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A PILOT STUDY ON THE SPATIAL DISTRIBUTION OF *DIAPREPES ABBREVIATUS* (L.) (COLEOPTERA: CURCULIONIDAE) AND *PHYTOPHTHORA* SPP. IN CITRUS

R. C. ADAIR, JR. AND N. K. MEHTA
Kerr Center for Sustainable Agriculture, Inc.
Vero Beach Research Station
7055 33rd Street
Vero Beach, FL 32966

J. H. GRAHAM
University of Florida, IFAS
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850

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Abstract. **This study describes the spatial distribution of adult *Diaprepes abbreviatus* and *Phytophthora* spp. levels in an East coast citrus grove using a Geographic Information System (GIS). Weevil density and distribution were determined by placing 99 Tedders traps in a grid pattern throughout the 12.5 acre weevil infested grove planted with two rootstocks, Swingle citrumelo and Cleopatra mandarin. All 1,492 trees and each of the Tedders trap locations were mapped on a digitized, georeferenced, aerial photograph using ArcView 3.1 GIS software. *Phytophthora* spp. levels were determined from soil samples taken from each tree with a Tedders trap and were similarly mapped. Tree condition was determined twice on 6 Oct. 1999 and 17 May 2000 for all trees by visual assessment, and were mapped and color coded as either healthy, slight, moderate, or severe decline. Topographical views with colored symbols were created using GIS, showing spatially the weevil density, *Phytophthora* spp. levels, and tree condition relative to tree location, variety, and rootstock. This approach permitted novel observations on the spatial distribution of not only pest and pathogen levels, but also displayed associations with the tree variety, rootstock and tree condition. GIS can be an important tool to elucidate the complex relationships between crop, pest, and pathogen levels, and aid in the improved management of a citrus horticultural system.**

The root weevil, *Diaprepes abbreviatus* L., is inflicting losses to the Florida citrus industry that exceeds $72 million annually (Stanley, 1996). Since its discovery in Apopka in 1964, *D. abbreviatus* has become established on many horticultural crops in Florida, including sugarcane, woody ornamentals, and approximately 10% of the citrus grown commercially (Woodruff, 1964, Simpson et al., 1996). It is an insidious pest of citrus because tree symptoms do not appear until the larvae are well established on the roots. Once established, *D. abbreviatus*-infested groves exhibit decline symptoms such as leaf yellowing, premature leaf drop, twig dieback, off-blooming, fruit drop, and alternate bearing (Quintela et al., 1998). Tree decline resembles symptoms caused by other tree diseases and root disorders such as citrus blight, tristeza, or *Phytophthora* spp. (Quintela et al., 1998). Larval feeding on citrus roots damages the root system and provides an avenue for pathogens such as *Phytophthora* spp. (Rogers et al., 1996). An industry-wide survey of citrus revealed that groves affected by both larval root feeding and *Phytophthora* spp. exhibited greater damage to the structural roots than when either the pest or pathogen was present alone (Graham et al., 1997). This relationship of pest and pathogen forms a complex, dubbed the Phytophthora-Diaprepes Complex (PDC) that can destroy citrus trees if not treated in a timely fashion (Graham et al., 1997). Furthermore, this same survey revealed that a new species, *Phytophthora palmivora* (E. J. Butler) E. J. Butler, was inflicting more damage to structural roots than root feeding by *D. abbreviatus*.

Rootstocks with trifoliate orange [*Poncirus trifoliata* (L.) Raf.] parentage such as Swingle citrumelo [*Citrus paradisi* Macf. H *Poncirus trifoliata* (L.) Raf.] typically are not susceptible to *Phytophthora nicotianae* (Breda de Hann), but when grown in wet, heavy soils in the presence of root feeding by *D. abbreviatus*, it readily succumbs to the more aggressive species, *P. palmivora* (Graham, 1998). Applications of systemic fungicides such as mefenoxam (Ridomil Gold® EC) have been shown to promote short-term beneficial tree responses under these conditions, but the vulnerability of the structural roots to *P. palmivora* makes extended control highly unlikely (Graham, 2000).

