Management of Almond Leaf Scorch Disease: Long-Term Data on Yield, Tree Vitality, and Disease Progress

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Abstract

Sisterson, M. S., Ledbetter, C. A., Chen, J., Higbee, B. S., Groves, R. L., and Daane, K. M. 2012. Management of almond leaf scorch disease: Long-term data on yield, tree vitality, and disease progress. Plant Dis. 96:1037-1044.

Almond leaf scorch disease (ALSD) has been a chronic problem for California almond growers. This disease is caused by the bacterial pathogen *Xylella fastidiosa* and is transmitted by xylem-feeding insects. Previous research suggested that retaining, rather than roguing, ALSD-affected trees may be more economically beneficial because ALSD-affected trees produced a reasonable yield and did not die over a 3-year period. Because almond orchards are kept in production for approximately 25 years, longer-term data are needed to fully evaluate the merits of retaining ALSD-affected trees. Extension of yield evaluations from 3 to 5 years demonstrated that yield loss due to ALSD was consistent over 5 years, with yields of ALSD-affected trees reduced by 20 and 40% compared with unaffected trees for 'Nonpareil' and 'Sonora', respectively. To assess risk of ALSD-affected trees serving as a source of inocula for secondary (tree-to-tree) spread and to evaluate vitality of ALSD-affected trees, previous surveys of two orchards were extended from 3 to 6 or 7 years. The relationship between disease incidence (percentage of trees infected) and survey year was linear for all cultivars examined at both orchards. Furthermore, at each orchard, the spatial location of infections detected after the first survey was random with respect to the spatial location of infections identified during the first survey, suggesting that ALSD-affected trees retained in orchards did not serve as a source for secondary spread. Over the 6- to 7-year study period, death of ALSD-affected trees was rare, with only 9% of ALSD-affected trees dying. Because orchards used in this study had relatively high disease incidence, 61 orchards containing Sonora were surveyed to determine typical levels of ALSD incidence. ALSD was widespread, with at least one infected tree in 56% of orchards surveyed, but incidence was typically low (mean incidence = 0.47%). Collectively, the results suggest that retaining ALSD-affected trees may be economically beneficial in older orchards.

The majority of the world's almond crop is produced in the Central Valley of California. For California almond growers, almond leaf scorch disease (ALSD) has been a chronic problem for more than 60 years (1). This disease is caused by the xylem-limited bacterium, *Xylella fastidiosa* (10). Various strains of *X. fastidiosa* cause disease in a wide variety of cultivated and ornamental plants, including grape, alfalfa, and oleander (11). In addition, *X. fastidiosa* can be found in weeds grown in and near agricultural sites (23). The pathogen is transmitted by xylem-sap-feeding insects (20), and the green sharpshooter (*Draeculacephala minerva*) appears to be the principal vector of *X. fastidiosa* in California's Central Valley (6). The green sharpshooter is abundant in permanent pastures and weedy alfalfa fields (6,19,25). Movement of green sharpshooters into almond orchards is incidental because almond is not a preferred host (19), although grassy weeds in and around almond orchards can sustain green sharpshooter populations (6).

Because almond trees are not a primary host for green sharpshooters, risk of ALSD may be reduced by distancing orchards from green sharpshooter habitats and by removing weeds that support vectors in and around orchards (6,25). Risk of ALSD can also be reduced by cultivar selection because cultivars vary in their susceptibility, with 'Sonora' being one of the more susceptible cultivars (4,24). If ALSD-affected trees are found in orchards, growers must decide to retain or remove infected trees. Historically, ALSD-affected trees were reported to decline and die over a 3- to 8-year period (1,18,21). If ALSD-affected trees die, the decision to remove them is simple because they have no value. However, Sisterson et al. (24) monitored yields of ALSD-affected trees over a 3-year period and found that ALSD-affected trees produced reasonable yields and did not die during the study period. If ALSD-affected trees produce reasonable yields over a long period, there is incentive to retain rather than remove ALSD-affected trees. The economic benefit of retaining ALSD-affected trees must be balanced against the risk of ALSD-affected trees serving as a source for secondary (tree-to-tree) spread of *X. fastidiosa*. Studies conducted over a 1- to 3-year period suggest that risk of secondary spread is low (4,9,24). Because orchards are kept in production for approximately 25 years (7), longer-term data are needed to better assess the risk of infected trees serving as a source for secondary spread and to determine whether ALSD-affected trees continue to produce a reasonable yield over the long term.

