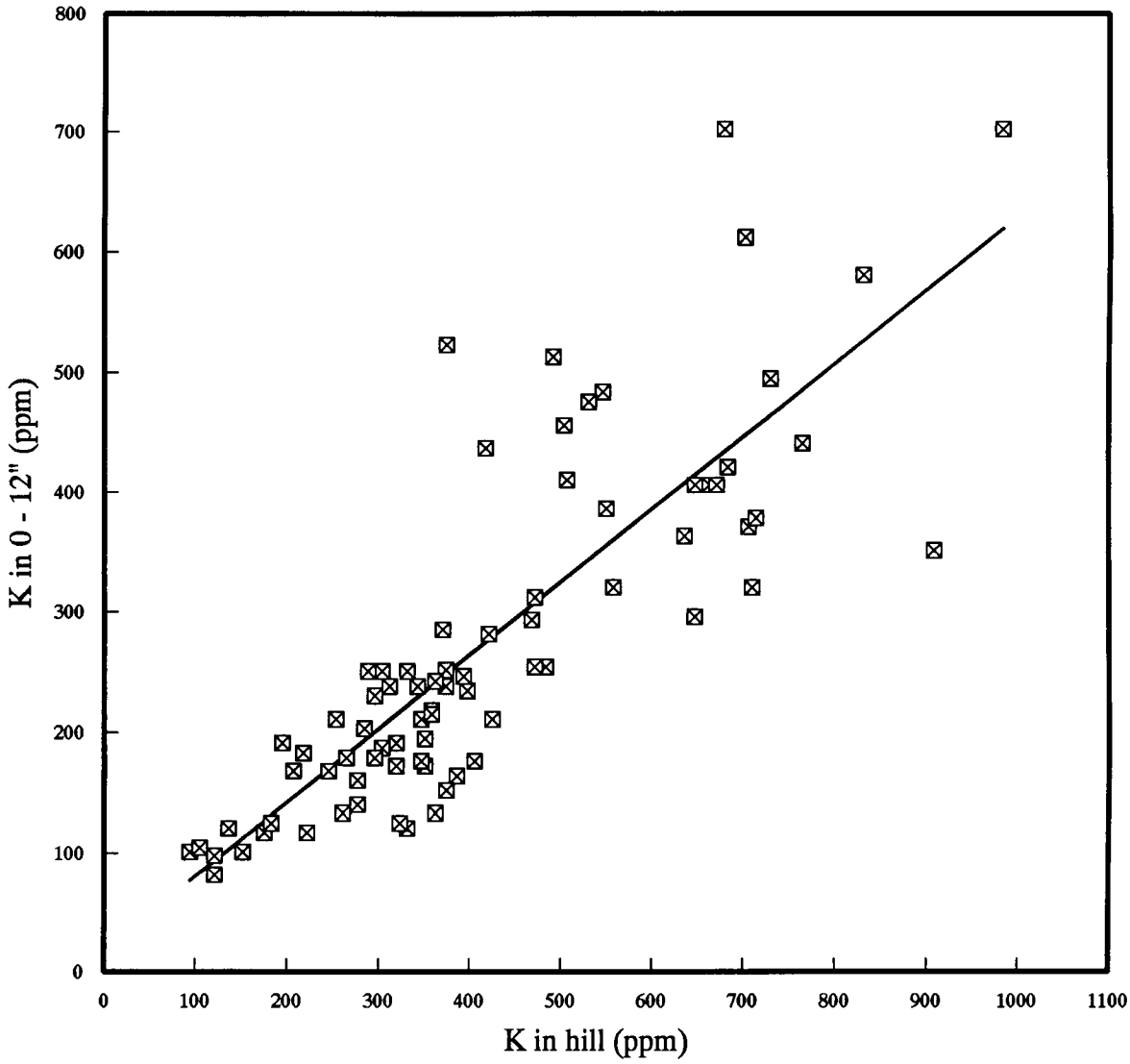

Figure 16. Correlation of potassium concentrations extracted from the hill with that extracted from the tilled surface soil between rows.

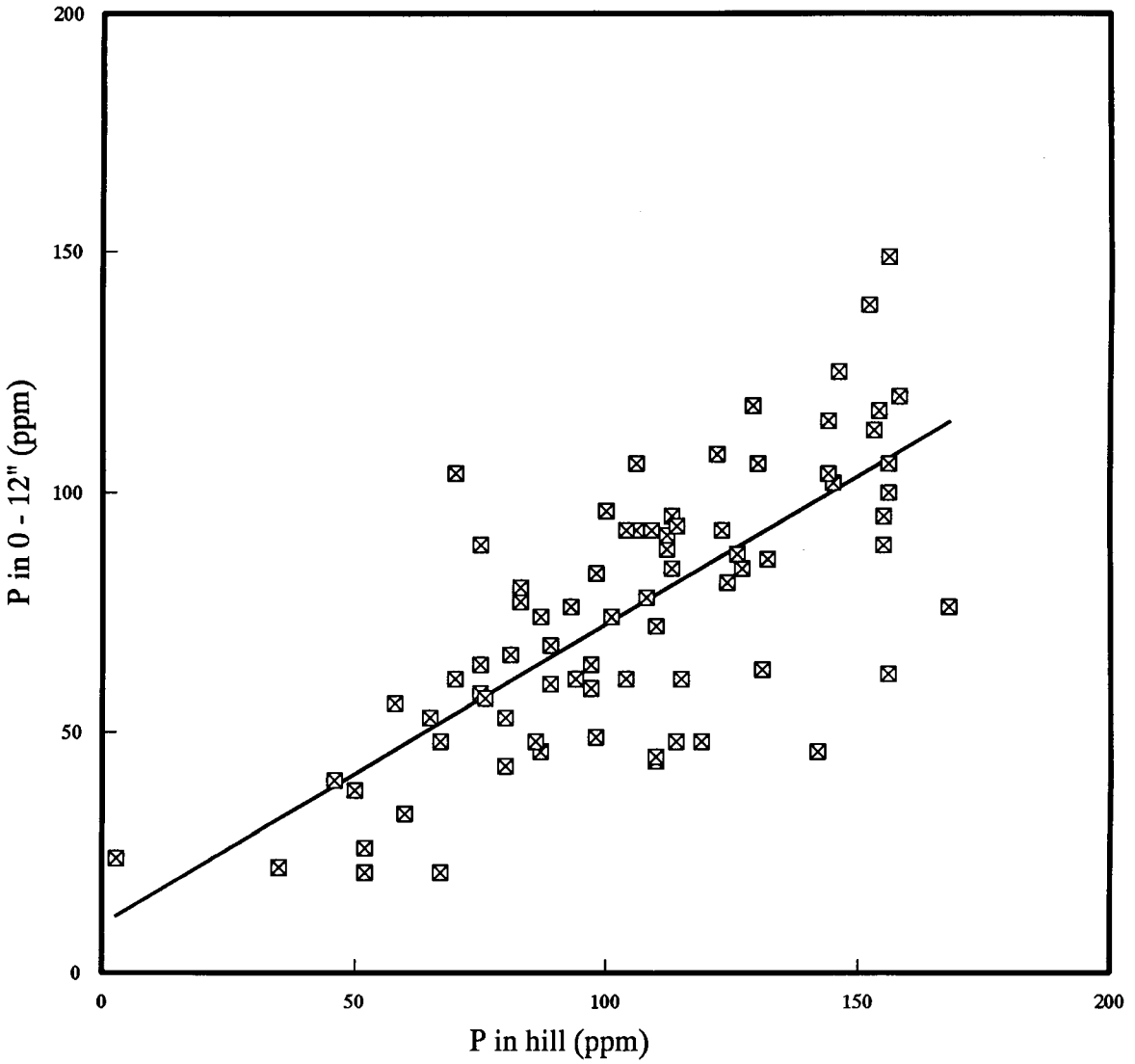

Figure 17. Correlation of phosphorus concentrations extracted from the hill with that extracted from the tilled surface soil between rows.

observed variation. However, if the phosphorus content in these two soil volumes were linearly related, then the hill would contain approximately 40% more of this nutrient (Figure 17). Thus, as with potassium and nitrogen, the hill should be analyzed separately from the tilled soil.