Visual observations of citrus trees on two different rootstocks at the Kerr Center citrus groves (Indian River County, Florida) treated in the last three yrs with mefenoxam showed contrasting responses for each rootstock (Adair, Personal observation). In addition, *D. abbreviatus* infested groves frequently appear to be very sporadic regarding tree condition, with healthy, vigorous trees located next to severely declined trees (Adair, Personal observation). Stansly et al. (1997) reported spatial variation in capture patterns of *D. abbreviatus* based on four trapping areas located within two grove sites using Tedders traps (PBE Graphics Warehouse, W. Palm Beach, FL), and commented that these variations may be due to interactions between tree condition and colonization patterns. Since a Geographic Information System (GIS) has the ability to integrate layers of spatial information and to uncover possible relationships that would not otherwise be obvious, it would be particularly applicable to elucidating a spatial pattern for the PDC. Numerous studies using GIS have been applied to agriculture for insect pests, weeds, and plant disease control (Nelson et al., 1999). However, no research has ever been conducted on the use of a GIS for study of *D. abbreviatus*, or PDC. The objective of this study was to explore the spatial distribution, on a tree by tree basis, of weevil density, *Phytophthora* spp. levels, and tree condition in relation to two rootstocks within a citrus grove using a GIS, and to determine if there are any relationships among these four variables.

Materials and Methods

The study site, named the K-20 block, is located at the Kerr Center groves, west of Vero Beach in Indian River County. *D. abbreviatus* was first detected in the southeast corner at this site in May 1990 and has spread throughout several surrounding sections of citrus (Adair, 1994). Drainage is accomplished by furrows between raised double beds running 1,200 ft to drainage ditches located at each end of the grove. The soil types are Winder (fine-loamy, siliceous, hyperthermic) and Manatee (coarse-loamy siliceous, hyperthermic), that vary widely in sand, clay and organic matter from the construction of raised beds. The K-20 block is 12.5 acres and was planted in Dec. 1989 with "Sunburst" tangerine (*C.* x *reticulata*) on Swingle citrumelo rootstock with a 15 ft × 25 ft spacing and "Nova" tangelo (*C.* x *tangelo*) on Cleopatra mandarin (*C. reticulata* Blanco) as pollinators with 12.5 ft × 25 ft spacing (Fig. 1). During periods of low rainfall, the grove was irrigated from an artesian well (1300-1500 ppm TDS) and water was supplied to the trees using a well maintained micro-irrigation system, with irrigation frequency determined by tensiometer readings. Daily rainfall records were maintained.

Due to high levels of *Phytophthora* spp. present, a low salt index liquid fertilizer (8-0-10) derived from urea, ammonium nitrate, and potassium nitrate was injected into the irrigation system only during dry periods (typically Mar.-June) and was boom-applied during rainy periods to minimize excessive irrigation. Additional applications of fertilizer were foliarly applied to the trees using an air-blast sprayer. *Phytophthora* spp. control was performed with two applications of mefenoxam per year, applied using a herbicide boom at 1 pint/grove acre/yr in the spring and fall, beginning in 1997 to the present. Additional applications of phosphorous acid products (Phos Might®) were applied two times per year. An aggressive IPM program for *D. abbreviatus* has been in practice for the last six yrs, which included applications of entomopathogenic nematodes, ovicidal sprays, and adulticides. Otherwise, conventional caretaking practices for fresh fruit were performed.

Ninety-nine Tedders traps were installed in a grid pattern, approximately 50 ft × 125 ft, throughout the grove. They were constructed with black corrugated plastic, 2 ft high with a 1.5 ft base and the upper portion of a Boll weevil trap (Great Lakes IPM, Inc., Vestaburg, MI) was affixed to the top (Riherd, 1996, Stansly et al., 1997). Each trap was placed on the south side of the tree at the drip edge, within the tree row. The traps were monitored weekly and the number of captured adult *D. abbreviatus* was recorded for each trap from 28 Feb. to 26 June 2000.

All 1,492 trees and each of the Tedders trap locations were mapped on a digitized, geo-referenced (State Plane 83-90), aerial photograph, taken in Mar. 1998, using ArcView 3.1 (Environmental Systems Research Institute, Inc., Redlands, CA) GIS software. Tree condition was determined twice for all trees on 6 Oct. 1999 and 17 May 2000, by visual assessment using a numerical ranking where 4 = healthy, 3 = slight decline, 2 = moderate decline, 1 = severe decline, and 0 = dead. The trees were similarly color coded and mapped for each assessment period. Composites of four 2.5 cm × 15 cm soil samples were taken from each tree with a Tedders trap on 5 Apr. 1999 (prior to the spring application of mefenoxam) using a standard soil probe. The soil samples were assayed by Dr. J. H. Graham's laboratory at the Citrus Research and Education

