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VX211, A Vigorous New Walnut Hybrid Clone with Nematode Tolerance and a Useful Resistance Mechanism

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Abstract: VX211 is a highly vigorous Paradox hybrid clone that outgrew other walnut seedlings in the presence of nematodes. A four-year macroplot trial involving Paradox VX211 and a standard Paradox selection, AX1, demonstrated that the damage threshold level of *Pratylenchus vulnus* on commercially available walnut rootstocks is < 1 nematode/250 cm^3 of soil. Using 1 as the initial population level (Pi) within an inoculation zone of 80 L of soil, the *P. vulnus* population level increased 2,500-fold in the first year of growth. Three years after inoculation soil population levels of *P. vulnus* on VX211 were significantly reduced compared to that of the moderately vigorous AX1. Growth of VX211 was 35% greater than that of AX1 regardless of the Pi. Examination of stained roots revealed that feeding and reproduction by *P. vulnus* on VX211 was primarily ectoparasitic. This is the first report on a new walnut rootstock that can be readily cloned, has high vigor, exhibits tolerance to low population levels of *P. vulnus,* reduces nematode feeding and reproduction within the root terminus, and is currently available to California growers.

Key words: asexual clones, host-parasite relationship, *Juglans*, lath house, macroplot, management, *Meloidogyne incognita*, *Pratylenchus vulnus*, resistance.

The English botanist Richard B. Hinds discovered a new species of walnut growing in the Sacramento Valley of California during his 1836 to 1842 global voyage. In honor of his discovery this new species of black walnut was named *Juglans hindsii*. A few decades later the more flavorful English-type walnuts, such as the Mission or Franquette cultivars of *J. regia* were introduced throughout northern California. Decades later it was noted that offspring of *J. hindsii* occasionally demonstrated high vigor but little or no fruit. In 1893, Luther Burbank gave the name Paradox to these puzzling trees which were the result of occasional hybridization of English walnut (*J. regia*) x California black walnut (*J. hindsii*) (Burbank, 1914). Paradox seedlings collected from *J. hindsii* trees were suggested as a source of seedling rootstocks that could invigorate the more desirable English scions. Throughout the 20th century the California walnut industry shifted from English walnut to Black walnut and then to Paradox hybrids as their favored walnut rootstocks.

In 1996 a team of scientists from the University of California and USDA-ARS set out to better understand the lineages, vigor differences, and pest resistance levels that resulted from open-pollinated Paradox seedlings (Potter et al., 2002). At that time there was only one clonal, own-rooted Paradox variety, a very vigorous natural hybrid from Modesto, CA, named 'Vlach' that was being tested by one commercial nursery.

From 1997-2002 our lab processed approximately 2,000 Paradox and 1,000 of maternal *J. hindsii* seedlings from numerous California nurseries. Using 10 seedlings from each source tree we conducted 2-year studies to determine the host status of each seedling to *Pratylenchus vulnus* and *Meloidogyne incognita*. Tissue culturing by Charles Leslie, Research Associate, and asexual propagation techniques by Wes Hackett, Horticulturist, both at University of California Davis, provided us with own-rooted clonal material of a number of putative resistant individuals from some of these seedling families adequate to conduct subsequent field and greenhouse studies.

This report summarizes our findings from Paradox clones of VX211. This rootstock hosted *P. vulnus* and *M. incognita* but appeared to have the potential to grow roots faster than nematodes could attack. We wanted to know if continued growth in the presence of nematode feeding was a result of extreme vigor expressed whether nematodes were present or not, or actual tolerance to nematode feeding.