In contrast to the macro-nutrients, both calcium and magnesium are not taken up by the plant in large quantities and are not annually applied. For this reason, concentrations in the hill and in the tilled soil are not expected to vary greatly. In fact, a regression of both cations produced an R^2 of 0.82 and 0.91 for Ca and Mg, respectively, providing evidence of a linear relationship. Furthermore, the regression coefficients were close to one (0.96 for Ca and 1.1 for Mg), indicating almost equal concentrations in both soil volumes (Figures 18, 19). Hence, unlike the macro-nutrients, soil testing for these basic cations can be made either from the hill or the tilled soil between the rows.

As pH is primarily a function of these basic cations, it may be expected that pH would not vary greatly among these soil volumes. However, a regression of the pH in the tilled soil on pH in the soil frm the hill produced an R^2 of only 0.59 (Figure 20). Although this suggests a linear relationship, some unexplained variation remains. This unexplained variation may be due, at least in part, to the soil ammonium-nitrogen content. It has previously been pointed out that a significant ($p = 0.0006$) negative

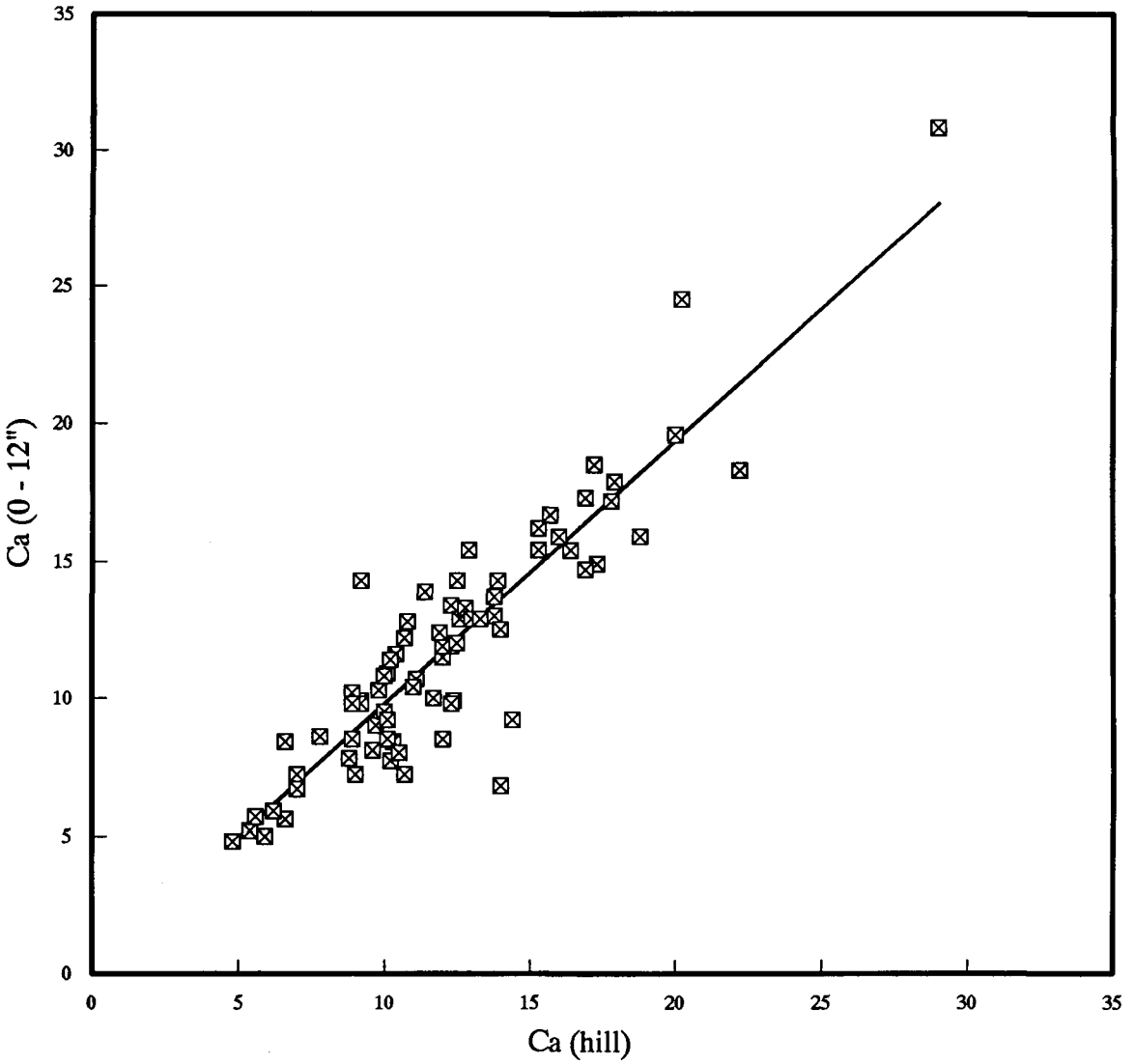

Figure 18. Correlation of calcium concentrations extracted from the hill with that extracted from the tilled surface soil between rows.