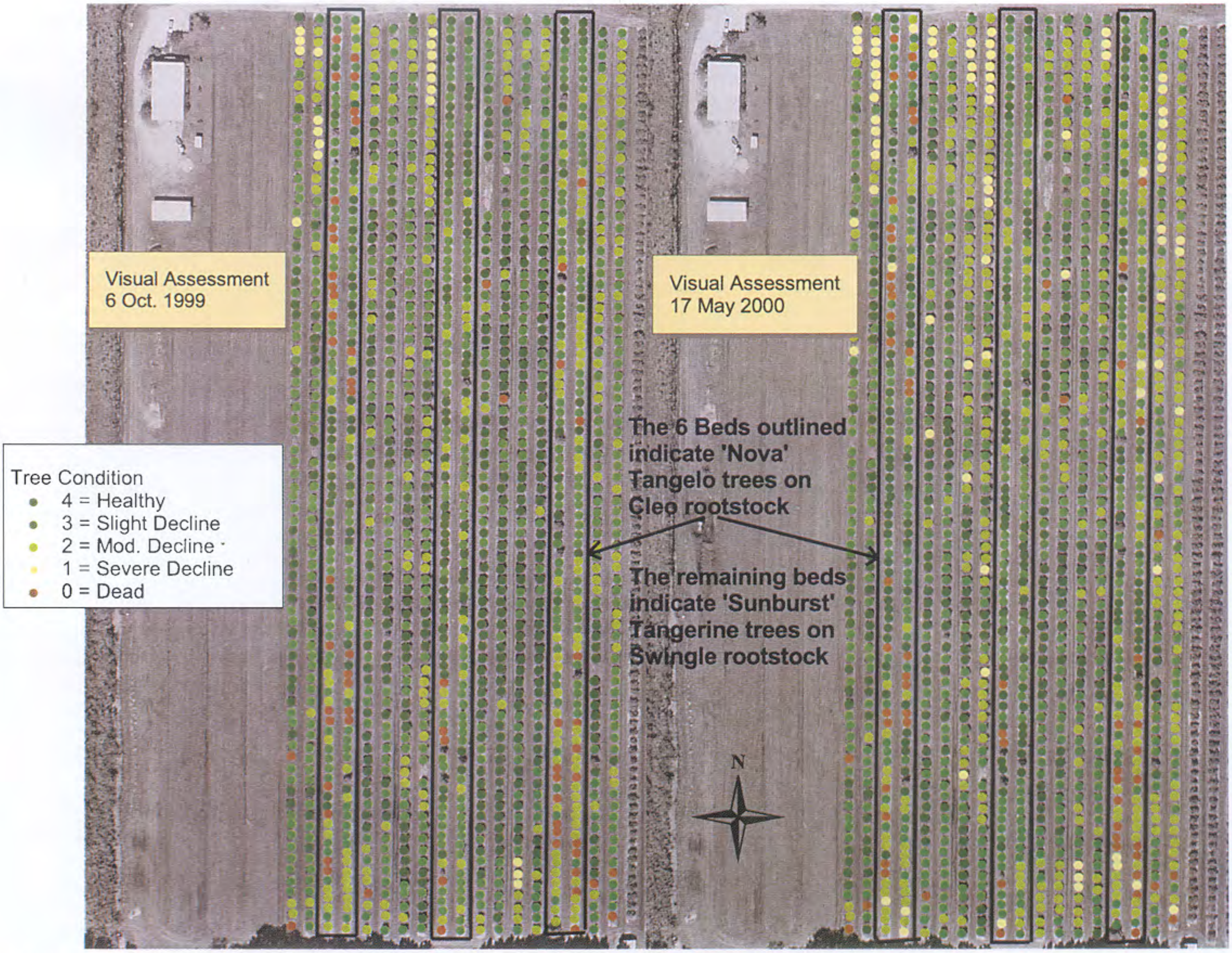

Figure 1. Two GIS views featuring visual comparisons of changes in tree condition over a six month period for "Sunburst" tangerine trees on Swingle citrumelo rootstock and "Nova" tangelo trees on Cleopatra mandarin rootstock (outlined) in a 10 yr-old grove infested with *Diaprepes abbreviatus* and *Phytophthora* spp., Kerr Center, Indian River County, Florida.