Materials and Methods

Resistance Screening: During 1997 and 1998, 3,400 1-yr-old walnut trees were planted in non-fumigated soil at a 4-acre site located at the Kearney Agricultural Center, Parlier, CA. Half the trees originated from seeds collected during the open pollination period of spring 1996, the other half during the open pollination period of spring 1997. The collected seeds were sprouted, grown for a year in several commercial nurseries, then phenotypically separated into those that were not hybridized (maternal Black walnut) from those that were hybridized (Paradox hybrids). Seedlings from 34 tree sources throughout California were bundled into groups of ten. These 34 groupings were randomly assigned two letters to indicate tree source and three numbers to designate each individual seedling. Each seedling was planted 2-m by 3-m apart along with 1 L of soil containing 200 *P. vulnus* and 100 *M. incognita* as inoculum. Every 6 mon for 2 yr, 20 g of roots were collected by shovel from one side of each tree and then diced and placed in a mist chamber for 5 d to extract endoparasitic nematodes. Nematodes were identified and counted under a dissecting microscope at ×40

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magnification. Trees with exceptionally high nematode counts, > 180/g of root, were generally eliminated by the second year of the study. Trees of continued interest were dug with a portable Vermeer tree spade (Vermeer Corporation, Pella, IA) and transferred to a Mother Block to further quantify their vigor and provide propagative cuttings for cloning.

Clones from tissue-culture: In a macroplot experiment, we evaluated the performance of 48 clonally propagated VX211 trees as well as 48 clonally propagated AX1 trees against a series of *P. vulnus* population levels. AX1 is a Paradox usually considered of moderate vigor when evaluated in field settings, thus was used in these experiments as a standard Paradox comparison. Nematode population levels were assessed in July and November of each year for four years. When additional clones became available from our studies or from associates at University of California Davis we initiated trials in a lath house setting or new field trials to improve our understanding of the differential preferred feeding sites of *P. vulnus* (Buzo et al., 2005).

Macroplot experiment: Responses of VX211 clones to *P. vulnus* were evaluated from 2004-2007 in 48 concrete-lined, open-bottomed macroplots, each 49-m^2 in size with walls extending 1.7-m deep into soil, established at UC Kearney Agricultural Center, Parlier CA. The Hanford sandy loam soil within the macroplots (65% sand, 27% silt, 8% clay, and < 1% organic matter) had been maintained in a fallow condition for 2 yr. No plant parasitic nematodes were detected in these plots prior to planting. Soil was leveled and smoothed with shovels and rakes. A micro-sprinkler irrigation system was installed and soil surface settled with 20-mm of water over a 12 h period after planting. Each tree was fertilized with 100 g of triple 15 fertilizer (Britz Fertilizers, Inc. (BFI), Stockton, CA) (200 g/plot of NPK) plus 8.3 ml of Britz SuperMicro (BFI) containing five micronutrients 15-d after planting. Macroplots were fertilized with annual applications of 55 kg/ha triple 15 fertilizers in each of the two succeeding years. Two sprays annually of 0.5% glyphosate were applied to keep plots free of summer and winter weeds.

Using a serial dilution method, 12 replicates of each of four *P. vulnus* population levels were imposed across the macroplots. Holes 60-cm-diam. and 30-cm deep were dug at each planting site and the soil placed onto a mixing board. This 80 L of soil was individually mixed with enough infested soil to result in nematode population levels of 0, 1, 20 or 500 *P. vulnus* juveniles, males and females/250 cm^3 of soil. Nematode inoculum was obtained from adjacent 2-yr-old *Prunus* spp. trees infested with *P. vulnus*. The different inoculum levels were placed in a randomized complete block design across the 48 macroplots.

In March 2004, 80-d-old plantlets consisting of 48 clones each of VX211 and AX1 were selected for uniform shoot and root size. One VX211 and one AX1 clone were planted 5-m apart in each macroplot. Plants were placed in the center of each inoculated area within 1 wk after nematode inoculation.

Soil samples were collected only from within the 80 L inoculated area in November 2004 and each subsequent July and November for a total of seven sampling periods per tree to assess nematode population levels. Using an Oakfield tube (2.5-cm-diam. x 30- cm long), seven soil cores were collected and composited into one sample from each tree. Soil samples were collected, placed into a plastic bag, labeled, transported to the lab and stored at 4°C until processed. Each sample was thoroughly mixed and a 250 cm^3 composite-subsample was processed through nested 20-, 100-, and 325-mesh screens followed by Baermann funnel extraction in a mist chamber for 3 d to collect nematodes (McKenry and Roberts 1985). Collected nematodes were identified and quantified under dissecting microscope at x40 magnification.