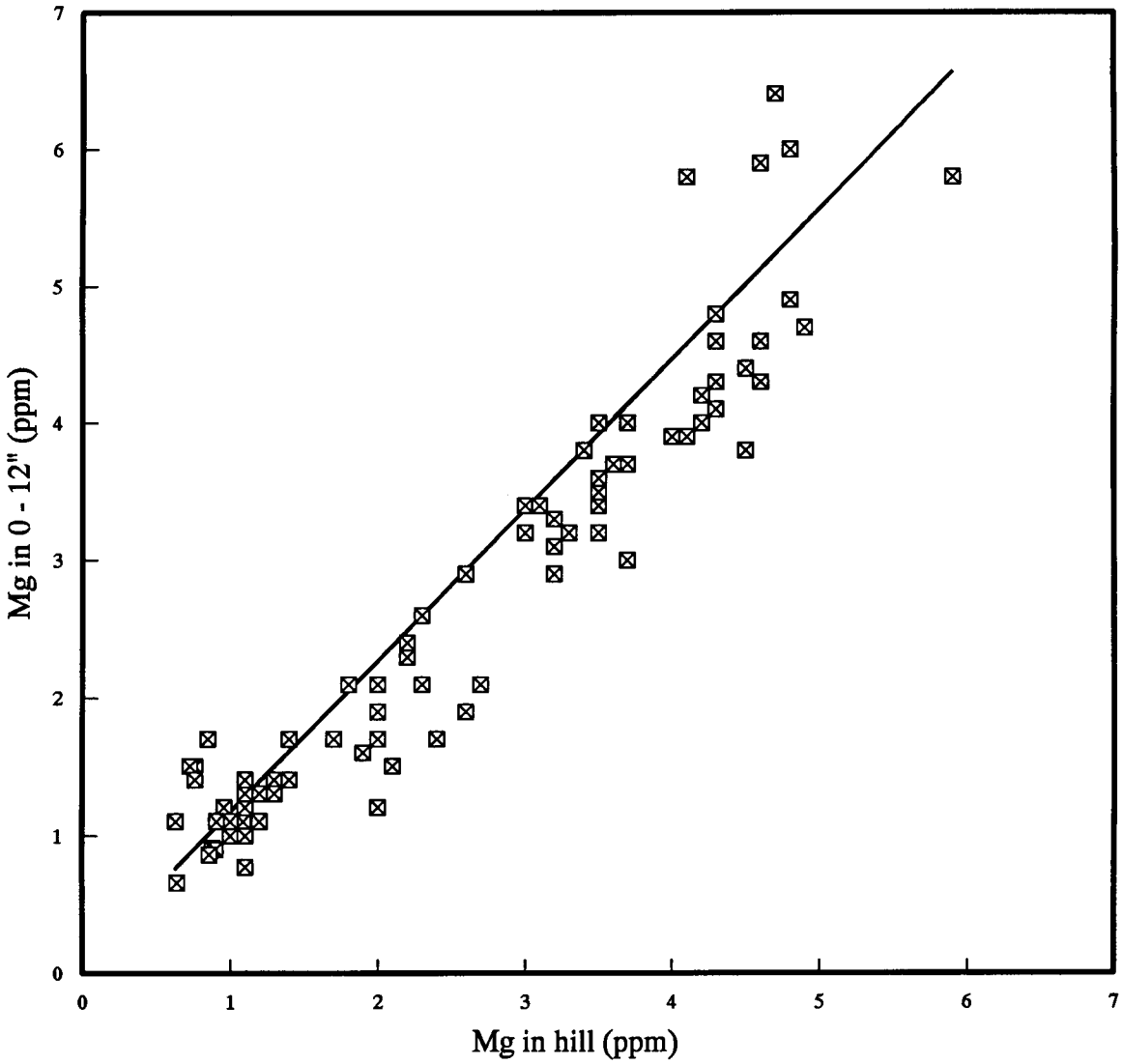

Figure 19. Correlation of magnesium concentrations extracted from the hill with that extracted from the tilled surface soil between rows.

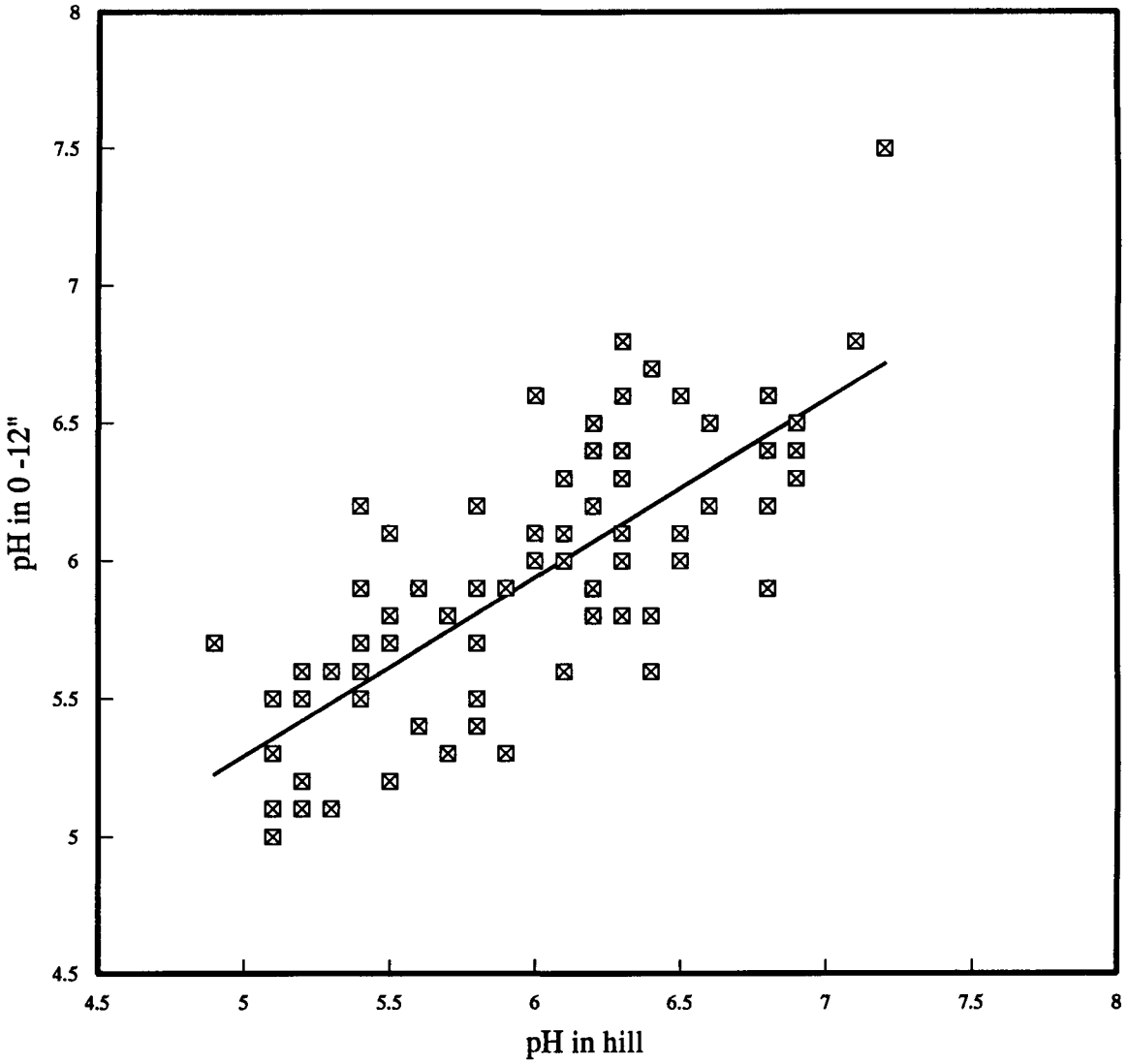

Figure 20. Correlation of pH in the hill with the tilled surface soil between rows.

correlation between the hill pH and the hill ammonium-N concentration existed. From this, it was speculated that the concentration of ammonium may have depressed pH, or that due to the pH, nitrification was reduced. In either case, an interdependency between these two variables could be established. For the 0 to 12" (0 - 30 cm) soil level, such an interdependency may have been weaker, due to the lower ammonium concentration there. Thus, ammonium may have influenced the pH in the hill differently than in the tilled soil (0 - 12", 0 - 30 cm), which may be one of the factors responsible for the unexplained variation observed. Therefore, it may be advisable to sample the hill and the surface soil separately for pH.

Tissue Nutrients

Nitrate-Nitrogen

Concentrations of petiole nitrates varied greatly among fields, from 0.16 to 1.13% NO_3-N (Figure 21). Since the amount of nitrate in other plants decreases exponentially with time, it may be assumed that this large range may be the result of the 32 day sampling period. In fact, a regression of petiole nitrate concentrations on the "date of sampling" is significant ($p = 0.0001$) and suggests a decrease of 0.14% NO_3-N per week (Figure 22). However, it must be emphasized that samples were collected only once during the growing season.