Center, University of Florida, in Lake Alfred, Florida for both *P. nicotianae* and *P. palmivora* propagules. *Phytophthora* spp. levels were reported as the number of propagules per cm^3 of soil. The *Phytophthora* spp. levels and the weevil density obtained from each of the 99 sample sites were entered into the GIS. This information was used to create topographical views with colored symbols showing the spatial distribution of weevil density, *Phytophthora* spp. levels, and both initial and final tree conditions for the entire grove. This data was spatially entered relative to the appropriate tree location, variety, and rootstock.

All data collected were checked for normality and homogeneity in variances using appropriate transformations and Hartley's (1950) Fmax test (Rohlf and Sokal, 1981, Sokal and Rohlf, 1981). Significant differences in tree condition between the two sampling periods were examined using the Wilcoxon Signed Rank Test; *D. abbreviatus* abundance between the two rootstocks was examined using the Student's t-test, and the relative abundance of the two *Phytophthora* spp. levels between the two rootstocks was tested using the Mann-Whitney U-test (Sokal and Rohlf, 1981). Regression and correlation analyses were used to test for significant relationships between selected dependent variables. The Kendall's Rank Correlation Coefficient (Tau) analysis was performed to determine possible associations for each rootstock, relative to the number of weevils captured, the population levels for *Phytophthora* spp., and tree condition (Sokal and Rohlf, 1981). All tests were carried out at the 5% significance level.

Results

The mean tree condition for the "Nova" trees on Cleo rootstock visually assessed on 6 Oct. 1999 and again on 17 May 2000 remained virtually the same while the "Sunburst" trees on Swingle rootstock declined significantly over the seven-month period (Table 1). Tree condition for this same period indicated an increase in healthy trees from 22% to 25% on Cleo rootstock, while the healthy trees on Swingle rootstock decreased from 31% to 21% (Fig. 2). There were increases in the moderate (17% to 24%) to severely declined

Table 1. Tree condition[z] of two citrus varieties in the K-20 citrus grove, Kerr Center, Indian River County, Florida.

Tree Variety/Rootstock	6 Oct. 1999		17 May 2000	
	No. of Trees	Mean Condition	No. of Trees	Mean Condition
'Nova' tangelo/ Cleopatra mandarin	537	3.00 (±0.70) a[y]	506	3.01 (±0.77) a
'Sunburst' tangerine/Swingle citrumelo	955	3.11 (±0.74) b	944	2.82 (±0.84) c

[z]Tree condition was determined twice by visual assessment using the following numerical index: 4 = Healthy, 3 = Slight decline, 2 = Moderate decline, and 1 = Severe decline.
[y]Means (±S.D.) followed by the same letter are not significantly different (Wilcoxon Signed Rank Test, 5% level).

(2% to 7%) trees on Swingle rootstock, while the same categories remained relatively unchanged for the trees on Cleo rootstock (Fig. 2). Careful inspection of Fig. 1 reveals minimal changes in the hue of the colored dots representing the tree condition of the trees in the outlined beds on Cleo rootstock. The areas that exhibited the greatest change in tree condition decline (from good to bad) occurred on the northern, eastern, and southern regions of the Swingle beds. This was especially pronounced in the northeastern quadrant and the two eastern most rows. This is best viewed by examining the increasing incidence of moderate (light green dots) and severe (yellow dots) declined trees from Oct. to May (Fig. 1).