Plant heights were measured only during fall 2004; however trunk diameters were measured with executive diameter steel tape (Lufkin®, Cooper Tools, NC) 15-cm above the field surface each fall over the duration of the experiment. Tops of all trees were removed by chain saw 80-cm above the soil surface spring 2005 and grafted to improperly collected English walnut scion wood of the cultivar 'Chandler', from which all grafts failed. New growth of the rootstocks was trimmed to a single limb and allowed to grow throughout 2005. During fall 2006 and 2007, trunk diameter of all new growth 90-cm above the soil surface and 10-cm above the new shoot trained in 2005 was measured to assess any negative impacts from the 2005 grafting activity. In spring 2007, all trees were cut down again by chain saw at the 90-cm height and English walnut 'Chandler' was successfully grafted confirming graft compatibility of the rootstocks.

Lath house experiment: In March 2006, 12 nematode-free shoot cuttings each of the Paradox clones PX1, VX211, RX1, AX1 and four 'Vlach' cuttings were available for this experiment. These selections were of interest because of their host status against nematodes, *Phytophthora* spp., and other soil pests. Nematode-infested sandy loam soil (65% sand, 27% silt, 8% clay, and < 1% organic matter) was collected from *Prunus* spp. located at the University of California Kearney Agricultural Center, Parlier CA and contained 1,300 vermiform *P. vulnus* and 145 second-stage juveniles (J2) of *M. incognita*/250 cm^3 soil. The infested soil was thoroughly mixed and placed undiluted into 4-L plastic pots. Individual shoot cuttings of each clone were transplanted into the pots and arranged on perforated steel benches. Three rows per bench provided 12 replications for each clone, except 'Vlach' which had only four replications. Transplants were watered to maintain the field capacity and fertilized with Miracle-Gro fertilizer (Scotts, Marysville, OH) at labeled rates every 14 d

throughout the active growth period. In March 2007, roots were separated from soil, weighed and carefully washed under tap water to remove adhering soil particles and towel dried. Nematodes were extracted by placing 20-g subsamples of chopped roots into a mist-chamber for 5 d.

Field Study for extensive root evaluation: One-year-old trees of VX211 and AX1 from lath house studies were transplanted into an open field at the Kearney Agricultural Center and adjacent to the macroplots. The field consisted of sandy loam soil (65% sand, 27% silt, 8% clay, and < 1% organic matter) and was treated in the fall of 2006 with 360 kg/ha Telone II (Dow Chemical, Indianapolis, IN). In this site a paired tree experiment was developed. Trees within each row were spaced 2 m apart down the row and 3 m between rows. In every other row, each tree was inoculated with 1 L of soil containing 1,400 vermiform *P. vulnus* and 1 *M. incognita* J2/250 cm^3 soil and the adjacent row planted without inoculum. Our goal was to focus greater attention on nematode counts within root initials, an activity too disruptive to conduct in the existing macroplot experiment. There were 14 replicates of each paired group of trees, but only four were used for destructive sampling. Root samples of both clones were collected by meticulously digging a trench 60-cm away from the trunk and then gently brushing the soil away until the peripheral root tips and their supporting root were found. Lengthy root pieces were collected intact, placed in plastic bags and kept cool in an ice chest. Roots were then washed, and cut by scissors into three distinct root sectors: 1) terminus 0-3-cm root tips 2) root parts 3.1 cm to 9 cm distal to the tip (fleshy roots) and 3) root parts 9.1-cm to 15-cm distal to the tip (fibrous roots). Roots in the latter grouping had usually begun to suberize. Half the root pieces of each size grouping were diced, weighed, and placed into a mist chamber as described above. The other half was stained with Schillings food coloring (Thies et al., 2002), destained with acetic acid and then placed between two glass plates for examination at x40 magnification.