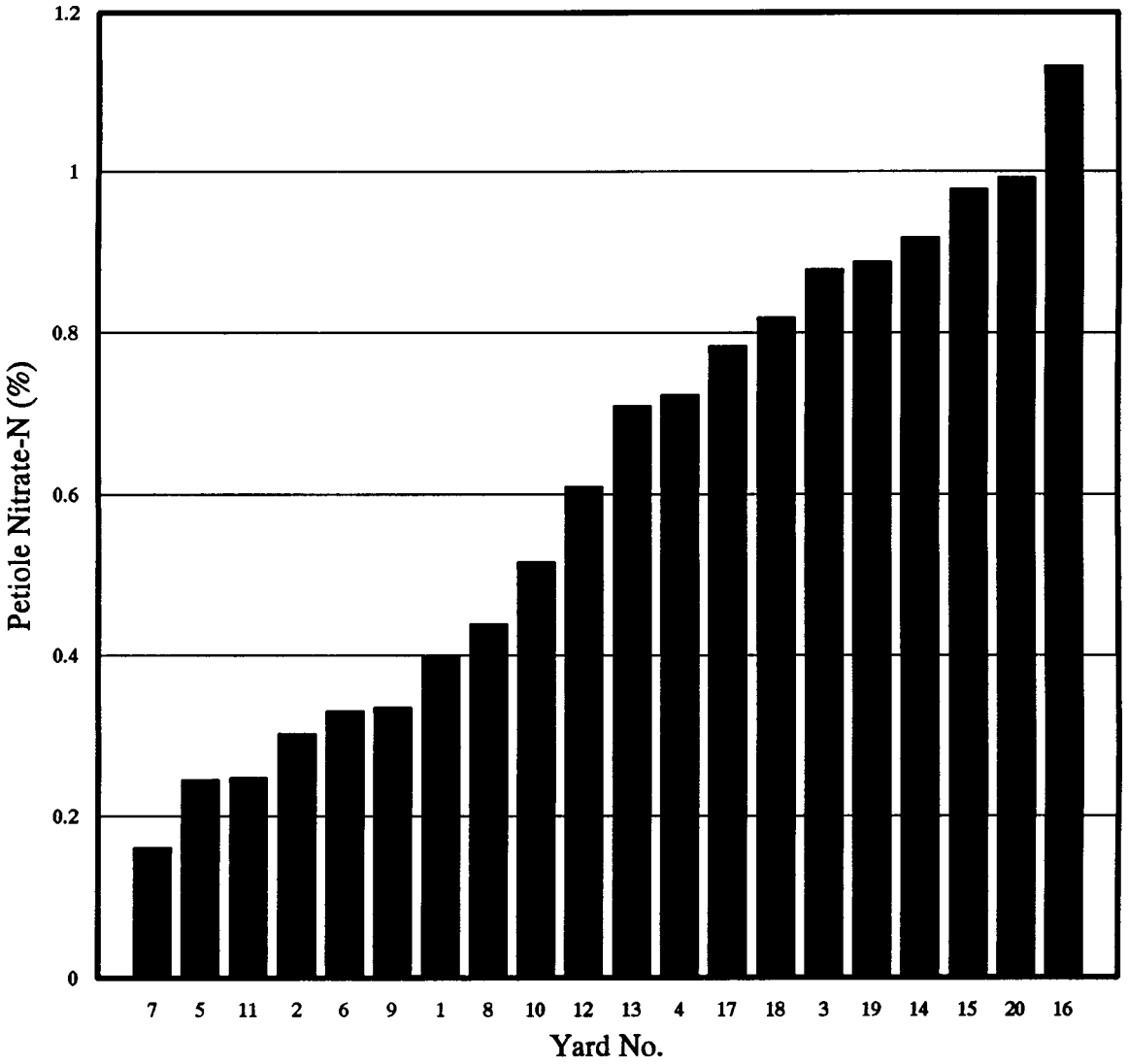

Figure 21. Petiole nitrate-nitrogen concentrations for 20 hop yards.

Without a second or third sampling of hop tissue to establish a decay curve, the large variation in nitrate concentrations may also be interpreted in another way. It is also possible that the observed large range of petiole nitrate values are representative of individual yards. If this were true, then the petiole nitrate content would be a reflection of management or environmental influences, or both.

In further analysis of this range of values, it should be noted that only three yards (15, 20, and 16) fall within the critical nutrient range established by Roberts et al. (1985). Although the term "critical nutrient range" implies that lower concentrations signal a nutrient deficiency, no symptoms of deficiencies were observed by the researcher or were reported by growers. Several important differences make the values established by Roberts et al. (1985) inapplicable to this data: 1) The two researchers based their findings on 'Bullion', 'E-2', 'L-8' and 'Comet', whereas this survey examined only 'Willamette'. 2) While petioles in their study were collected during the first week in May, after vines reached the wire, petioles in the present study were sampled in late July to early August. It is probably this difference that accounts for the greatest discrepancy between the publsihed critical ranges and the concentrations measured in this study.