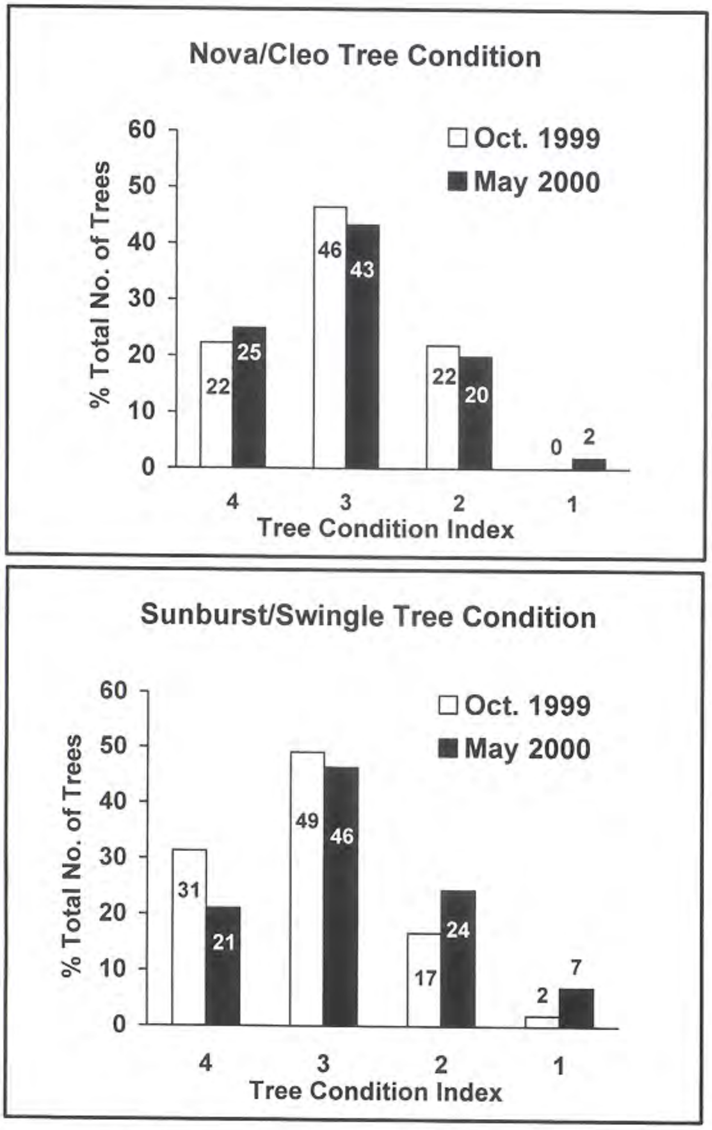

Figure 2. The percent changes in tree condition by category from 6 Oct. 1999 to 17 May 2000 of "Sunburst" tangerine trees on Swingle citrumelo rootstock vs. "Nova" tangelo trees on Cleopatra mandarin rootstock growing in a 10 yr-old grove infested with *Diaprepes abbreviatus* and *Phytophthora* spp., Kerr Center, Indian River County, Florida. Tree condition categories are represented by the following numbers: 1 = Severe decline, 2 = Moderate decline, 3 = Slight decline, 4 = Healthy.

The mean number of captured adult *D. abbreviatus* per trap/week from each citrus variety/rootstock failed to demonstrate any significant difference for either variety/rootstock, although more adults/trap/week were captured from the "Sunburst" on Swingle (0.38) trees vs. the "Nova" on Cleo (0.27) trees (Table 2). The spatial distribution of trapped *D. abbreviatus* depicts below average abundance for the traps located at the extreme north and south ends of the grove as well as at the center (Fig. 3). All seven traps located next to dead or missing trees captured adult weevils and only four of the 99 traps failed to capture any adults.

There was a very uniform, spatial distribution of excessively high levels of *P. nicotianae* (>60 propagules/cm^3, Graham 2000) throughout the grove (Fig. 4). Neither rootstock indicated any significant differences in the mean no. of *P. nicotianae* propagules/cm^3 soil sampled from the 92 live trees, although Swingle had a higher concentration than Cleo at 262/propagules/cm^3 soil and 193/cm^3 soil, respectively (Table 3). This is in contrast with other studies which reported higher levels of *P. nicotianae* associated with Cleo than with trifoliate orange rootstock which is a parent of the hybrid Swingle (Rogers et al., 1996, Graham, 2000). However, the mean no. of *P. palmivora* levels for the Swingle rootstock of 22 propagules/cm^3 soil was significantly higher than the mean no. of 4 propagules/cm^3 soil obtained from Cleo rootstock (Table 3). This clearly suggests a difference between Cleo and Swingle with respect to rootstock susceptibility to *P. palmivora* under the conditions at this study site.

This observation of low *P. palmivora* levels for Cleo was visually apparent in Fig. 5, where *P. palmivora* was detected only in one sample on Cleo rootstock. All other detections of *P. palmivora* occurred on Swingle rootstock. There was a dramatic difference in the spatial distribution between *P. palmivora* which was detected at only 19 sites as opposed to the 93 sites where *P. nicotianae* was detected (Figs. 4 and 5). Most of the detections, as well as the higher levels of *P. palmivora*, occurred in the northern and northeastern regions of the K-20 grove (Fig. 5). Only the moderate and severely declined trees assessed on 17 May 2000 are shown in Fig. 5 to illustrate their incidence in these same areas. Interestingly, this corresponds to the same general area which exhibited a large incidence in declining trees described above.