Data analysis: Data were subjected to analysis of variance with equal and unequal replication using SAS (SAS Institute, Cary, NC). Nematode data in Table 4 were log transformed and means expressed as an inverse of $[\log_{10}(x + 2)]$. Significant differences in means of nematode reproduction were separated using Duncan's multiple range test at ($P \leq 0.05$), where applicable.

Results

Resistance screening: Several Paradox seedlings from a few families emerged as unique. One seedling out of ten, from the family coded as VX, demonstrated high vigor and apparent tolerance to the presence of *P. vulnus* and *M. incognita*. Four of the 10 NX seedlings and three of the 10 UZ seedlings screened appeared to avoid nematode penetration during the first year, but not the second, suggesting some type of pre-infection resistance that was eventually overcome. Selections from the pure black walnut *J. hindsii* commonly expressed resistance to *M. incognita*, but one selection, AW269, also appeared to be a poor host for *P. vulnus*. The trees that contained these three different pest protection mechanisms were set aside for clonal propagation and more intensive experimentation. Clones of VX211 became available in 2004.

Macroplot experiment; nematode levels in soil: Pratylenchus vulnus developed and reproduced at all inoculum levels regardless of walnut clone (Table 1). First-year reproduction rates (Pf/Pi) were greatest on both walnut clones at Pi 1. In July of 2005, clone AX1 supported 82% more *P. vulnus* than clone VX211 at Pi 500. The population levels in clone AX1 at Pi 1 increased in July and November of 2005 compared to that of clone VX211 ($P \leq 0.05$). In the July 2006 sample, AX1 exhibited a 3.4-fold increase at Pi 1 compared to VX211 (Table 1). During the first 2 yr few nematodes were detectable from non-inoculated trees of either clone. Population levels generally decreased in the third and fourth years, however these declines were greatest around the VX211 rootstock (Table 1). Meanwhile,

Table 1. Reproduction of *Pratylenchus vulnus* on Paradox walnut clones from 2004 through 2007.

		Nematode population per 250 cm^3 soil during a 4-yr study[a]							
		2004	2005		2006		2007		
Walnut clones	Pi	Nov.	July	Nov.	July	Nov.	July	Nov.	RF[b]
VX211	0	0c[c]	31d	142c	30d	426bc	263cd	224b	0b
	1	2753a	2710ab	1133b	872bc	672abc	624abc	538ab	538.1a
	20	2164ab	1742bc	1213b	1002bc	1150a	625abc	628a	31.4b
	500	1333bc	1242c	755bc	983bc	762abc	570cd	392ab	0.78b
AX1	0	0c	0d	7c	184cd	260c	180d	221b	0b
	1	2273ab	3256a	2050a	3012a	1208a	1031a	568ab	568.1a
	20	2132ab	1731bc	1583ab	1640b	1201a	809ab	655a	32.7b
	500	708c	1519c	1265ab	1169b	927ab	804ab	439ab	0.88b

[a] Each mean consists of an average of 12 replications.
[b] Reproduction factor = Pf (final population) / Pi (initial population) for 2004.
[c] Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P \geq 0.05$.

some replicates of the non-inoculated trees were gradually becoming contaminated. In November 2006, nematode detections on non-inoculated VX211 trees increased 3-fold from November 2005 compared to 37-fold on non-inoculated AX1 trees (Table 1). At the July 2007 sampling, VX211 at Pi 500 had fewer ($P \leq 0.05$) *P. vulnus* than AX1 whether inoculated at Pi 1, 20 or 500. By November 2007 nematode populations within the three inoculated treatments had generally reduced from their highest levels down to a pooled average for both clones of 536 *P. vulnus*/250 cm^3 soil. Walnut clones VX211 and AX1 in non-inoculated soil did not reach high nematode soil population levels because by the third year most of the roots within the 80 L inoculated zone were larger and suberized, not the preferred feeding site of *P. vulnus* (Buzo et al., 2005).