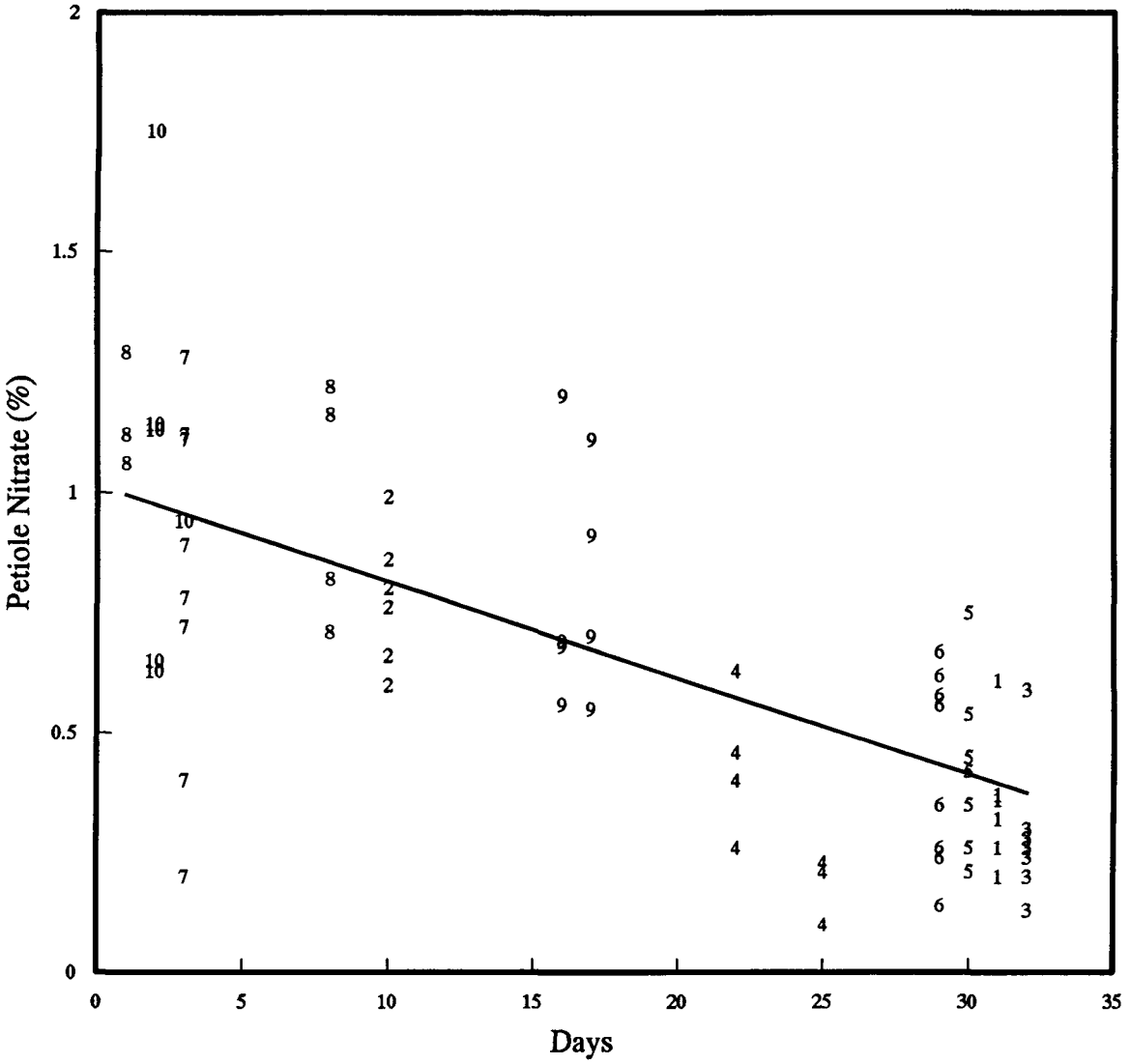

Figure 22. Petiole nitrate-nitrogen concentrations for plots according to time of sampling. Values of data points indicate grower number.

Statistical Analysis

In testing the data for associations with the main effects or their interactions, an analysis of variance of NO_3-N in the petioles detected significant differences ($p < 0.001$) among growers (Table 19). It is not surprising that individual yards were not significantly different, since, in most cases, both yards from one grower were sampled within a 24 hour period and had received similar management. Conversely, time may have been responsible for accentuating the differences among growers. Significant differences were most pronounced when several days had passed between sampling.

Of greater importance for this study, however, is the fact that "plot quality" proved to be a significant main effect ($p = 0.039$). Averaged across growers and yards the "good" plots contained 0.672% NO_3-N in their petioles, which were 0.105% more than in the "not-so-good" plots (0.567%). Therefore, vines in the "good" plots may have taken up more nitrogen, which may have resulted in an increased vegetative development. If so, plants in the "good" plots produced more foliage of a darker green color. Since plots were chosen, in part, for their foliage and color, it may be that the decision to categorize individual plots into "good" and "not-so-good" was influenced by a nitrogen fertility effect.

Petiole nitrate-N concentrations were also significantly different among infected (at least one vine colonized by Verticillium) and non-infected plots. The mean

Table 19. Tissue concentrations in the petiole and the leaf as influenced by "Grower".

Grower	NO_3	K	K	P
	petiole	petiole	leaf	leaf
	%	%	%	%
1	0.35 cde[1]	4.88 de	1.42 bcde	0.32 a
2	0.80 b	5.36 cd	1.35 cde	0.25 c
3	0.29 e	5.22 cd	1.24 def	0.28 bc
4	0.30 de	4.16 e	1.22 ef	0.28 bc
5	0.43 cd	5.54 bcd	1.87 a	0.27 c
6	0.43 c	1.88 f	1.04 f	0.28 bc
7	0.81 b	6.08 abc	1.71 ab	0.28 bc
8	1.05 a	6.69 a	1.59 abc	0.31 ab
9	0.80 b	5.32 cd	1.30 cdef	0.26 c
10	0.94 a	6.39 ab	1.53 bcd	0.26 c
LSD @ %	0.12	0.93	0.30	0.032

[1] numbers followed by the same letter in one column are not significantly different at p = 0.05

of the infected plots was higher with 0.73% compared to the non-infected plots that contained only 0.56% nitrate-N in their petioles. Due to the observational nature of this study, it cannot be said whether the higher nitrogen content in diseased plots is the result of fungal attack or whether it predisposed vines to become more susceptible. However, increased nitrogen in wilt affected plants may implicate the importance of this nutrient in the disease complex.

Potassium

Potassium concentrations in the petioles were, generally, three to four times greater than in the leaves (Figure 23). While the petiole potassium exhibited a large range (from 1.26 to 6.84%) the leaf K varied only from 1.04 to 1.97%. The lowest petiole K concentrations were observed in fields 11 and 12, both belonging to grower # 6. Although the leaf values for these two yards were the lowest, they were not disproportionately low compared to leaf K concentrations from other yards. In comparing the leaf K concentrations from yards 12 and 11 with those from yards 16 and 20, which exhibited the highest petiole potassium, the difference was only 0.65%. On the other hand, when the same yards are compared on the basis of their petiole K concentrations, the difference was in excess of 3%. It, therefore, appears that the measurement of petiole potassium may be a more sensitive indicator of the potassium status in hops than leaf potassium.