Only two significant associations were determined between the number of weevils captured, *Phytophthora* spp. levels, and tree condition relative to each rootstock. (Table 4).

Table 2. Relative abundance of adult *Diaprepes abbreviatus* captured using Tedders Traps from 28 Feb. to 26 June 2000 in two citrus varieties in the K-20 citrus grove, Kerr Center, Indian River County, Florida.

Tree Variety/Rootstock	No. of Tedders Traps[y]	Total No. of Adults	No. of Adults/Trap/Week[z]
'Nova' tangelo/Cleopatra mandarin	27	132	0.27 (±0.25) a
'Sunburst' tangerine/Swingle citrumelo	65	448	0.38 (±0.35) a

[z]The No. of Adults/Trap/Week represents the mean of 18 weekly collections from the Tedders Traps. Means (±S.D.) followed by the same letter are not significantly different (Student's t-Test, 5% level).
[y]Traps located at the 7 dead or missing trees along with the Adults captured in them were not included in this table.

The "Sunburst" tangerine trees on Swingle citrumelo rootstock exhibited a significant positive association between high densities of captured *D. abbreviatus* and high soil levels of *P. nicotianae*. This would mean that where a high incidence of *D. abbreviatus* was observed, there would likely be high soil levels of *P. nicotianae* with "Sunburst" tangerine trees on Swingle citrumelo rootstock. Also significant was the negative association between tree condition and *P. palmivora* levels for the trees on Swingle, meaning trees in poorer condition would likely have higher levels of *P. palmivora*. There were no significant relationships between weevil density, Phytophthora spp. levels, and tree condition for the two rootstocks.

Discussion

A general decline in tree condition, over time, was predicted for both citrus tree varieties due to the PDC. However, the trees on Cleo rootstock failed to demonstrate any decline. Even though the trees on Cleo rootstock clearly exhibited the greatest number of dead tree sites, all the mortality occurred prior to this study and most mortality was located at both ends of the beds where drainage was most difficult to accomplish. The trees on Swingle rootstock demonstrated a dramatic decline both statistically and visually. It is well documented that Swingle rootstock exhibits a tolerance to *P. nicotianae*, while Cleo is susceptible, so any decline by Swingle would be less likely due to this pathogen (Castle et al., 1993; Widmer et al., 1998). The trees planted on Swingle citrumelo had higher *P. palmivora* levels and declined significantly over the six-month period, while the condition of the trees on Cleopatra mandarin remained the same with relatively low levels of *P. nicotianae* (Tables 1 and 3). This observation is consistent with reports indicating that Swingle rootstock is very susceptible to *P. palmivora* in heavy, wet soils where *D. abbreviatus* is abundant, which were the conditions found at this study site (Graham, 1998). However, Cleo rootstock was also reported to be susceptible (Graham et al., 1997). Since all the trees in the K-20 study site received semi-annual, full label rate applications of mefenoxam for *Phytophthora* spp. control, this could explain the lack of any changes in tree condition due to *P. nicotianae*. The likelihood that *P. nicotianae* was not a causal factor in the differences in tree condition between the two rootstocks is consistent with the low Tau values for the degree of association of this pathogen vs. tree condition for both rootstocks. Likewise, an asymmetrical abundance of *D. abbreviatus* within each of the two rootstocks was not observed and therefore not considered as a possible factor affecting tree condition. The explanation for the apparent lack of susceptibility by Cleo rootstock to *P. palmivora* is puzzling unless this rootstock is selectively tolerant, or less susceptible, to *P. palmivora* while susceptible to *P. nicotianae*. Thereby, fenoxam would control *P. nicotianae* while relying on resistance for *P.*

Table 3. Relative abundance of *Phytophthora* spp in soil from two citrus rootstocks in the K-20 citrus grove sampled 5 Apr. 2000, Kerr Center, Indian River County, Florida.