Sisterson et al. (24) compared yields of ALSD-affected and unaffected trees over a 3-year period, short of the 3- to 8-year period over which ALSD-affected trees were anecdotally reported to decline (1,18,21). In this study, yield evaluations of Sisterson et al. (24) were extended to 5 years. In addition, some growers reported that yields of unaffected trees located next to ALSD-affected trees compensated for yield loss due to ALSD. In this scenario, unaffected trees located next to ALSD-affected trees developed larger canopies due to reduced growth of ALSD-affected trees. To test this hypothesis, yields of unaffected trees located next to ALSD-affected trees were compared with yields of unaffected trees that were located next to unaffected trees.

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Accepted for publication 10 February 2012.

http://dx.doi.org/10.1094/PDIS-08-11-0693-RE

If ALSD-affected trees are retained in orchards, it is critical that they do not serve as a source of inocula for secondary pathogen spread. Sisterson et al. (24) reported results of orchard surveys conducted from 2003 to 2005, providing data on disease progress over 3 years. For this study, orchards were resurveyed in 2008 and 2009, extending these surveys to a period of 6 or 7 years (depending on year of first survey). Results were analyzed to describe disease progress, assess effects of retaining infected trees on pathogen spread, and assess extent of tree mortality over the study period. The orchards used in this study were selected due to their relatively high incidence of ALSD. To better describe typical levels of ALSD incidence in the San Joaquin Valley of California, results from surveys assessing incidence of ALSD at 61 orchards over a 2-year period are reported.

Materials and Methods

Study sites. Studies were conducted at the same three orchards using the same site names (orchards A, B, and C) as used by Sisterson et al. (24). All three orchards were located in the San Joaquin Valley of California. Orchard A was located in Fresno County, established in 1989, and removed from production in 2009. Orchard A consisted of alternating rows of almond, *Prunus dulcis* (Mill.) D. A. Webb, 'NePlus' and Sonora planted with 6.7 m between rows and 4.9 m within rows. Orchard B was located in Fresno County, established in 1990, and removed from production in 2007. Orchard B consisted of rows of 'Nonpareil' between rows of 'Carmel' and Sonora planted with 7.9 m between rows and 6.7 m within rows. Orchard C was located in Kern County, established in 1996, and remains in production. Orchard C consisted of rows of Nonpareil between rows of 'Fritz' and Sonora planted with 7.3 m between rows and 6.1 m within rows. Orchards were managed by growers using conventional practices.

Yield evaluations. Yield evaluations were completed at all three sites (orchards A, B, and C) in 2007. Because orchard B was removed from production at the end of the 2007 production cycle, yield evaluations in 2008 were completed at orchards A and C only. Yields of Sonora were evaluated at all three orchards. Yields of Nonpareil were evaluated at orchard C only. Within cultivar, yields of three categories of trees were evaluated: yields of ALSD-affected trees, yields of unaffected trees located next to unaffected trees, and yields of unaffected trees located next to ALSD-affected trees.

For each year (2007 and 2008), site (orchard A, B, and C), cultivar (Sonora and Nonpareil), and tree category (ALSD-affected, unaffected next to unaffected, and unaffected next to ALSD-affected), kilograms of kernel produced per tree was estimated for 10 trees. This was accomplished by hand harvesting, largely following the methods described by Sisterson et al. (24). Kilograms of kernel produced per tree were compared within cultivars and years using analysis of variance (22). Yield data were log transformed to homogenize variances. For Sonora, the statistical model included tree category, orchard, and their interaction. For Nonpareil, the statistical model included only tree category. Contrasts were used to evaluate the significance of planned pairwise comparisons. To evaluate yield loss due to ALSD, yields of ALSD-affected trees were compared with yields of unaffected trees located next to unaffected trees. To evaluate compensation effects, yields of unaffected trees next to unaffected trees were compared with yields of unaffected trees next to ALSD-affected trees.

Disease progress. Orchards A, B, and C were initially surveyed for ALSD-affected trees between 2003 and 2005. Results from the first survey were reported by Groves et al. (9) and results from all 3 years were reported by Sisterson et al. (24). Orchards A and C were resurveyed in 2008 and 2009. Orchard B was not resurveyed because it was removed from production in 2007. Because orchard A was first surveyed in 2004, resurveying in 2008 and 2009 extended this analysis to 6 years. Because orchard C was first surveyed in 2003, resurveying in 2008 and 2009 extended this analysis to 7 years.