Macroplot experiment; tree growth: Inoculations with 1, 20 or 500 nematodes/250cm^3 suppressed height and trunk growth of VX211 in the first year of growth (Table 2). However, growth differences among the three inoculation levels were not evident during year 1. Clone AX1 plant heights were reduced by 22% at Pi 20 in 2004 compared to the non-inoculated control ($P \leq 0.05$). However, there was no significant height reduction on AX1 at Pi 1 or 500 during the first year of growth. At all inoculation levels the effect of nematode feeding significantly ($P \leq 0.05$) reduced trunk expansion of AX1 compared to that of VX211, although differences among the initial inoculation levels for each clone were not always evident.

In the second year, 2005, trunk diameter of both clones was smaller ($P \leq 0.05$) on trees inoculated with *P. vulnus* at all Pi levels, except AX1 at Pi 1 (Table 2) compared to its respective non-inoculated control. The Pi levels had no significant effects on growth of VX211, except all were smaller than that of the control. The AX1 clone showed significantly ($P \leq 0.05$) more damage when inoculated with Pi 20 compared to that of Pi 1. Trunk diameters at 15-cm were significantly greater ($P \leq 0.05$) for VX211 non-inoculated trees than for any other trees in this experiment. *Pratylenchus vulnus* suppressed growth of VX211 trees at all inoculation levels. By 2005 the non-inoculated trees of AX1 had not grown better ($P \geq 0.05$) than those inoculated with Pi 1. In the third year, 2006, VX211 continued to outgrow AX1 in the presence or absence of *P. vulnus* ($P \leq 0.05$). In the final year, 2007, the non-inoculated VX211 trees grew larger compared to all other inoculated treatments regardless of clone. Compared to AX1, VX211 tolerated *P. vulnus* infection and associated impact on trunk expansion at each inoculation level and in each consecutive year.

Trunk diameters at 90-cm in 2006 provided evidence that VX211 inoculated with Pi 1 and 500 tolerated extreme shoot pruning better than the standard AX1 in the presence of heavy *P. vulnus* pressure. In fact, VX211 at Pi 1 and 500 were numerically similar to the non-inoculated VX211 at the 95% confidence level and were also statistically larger than any of the AX1 inoculated treatments (Table 2). Using this same metric in 2007, non-inoculated VX211 was statistically different from all other inoculation treatments. By contrast, AX1 was severely impacted by severe pruning even in the absence of inoculum and its trunk growth improvement from 2006 was only 0.1 cm compared to a 1.1 cm growth increase by VX211.

Lath house experiment: The nematode host status of various Paradox clones was compared in a one year study. 'Vlach' supported *P. vulnus* populations that were 3.4 to 9.0 times higher than those observed in all other Paradox ($P \leq 0.05$) (Table 3). Several rootstocks including VX211 and AX1 appeared to be poorer hosts for *P. vulnus*, as compared to 'Vlach' rootstock (Table 3). Against *M. incognita*, PX1 was a better host ($P \leq 0.05$) compared to 'Vlach', VX211, or UZ229. The standard clone, AX1, supported numerically higher *M. incognita* population densities than 'Vlach', VX211, or UZ229 (data not shown). All rootstocks were poorer hosts for

TABLE 2. *Pratylenchus vulnus* impact on tree growth of Paradox walnut clones.

		Growth of walnut clones (cm) during fall[a]						
			Trunk diameter (cm) at distance from soil surface					
		Height (cm)	15 cm				90 cm[b]	
Walnut clones	Inoculum levels	2004	2004	2005	2006	2007	2006	2007
VX211	0	289.09a	3.95a[c]	9.28a	15.0a	15.9a	11.7a	12.8a
	1	231.1b	3.04b	7.85b	12.9bc	14.0b	10.7ab	11.4b
	20	221.46bc	3.11b	7.33b	12.4c	13.9b	10.2b	11.2b
	500	216.52bc	3.04b	7.90b	13.7b	14.4b	10.8ab	11.4b
AX1	0	207.85bc	2.38c	5.85c	10.6d	10.9c	8.4c	8.5c
	1	197.15bcd	2.2 1c	5.25cd	9.3e	9.5cd	7.3d	7.2cd
	20	163.48d	2.01c	4.47e	8.1e	8.9d	6.1e	6.2d
	500	181.47cd	2.07c	5.00de	8.8e	9.9cd	7.0de	7.7c

[a] Each mean consists of an average of 12 replications.
[b] 2nd year re-growth following grafting failure.
[c] Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P \geq 0.05$.