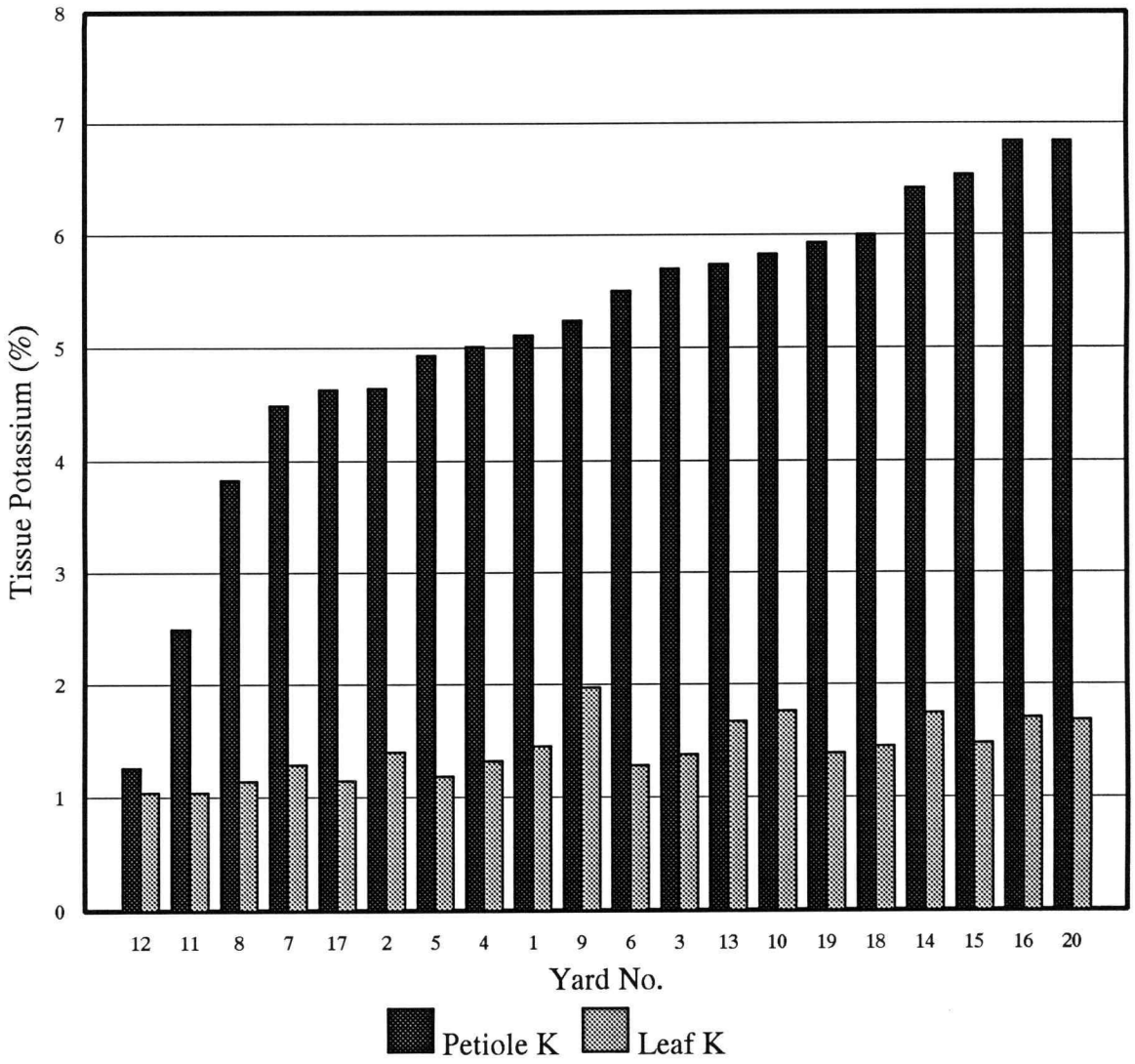

Figure 23. Concentrations of potassium in the petioles and the leaves for 20 hop yards.

Similar to petiole nitrate, the content of potassium in the petiole may also decrease with time. Disregarding the last three sampling dates, a definite decrease in petiole K concentrations occurred. Yet, surprisingly, the last six yards from growers # 5, 1, and 3 exhibited concentrations that were just as high as yards sampled at the beginning of the study. This fact reemphasizes the need for caution in imposing any decay curve on petiole concentrations based on only one date sampling for both potassium and nitrate.

Statistical Analysis

In analyzing the influence of the main effects or their interactions on petiole K, significant differences ($p < 0.001$ were found for the "Grower" effect (Table 19). Clearly, the grower with the lowest concentrations was grower # 6 with only mean of 1.88% K for all plots. Petiole concentrations were significantly lower by 2.28% K (LSD @ 0.05 = 0.93%) than the next lowest values from grower # 4. That this was probably not due to a time effect can be seen by comparing grower # 5 with growers #'s 10, 7, 2, and 9. Although sampled up to 28 days apart, no significant differences were observed among these growers.

An analysis of variance also found the "Grower" effect on leaf potassium to be significant ($p = 0.040$). As previously discussed, the leaf potassium concentrations from grower # 6 (1.04%) were the lowest, however, they were not significantly different (LSD @ 0.05 = 0.30%) from

concentrations observed in growers #'s 3, 4, and 9. Hence, significant differences in petiole K concentrations did not, for the most part, translate into significant differences for leaf K. This may strengthen the aforementioned hypothesis that leaf potassium concentrations may be insensitive to the overall potassium budget in the plant.

Perhaps more important than the differences among growers is the fact that "Plot Quality" was significant for the petiole potassium concentrations ($p = 0.011$). While the "good" plots contained an average of 5.49% K, the "not-so-good" plots contained only 4.81% K, or 0.68% less. This may be yet another indication that the selection of the "good" versus the "not-so-good" plots was influenced by the nutrient status of plants.

Phosphorus

Concentrations of total phosphorus in the leaves ranged from 0.25 to 0.32% (Figure 24). A comparison with recommended leaf phosphorus values (Roberts et al., 1985) indicated that these concentrations were well within the critical nutrient range of 0.18 to 0.25%. Unlike petiole nitrate-N, the recommended concentrations for leaf phosphorus are probably very much applicable to this survey. Sampling by Roberts et al. occurred in mid-July at the early bloom stage, while samples for the present survey were collected during and after bloom. Concentrations of petiole phosphates ranged from 0.06 to 0.12% (Figure 24). Extreme

variations among yards were not obsereved in either leaf phosphorus or petiole phosphate concentrations.

Statistical Analysis

An analysis of variance of the petiole P content resulted in a significant "Grower" x "Yard" interaction ($p = 0.014$) (Table 20). Means were separated to produce only two significant differences (LSD @ 0.05 = 0.026%) among yards for grower #'s 1 and 3. In both cases, the "not-so-good" yard contained the higher petiole concentrations. An explanation for these differences could not be found.

The same analysis of leaf P produced significant differences among "Growers" ($p = 0.040$). This outcome is representative of most tissue variables, which may reflect either the growers particular management or the contribution of the soil on his farm. However, it may also have been a reflection of the differences in sampling dates.

An analysis of variance further revealed a "Plot Quality" effect ($p = 0.016$). The concentrations of leaf P in the "good" plots averaged 0.27% and were 0.02% lower than the "not-so-good" plots with 0.29%. Although it may at first appear to be contradictory that the weaker looking plots contained the higher concentration of P in their leaves, it must be remembered that non-vigorously growing plants do not produce as much biomass as rapidly developing vines. Hence, the higher P content in the "not-so-good"

Table 20. Petiole phosphorus concentrations as influenced by "Grower" and "Yard".

Grower	Yard	Petiole P
		%
1	good	0.06 ef[1]
	not-so-good	0.10 ab
2	good	0.09 bcde
	not-so-good	0.07 cdef
3	good	0.08 bcdef
	not-so-good	0.12 a
4	good	0.05 f
	not-so-good	0.08 bcdef
5	good	0.06 ef
	not-so-good	0.07 cdef
6	good	0.08 bcdef
	not-so-good	0.05 f
7	good	0.09 abcd
	not-so-good	0.09 abcd
8	good	0.08 bcdef
	not-so-good	0.10 bc
9	good	0.07 def
	not-so-good	0.07 def
10	good	0.08 bcdef
	not-so-good	0.09 abcd
LSD @ 0.05		0.026

[1] values followed by the same letter are not signifciantly different at p = 0.05

plots may have been the result of a slight concentration effect.

Zinc

The analysis of zinc in the leaf tissue was carried out only to determine whether any zinc deficiencies had occurred during the 1989 season. All leaf tissue contained at least 13 ppm more Zn than the recommended 12 to 20 ppm concentration (Roberts et al., 1985). In addition, no reports were received from participating growers that they had observed a deficiency.

Soil and Tissue Nutrient Relationships

A visual appraisal of scatterplots relating concentrations of nutrients in the soil with those in the tissue resulted in only one satisfactory association: hill potassium concentrations with petiole potassium (Figure 25). The exponentially shaped curve suggests that soil concentrations exeeding 300 to 350 ppm do not result in higher petiole potassium values. Conversely, soil potassium levels below 300 ppm may be less than adequate for optimum plant growth. It is interesting to note that most plots containing concentrations less than 250 ppm belonged to grower # 6.