Methodology for surveys in 2008 and 2009 was similar to those used by Groves et al. (9) and Sisterson et al. (24). Briefly, near harvest time, two observers walked each row of the orchard, observing each tree from opposite sides of the row. Each tree was evaluated for presence of visual leaf scorching symptoms. In addition, if trees were removed, replanted, or dead, this was recorded. Leaf samples from trees displaying leaf scorching symptoms were collected and returned to the laboratory to confirm the presence of *X. fastidiosa* using polymerase chain reaction (PCR) methodology similar to that reported by Sisterson et al. (25). All samples collected from trees displaying leaf scorching symptoms but not previously identified as infected with *X. fastidiosa* were subjected to diagnostic testing (136 trees). To limit the number of samples screened, only a subset of samples taken from trees identified as infected by Groves et al. (9) and Sisterson et al. (24) were reevaluated. Specifically, if a tree was previously confirmed as infected with *X. fastidiosa* and leaf scorching symptoms were clearly present, testing was considered unnecessary. Nonetheless, 93 (44% of all previously identified ALSD-affected trees) samples from trees previously identified as infected with *X. fastidiosa* were collected and used as internal positive controls for PCR-based screening. If symptoms were absent from a tree previously identified as infected with *X. fastidiosa* (six trees), testing was considered necessary. Finally, 109 samples were randomly collected from trees without ALSD symptoms to serve as internal negative controls.

To describe disease progress, a linear model was fit to the relationship between survey year and disease incidence (percentage of trees infected) for each cultivar–orchard combination (22). To evaluate the extent to which primary versus secondary spread of *X. fastidiosa* may have occurred, results for Sonora were further analyzed. Specifically, the fit of a monomolecular and an exponential model to the disease progress data for Sonora at orchards A and C were compared. The monomolecular model describes disease progress in systems with only primary spread, whereas the exponential model describes disease progress in systems with secondary spread (16). Models were fit using JMP (22) and the model that produced the lower value for the sum of squares for error (SSE) was deemed to better describe the data. During the model-fitting process, assumptions regarding disease incidence at time of orchard establishment were made. To fit the monomolecular model, disease incidence was assumed to be zero at time of orchard establishment. To fit the exponential model, orchards were assumed to contain a single infected tree at time of orchard establishment. The robustness of results generated under assumptions of low or zero initial disease incidence was evaluated by incrementally increasing initial disease incidence and refitting the models.

To assess risk of tree death due to ALSD and to evaluate the extent to which growers removed infected trees, the status of trees identified as ALSD-affected or unaffected in the first survey (2003 for orchard C and 2004 for orchard A) was compared with their status at the end of the study (2009). Specifically, the percentages of trees identified as healthy or infected during the first survey that subsequently died or were removed were compared for each cultivar at each site using Fisher's Exact Test.

To determine whether retaining ALSD-affected trees affected the risk that neighboring trees became infected, the proximity of infections detected after the first surveys to trees identified as infected during the first surveys was evaluated at each orchard using a Monte-Carlo simulation (17). To accomplish this, ALSD-affected trees were placed into two categories: initial infections and new infections. Initial infections consisted of ALSD-affected trees that were identified during the first surveys (2003 for orchard C and 2004 for orchard A) and retained in orchards throughout the study. New infections consisted of ALSD-affected trees identified after the first surveys. This information was used to calculate the observed number of new infections that arose in the same row within a distance of one to five trees from an initial infection. The observed values were compared with distributions generated, assuming that new infections were distributed randomly throughout each orchard. To generate such distributions, a grid consisting of the x,y spatial coordinates of all trees in each orchard was created in a program written in C++ (Microsoft Visual C++). The locations of

trees in the initial infection category were then input into the program. Subsequently, the program assigned the appropriate number of new infections to randomly selected x,y coordinates. The number of randomly assigned new infections that occurred in the same row within a distance of one to five trees from an initial infection was determined. This process was repeated 5,000 times for each distance and 95% confidence intervals were constructed. The 95% confidence intervals represent the range of values expected if new infections were distributed randomly throughout orchards. If the observed number of new infections that were in proximity to initial infections falls within the 95% confidence interval, the hypothesis that the spatial location of new infections was random with respect to the spatial location of initial infections cannot be rejected.

Large-scale evaluation of ALSD incidence. To better understand typical levels of ALSD incidence, 61 orchards in Kern County were surveyed for the presence of ALSD-affected trees in fall 2006 and fall 2007. Some orchards were surveyed in both years and some in only 1 year. All surveyed orchards contained Sonora and only rows containing Sonora were surveyed. In total, 46,159 trees were examined. Due to the number of trees examined over a short period of time, survey methodology was streamlined. Workers drove slowly down each row on an all-terrain vehicle, observing each tree. If symptoms of ALSD were present, a sample was collected and returned to the laboratory. To determine whether trees showing symptoms of ALSD were infected with *X. fastidiosa,* the collected leaf samples were subjected to culturing following the methods of Chen et al. (5).