TABLE 3. Reproduction *Pratylenchus vulnus* on five Paradox clones after 1 yr in a lath house[a].

Walnut clones	Pf /g of root[b]
Vlach	6580a[c]
PX1	1944b
AX1	1426bc
VX211	1307bc
UZ229	730c

[a] Initial inoculum was 1,300 nematodes/250 cm^3 within 4 liters soil.
[b] Each mean consists of 12 replications except the Vlach (4), with unequal reps.
[c] Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P \geq 0.05$.

P. vulnus, as compared to 'Vlach' rootstock ($P \leq 0.05$) (Table 3). When studying walnut clones growing in 4 L pots the small pot size can become a limiting factor by preventing root exploration within the soil.

Root examinations in the field: Six parameters were assessed to determine *P. vulnus* feeding and reproduction preferences among Paradox clones VX211 and AX1 (Table 4). First-year measurements of tree height and diameter indicated VX211 grew better than AX1 ($P \leq 0.05$). Examinations of the terminus 15 cm of VX211 roots indicated they were less infected by *P. vulnus* compared to AX1. Root sections 3.1 cm to 9.0 cm indicated AX1 supported 44 times more *P. vulnus* per gram of root than VX211. Distances 0 to 9.0 cm distal from the root tip were the preferred nematode feeding sites of the more susceptible AX1. However, quantification of first-year soil populations confirmed the macroplot findings that *P. vulnus* was in abundance regardless of clone selection.

DISCUSSION

Pratylenchus vulnus is primarily a parasite of tree and vine roots (Lownsbery, 1956, McElroy, 1972; Scotto et al, 1989; McKenry, 1989). This nematode is a primary causal agent of tree decline in addition to orchard replant problems in many parts of the world (Nyczepir and Halbrendt, 1993; McKenry, 1999). The current study confirms the parasitism of *P. vulnus* on Paradox hybrids of walnut. The reduction of plant growth appears similar to the pathogenic effects of *P. vulnus* on other rootstocks including pear and apple (Fernández et al., 1992), almond, peach, peach-almond hybrid, and plum (McKenry and Kretsch, 1987; Pinochet et al., 1993). The exception however is that on these walnut rootstocks *P. vulnus* population levels can easily double those found on the other crops.

Our previous research (Buzo et al., 2005) indicated that nematode attraction and reproduction could be 10^3-fold greater at the walnut root terminus rather than in roots pencil-sized or larger. In the presence of young expanding root systems the damage caused by this nematode plus the replant problem can completely halt tree growth, even with a Pi of only 1 nematode/250 cm^3 soil (observations by M. McKenry). Once a far-reaching root system is adequately developed this nematode's assault occurs as they await production of new feeder roots along the existing main root system.

Walnut orchards established across California commonly support 500 *P. vulnus* /250 cm^3 soil. Preplant soil fumigants when poorly applied can reduce population levels to 1 *P. vulnus*/250 cm^3 soil whereas quality fumigation can reduce their numbers to 0.01/250 cm^3 soil throughout the surface 1.6-m of soil (McKenry, 1987). Application of a systemic herbicide (Garlon 4, Dow AgroSciences LLC, IN) prior to removal of walnut trees can kill all roots and reduce root dwelling populations by 99% within 1 yr (McKenry, unpublished data). However, soil-dwelling populations after 5 yr of fallow remain at 20 *P. vulnus*/250 cm^3 soil when quantified at 1- to 3-m in depth (McKenry, unpublished data). The Pi levels utilized in this experiment represent actual nematode situations common to the replanting of walnuts in California.