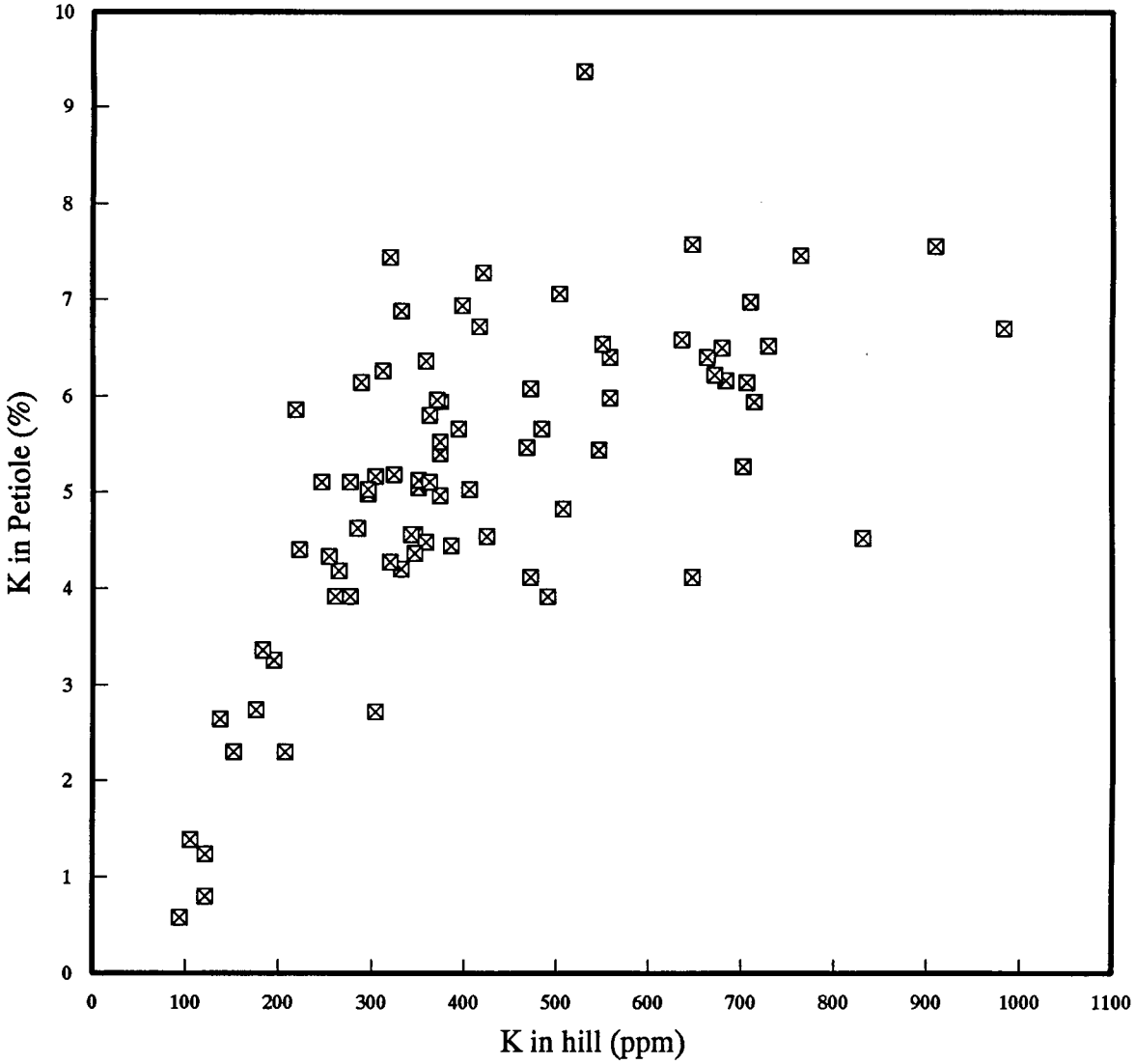

Figure 24. Realtionship of the soil potassium content with petiole potassium concentrations.

Responses to the Questionnaire

In the questionnaire, participating growers reflected the industry's general opinion that 'Willamette' produces lower yield with time. Strong yield declines were reported for four yards that were planted 6 and 8 years ago, while all four younger yards (1 to 3 years of age) appeared to yield normally. Also, according to the questionnaire, this decline in yield seems to commence after the fourth or fifth year, as was indicated by moderate reductions observed in 5 yards. However, it must be emphasized that of the 9 yards that exhibited no apparent yield declines, 5 were six or eight years old.

In contrast to yield declines, reduced vigor does not seem to follow a time trend. Growers indicated that half of the yards, which included all ages in roughly equal proportions, experienced moderately reduced vigor. Furthermore, grower observations showed that decreased vigor does not necessarily translate into lower yields, as is seen in some fields that had a lowered vigor while still maintaining normal production.

Growers had a surprisingly good perception of Verticillium wilt. Sixty percent of them believed that they were able to identify the disease and, of these, 40% indicated prior incidences of Verticillium wilt in at least one of their yards. Growers # 5 and # 6 were certain that their 'Willamette' yards were not infected and had the lowest percentage of *Verticillium* isolated from their yards.

On the other extreme, the only grower who indicated a strong incidence of Verticillium wilt in one yard (# 16) had the only case of *V. albo-atrum*.

The majority of the growers planted 'Willamette' in soil that had not previously grown hops. In fact, grass seed, wheat, and mint comprised 50% of the yards prior crops. However, three growers did establish 'Willamette' in fields that were 20 and even 45 years in continuous hops.

The management of the soil differed both among growers and among fields of the same grower. In 11 cases, waste (chopped, harvested vines) was returned to the field. It is not clear, however, whether the original vines came from the same field. Differences were also noted in the use of cover crops. Thirteen yards were planted with predominantly barley, while the other seven yards were left bare. Philosophies also differed on crowning: 13 fields were crowned only lightly, two moderately, and three heavily.

SUMMARY AND CONCLUSION

It is the long term goal of this study to investigate the cause, the frequency and the severity of Verticillium wilt in hops, and to identify factors that promote plant infection, or enhance symptom severity. Knowledge of such factors can help farmers to employ strategies that either reduce the wilt severity or control the disease.

Cause of Verticillium Wilt

In 1989 Verticillium wilt was caused primarily by *Verticillium dahliae*, and in one case by *V. albo-atrum*. Although the *V. albo-atrum* occurred in only one yard, it should, nevertheless, be taken seriously. First, since only 20 yards were included in this survey, it may be possible that other *V. albo-atrum* infected yards exist. If this is the case, then this fungus may be inadvertently spread to other yards located on the same farm or even transported to other growers in the form of infected rhizomes (root stock). Second, with the constant introduction of new varieties, especially the German aroma types, *V. albo-atrum* may be able to find a more suitable host. Temperatures permitting, more susceptible hosts could facilitate the establishment of V. albo-atrum in yards and allow for an increased rate of infection. This could, in turn, raise the possibility of mutations which may result in more virulent strains. For

this reason, the presence of *V. albo-atrum* requires close monitoring.

Frequency of Plant Infection

Results from the study suggest that Verticillium wilt in hops can be found in almost every hop growing district in the valley. Although striking, this finding is not surprising when management practices and cropping histories of yards are considered. Since the primary cause of Verticillium wilt in the Oregon 'Willamette' is *V. dahliae*, infection occurs via its soil borne resting structure, the microsclerotium. This resting structure can remain viable for up to 10 years or more in the absence of a host. Thus, once the ground becomes contaminated with *V. dahliae* it is likely to stay that way.

Contamination of hop yards can occur in two ways. First, hop yards may be established on soil that had previously grown an infected mint, strawberry, or potato crop. Horner (1967; 1968) suggested that hops are susceptible to *V. dahliae* strains specialized on all of these crops. Second, chopped vine waste from an infected yard may be distributed to a non-infected field, and hence, the inoculum may be spread throughout the farm.

As it is unlikely that the return of vine waste will be abandoned or that these chopped vines will be composted before they are deposited on fields, increases in wilt incidence in susceptible varieties are foreseeable.

Furthermore, with the annual fluctuation of the hop acreage, there is an increasing probability that new hop yards will be established on land infested with *V. dahliae*.