		Propagules/cm³ Soil[z]	
Tree Variety/Rootstock	No. of Trees	*Phytophthora nicotianae*	*Phytophthora palmivora*
'Nova' tangelo/Cleopatra mandarin	27	193 (±169) a	4 (±14) b
'Sunburst' tangerine/Swingle citrumelo	65	262 (±234) a	22 (±59) c

[z]Means (±S.D.) followed by the same letter are not significantly different (Mann-Whitney U-test, 5% level).

Table 4. Associations with *Diaprepes abbreviatus* abundance, *Phytophthora palmivora* and *P. nicotianae* propagule levels, and tree condition (17 May 2000) for two citrus varieties/rootstocks[z], Kerr Center, Indian River County, Florida.

	Kendall's Rank Correlation Coefficient (Tau)	
Variables	'Sunburst' tangerine/Swingle citrumelo	'Nova' tangelo/Cleopatra mandarin
D. abbreviatus vs. *P. nicotianae*	0.246[y]	0.081
D. abbreviatus vs. *P. palmivora*	-0.157	0.040
Tree condition vs. *D. abbreviatus*	0.011	0.152
Tree condition vs. *P. nicotianae*	0.024	-0.093
Tree condition vs. *P. palmivora*	-0.327[y]	-0.210
P. palmivora vs. *P. nicotianae*	0.003	-0.008

[z]n = 65 for 'Sunburst' tangerine, n = 27 for 'Nova' tangelo.
[y]Kendall's Rank Correlation Coefficient (Tau), significant at 5% level.

Figure 3. GIS view showing tree condition assessed on 17 May 2000 as colored dots superimposed over colored asterisks representing three levels of adult *Diaprepes abbreviatus* density determined by Tedders trap capture from 99 sites, 28 Feb. to 26 June 2000, Kerr Center, Indian River County, Florida.

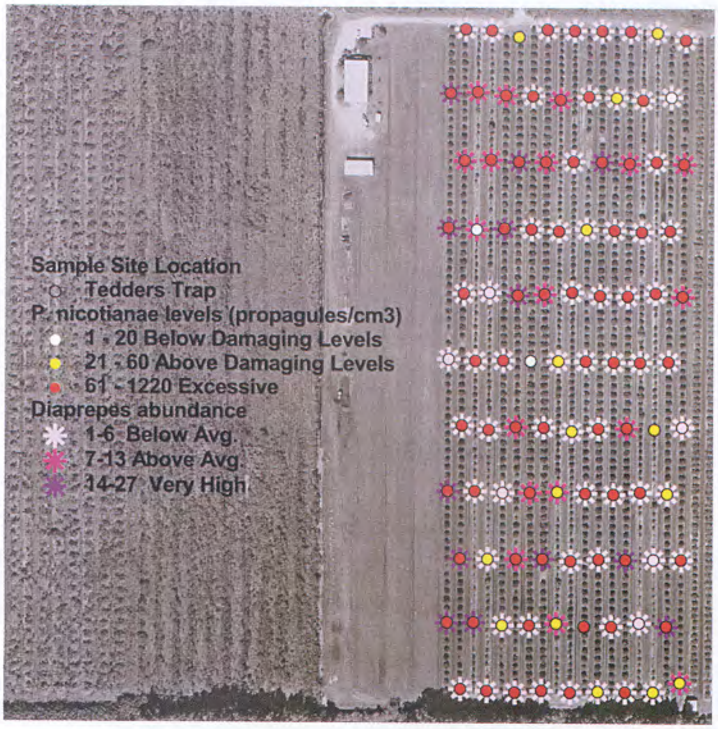

Figure 4. GIS view representing three increasing levels (propagules/cm^3 of soil) of *Phytophthora nicotianae* sampled 5 Apr. 2000 from 99 sites, as colored dots superimposed over colored asterisks representing three levels of adult *Diaprepes abbreviatus* density determined by Tedders trap capture from 28 Feb. to 26 June 2000, Kerr Center, Indian River County, Florida.

palmivora. Prior to this study, the trees on Cleo rootstock were rapidly declining and several died, but when semi-annual applications of fenoxam commenced in 1997, they responded