Clonal rootstocks provide a tool for avoiding seedling variability and improving control of root pests and diseases including resistance or tolerance to *P. vulnus*, *M. incognita*, *Phytophthora* spp., and *Agrobacterium tumefaciens*. In this paper we have focused on nematode resistance and tolerance, qualities that have not been previously available among *Juglans* spp. rootstocks. For each of the 4 yr of our macroplot study the poorest growing VX211 Paradox always grew larger than the best growing non-inoculated AX1 Paradox, thus hybrid vigor is a primary quality of VX211.

TABLE 4. Six distinct parameters of two lath house walnut Paradox clones 1 yr after transplanting into the field.

			P. vulnus / gram of root[a]			*P. vulnus* / 250 cm^3 soil[a]
	Growth*		Root partition			Soil profile
Clones	Plant Height (cm)	Trunk Diameter (cm)	0-3cm root tips	3.1-9 cm root sections	9.1- 15 cm root sections	0-30 cm soil depth
VX211	114.5a[b]	2.1a	5.9a[c]	7.9a	4.0a	881ns[d]
AX1	79b	1.4b	271.6b	345.1b	103.7b	540.7

[a] Each mean consists of an average of four replications.
[b] Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P \geq 0.05$.
[c] Nematode data represents log-transformed data and means are expressed as an inverse of $[\log_{10}(x + 2)]$.
[d] ns = $P \geq 0.05$.

First-year growth of VX211 was significantly better where *P. vulnus* was present at fewer than 1 nematode/250 cm^3 soil. During the first year of our study the ability of AX1 to tolerate various nematode population levels appeared similar to that of VX211. We questioned our initial observations about nematode tolerance in VX211 because it was damaged by nematode feeding in terms of plant growth. However, tolerance comes in many forms (McKenry and Anwar, 2006). With walnut, tolerance could emanate where the nematodes primarily feed and reproduce outside of root initials in the soil compared to feeding inside of root initials. In fact, in the second and third year after inoculations at the low Pi level, the eventual population levels of nematodes in root tissue were significantly less on VX211 compared to AX1. Tolerance can also emanate from a modicum of nematode resistance at the root tips because well established primary roots are relatively nematode free. In fact, in the second and third years after inoculation at the low Pi level, the Pf was significantly less on VX211 than that on AX1. Based on the 1-yr comparison of five paradox hybrid clones it appears that VX211 could be a better choice than 'Vlach' but neither clone exhibited adequate nematode resistance in a pot study. Our future search for resistance among Paradox could include the pre-infection resistance we noted in clones of UZ229 coupled with the mechanism in VX211 that halts feeding within root tips. The finding of fewer *P. vulnus* in VX211 root terminals compared to their numbers within rhizosphere soil indicates there may be a resistance mechanism in VX211 compared to AX1.

Understanding the relationship between initial nematode population densities and plant growth is essential to predict crop losses by plant-parasitic nematodes and to utilize defined management strategies. Nematode reproduction was affected by initial inoculum density on both clones; however, reproduction rates were inversely related to initial inoculum density (Table 1), indicating that the carrying capacities for this nematode can be exceeded. Competition for food resources is less at low Pi, allowing a more striking increase in nematode development (Davide and Triantaphyllou, 1967; Seinhorst, 1967). As Pi increases, competition increases, and a smaller proportion of the inoculum develops successfully. This population equilibration occurs when Pf = Pi, after which further increases in Pi result in reduced reproduction rates (Seinhorst, 1967). The vigor component in VX211 in the non-inoculated soil contributed to statistical separation in trunk growth compared to Pi levels 1, 20, and 500. In contrast, the absence of vigor within AX1 in similar soil did not separate statistically until the second year when nematode population densities reached damaging levels (Table 1). However, at Pi 500 nematodes were competing for feeding sites during the first year but were able to develop sufficient feeding sites to inflict the damage the following years (Table 1). These studies provided the impetus to release VX211 as a vigorous nematode tolerant Paradox rootstock clone to California growers in 2007.

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