Severity of Verticillium Wilt

A rating of Verticillium wilt symptoms within individual plots has not been performed. However, when vascular necrosis was observed during the preliminary stem sampling, symptoms were noted (see Results and Discussion).

In comparison with observations made by farmers in 1988, Verticillium wilt symptoms in 1989 were not severe. While some farmers during the 1988 season reported vines that exhibited strong wilting of leaves, premature ripening of cones and actual dropping of cones, this study found only vines with mild to moderately wilted leaves (when infected with *V. dahliae*). Severely wilted vines and dried cones were observed only in the yard infected with *V. albo-atrum*.

There are several reasons that may explain the discrepancy between the severity of symptoms reported in 1988 and those observed in 1989. 1) Yellow or necrotic leaves may have been mistaken for wilt symptoms when in fact they were the result of pesticide sprays or of natural senescence which could have been accelerated under stress conditions. Especially along roadsides, automobile exhaust, dust, and heat may have induced unfavorable growing conditions which may have resulted in wilt-like symptoms. 2) Unlike the summer of 1988, temperatures during the 1989

season were moderate and direct sunlight was usually blocked by overcast skies. Hence, one may speculate that vines were not subjected to the same heat stress as in the previous year and that this could have resulted in a weaker symptom expression in 1989.

Based on observations taken in 1989, and on reports from growers and from formal documentation of Verticillium wilt in Oregon, it is concluded that the hop wilt, caused by *V. dahliae*, does not severely affect development of 'Willamette' or other equally susceptible varieties. However, in years when environmental conditions favor symptom expression, some yield losses due to the disease may be experienced. In these years, plants infected with *V. dahliae* may exhibit strongly wilted leaves and prematurely ripened or even completely dried cones. Thus, losses may be in the form of lower cone quality or reduced picking efficiency.

Yards infected with *V. albo-atrum*, on the other hand, may exhibit substantial yield losses. Grower # 8 reported a 2 to 2.5 bale/A reduction in yield when wilt symptoms emerged in the mid to late 1980's. Since then, both symptoms and yields appear to have remained unchanged.

Factors that Promote the Incidence of Wilt

To investigate wilt promoting factors, the study focused on plant pathogenic nematodes and on soil nutrients. Although other predisposing variables such as soil moisture

and soil temperature may exist (Rintelen, 1974), they cannot be easily managed by the grower.

Plant Pathogenic Nematodes

Of the nematodes recovered, *Heterodera humuli* occurred in the highest numbers both in the soil and the roots. Because of the root damage that takes place when the nematode enters young rootlets and, more importantly, when maturing females break through the epidermal layer of roots, *H. humuli* may act as "path making" organisms. Correlations between population levels of nematodes in the soil and in the root, however, were not significant. In addition, no improvement in significance was obtained when both nematode variables were regressed on "isolated Verticillium". The same was true when "stem necrosis" was used as the dependent variable.

These findings suggest that population levels of *Heterodera humuli* do not promote increased infection of *Verticillium*. Similar results had been obtained by von Mende (1985), who found little differences in the *Verticillium* incidence when wilt susceptible vines were inoculated with the fungus (*V. albo-atrum*) alone and with a combination of the fungus and the nematode.

It should be noted, however, that while intensive sampling of two plots in the yard infected with *V. albo-atrum* did not demonstrate a significant correlation between *Verticillium* and nematodes, the incidence of *Verticillium*

was greater in the plot with higher nematode counts. As *H. humuli* populations for the plot with the higher wilt incidence were extremely high, one may speculate that an increase in *Verticillium* infection does occur, once populations have reached a certain threshold level.

Due to the importance of *Pratylenchus* on the wilt complex in other crops, its influence on the *Verticillium* incidence in hops was also explored. However, results from correlations and regressions of both soil and root populations do not indicate any significant association with the occurrence of wilt at population levels present in the yards surveyed.

It is, thus, implied by the data that plant pathogenic nematodes alone are not associated, i.e. do not promote, infection of hops by *Verticillium* and therefore do not seem to act as "path-making" organisms. Yet, since samples for *Verticillium* and for nematodes were not necessarily collected from the same hills, and since the variability of nematode populations among plots and also among individual hills were so great, only very strong associations would have been detected statistically. Therefore, the foregoing conclusion does not exclude the existence of more subtle associations between nematodes and infection by *Verticillium*.

Soil Nutrients

In order to determine the influence of soil nutrients (including pH) on the incidence of wilt, each element occurring in the top soil (hill - 12", hill - 30 cm) was correlated with the variables "isolated Verticillium" and "stem necrosis". The most significant correlation ($p = 0.004$; $r = 0.319$) occurred with phosphorus at the 0 - 12" (0 - 30 cm) depth. Isolation of *Verticillium* and stem necrosis were also significantly correlated with phosphorus in the hill ($p = 0.046$; $r = 0.223$). These data suggest that the frequency of *Verticillium* isolation increased as more phosphorus became available to the plant.

Although the same relation was also observed in a correlation ($p < 0.001$; $r = 0.347$) of "stem necrosis" with phosphorus at a depth of 0 - 12" (0 - 30 cm), its value in helping to explain the incidence of Verticillium remains questionable. However, it is conceivable that hop roots respond positively to increased soil phosphorus concentration by developing a larger root system. If so, roots may ramify a larger soil volume which could increase the probability of coming into contact with microsclerotia. Still, a positive association of P on the frequency of infection has never been reported in the literature. Furthermore, if there existed a cause-effect relationship between this element and frequency of infection, then growers would still not be able to influence the incidence significantly. This is because of the soil's inherent

buffering capacity that strongly controls the availability of P in solution and hence does not allow the grower to manipulate phosphorus concentrations easily.

In expanding this association into a multiple regression model, the variable pH (0 - 12", 0 - 30 cm) was added. Although this model only explained 16% of the variation on a linear basis, pH was significant ($p = 0.026$). The regression coefficient of 0.003 indicates an increase in *Verticillium* incidence with an increase in pH. The same model was obtained using "stem necrosis" as the dependent variable. In this case, a slight improvement in R^2 (0.23) was observed and the significance of both P and pH were also enhanced. However, as with phosphorus, an association of the disease frequency with pH has not been reported on hops. Furthermore, as the availability of phosphorus increases with increased soil pH, these two variables may be strongly dependent on each other.

Conclusions

Results from the 1989 survey of Oregon 'Willamette' hop yards suggest that yards, in general, exhibit great variability in soil and tissue nutrient status and in nematode populations. In contrast, Verticillium wilt was isolated in 14 of 20 yards, although observations on stem necrosis suggest that some yards are more severely affected. This was especially noted in those yards which have had a history of the disease.

Variations within individual yards, as was determined by contrasting "good" with "not-so-good" looking plots, may have been due to a fertility effect. Concentrations of nitrate-N and potassium in the petioles were significantly higher in the "good" plots, while leaf phosphorus concentrations were significantly lower in the "not-so-good" plots. Populations of nematodes, especially populations of *Heterodera humili*, may have also been involved in contributing to the variability within yards, although confirmation of this effect lacks statistical evidence.

The study did not find any particular variable that was associated with "good" and "not-so-good" yards. (This classification of yards was made by the grower, and in most cases was based on previous yield). However, unconfirmed data suggest that a soil pH below 6 could be associated with "not-so-good" yards.

Although no concrete associations between plant pathogenic nematodes or between soil nutrients with either incidence or severity of wilt were determined, this study does not exclude their possible involvement in Verticillium wilt in hops.

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