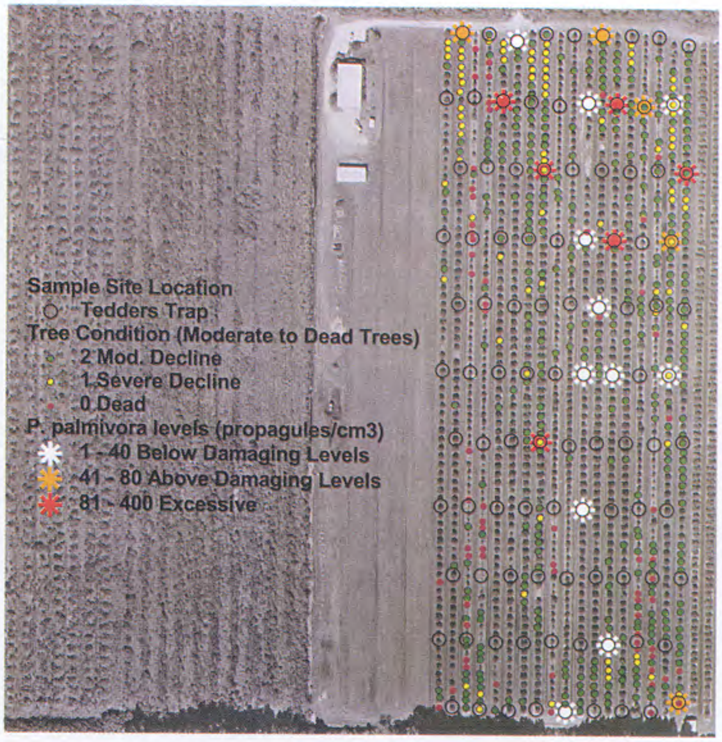

Figure 5. A GIS view representing dead, severe, and moderate declined trees (17 May 2000) located as colored dots superimposed over colored asterisks representing three increasing levels (propagules/cm^3 of soil) of *Phytophthora palmivora*, sampled 5 Apr. 2000 from 99 sites, Kerr Center, Indian River County, Florida.

dramatically and improved in appearance and production. By contrast, the trees on Swingle rapidly declined due to attack of their structural root system by *P. palmivora*. This is confirmed by the association for tree decline and high populations of *P. palmivora* and by the apparent lack of control of the structural root damage by mefenoxam.

The observed higher incidence of tree decline by the trees on Swingle rootstock near the end of the furrows, as evident by the GIS views, is probably due to poor drainage typically observed in this area of bedded groves. This was also the same region that the trees on Cleo rootstock died prior to 1997. The general decline observed in the northeast corner was also the same area exhibiting both a wide occurrence and high levels of *P. palmivora*. This could be due to a soil characteristic common to this area and should be investigated further. In contrast, *P. nicotianae* levels were uniformly high throughout the grove. This can be explained by the exceptionally dry Winter-Spring 2000 conditions which created unfavorably dry conditions that were not conducive for *P. palmivora*, while *P. nicotianae* was relatively unaffected. The dry weather which is unfavorable for *P. palmivora* would also explain why there was no association or competition between the two *Phytophthora* spp. This explanation will be confirmed by resampling for both *Phytophthora* spp. at the end of the summer rainy season.

The explanation for trees on Swingle rootstock expressing a negative association between tree condition and *P. palmivora* is probably due to the aggressive root damage inflicted on larger structural roots by *P. palmivora*, resulting in girdling and leading to the collapse of all the roots outward from the crown of the tree. At this stage, the moderate and severely declined trees cannot support high levels of *P. palmivora* due to a lack of a food source. Likewise, similarly infected trees with *P. palmivora* would not be able to support high populations of *D. abbreviatus* due to a lack of root tissue for larval feeding. This association was not significant in this study, possibly because of the low occurrence of *P. palmivora* caused by the unusually dry weather encountered during this study. The spatial distribution pattern for *D. abbreviatus* revealed lower abundance at both the North and South ends of the grove. Both these ends abut up to dirt access roads with daily farm equipment traffic and are bordered with *Casuarina* spp. wind breaks, either of which might deter weevils.

Our results attest to the importance of the development of alternative rootstocks with greater resistance than Swingle to *Phytophthora* spp., for the management of the *Phytophthora*-*Diaprepes* complex in flatwoods soils. This pilot study demonstrated the capability of a GIS to view spatial relationships between *D. abbreviatus*, two pathogens, and tree condition for two rootstocks. Geo-statistical analysis is planned to determine the spatial significance of these relationships. Future work is required to increase the agricultural database by including more biotic and soil parameters into the GIS to determine their interactions and relationships as a means to develop an effective IPM strategy for *D. abbreviatus*.

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