Results

Yield evaluations. The yield study was designed to make two comparisons. The first comparison evaluated yield loss due to infection by comparing yields of ALSD-affected trees to unaffected trees. The second comparison evaluated compensation effects and compared yields of unaffected trees that were next to ALSD-affected trees with unaffected trees next to unaffected trees. For Sonora, there was a significant effect of orchard in both years of study, with greater yields at orchard C compared with orchards A and B (2007: $F = 47.4$, df = 2, 78, $P < 0.0001$; 2008: $F = 236.3$, df = 1, 53, $P < 0.0001$; Fig. 1A–C). Orchards A and B were in their final years of production during the study and were established 5 to 6 years before orchard C. Tree category significantly affected yield of Sonora trees during both years of study (2007: $F = 20.5$, df = 2, 78, $P < 0.001$; 2008: $F = 16.5$, df = 2, 53, $P < 0.0001$), with a significant tree category–orchard interaction in 2007 ($F = 4.84$, df = 4,

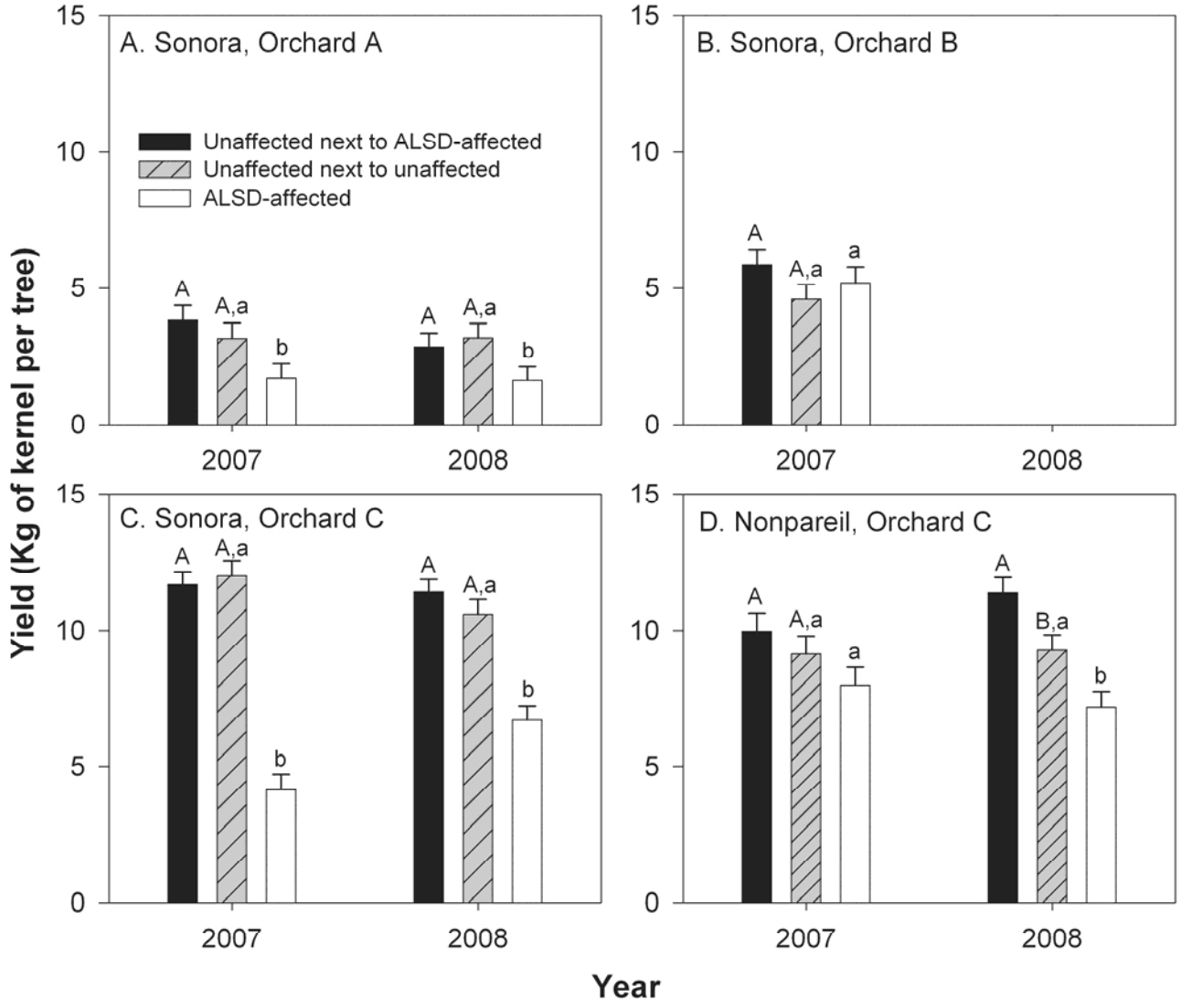

Fig. 1. Least squares mean (± standard error) kilograms of kernels produced by unaffected trees located next to almond leaf scorch disease (ALSD)-affected trees, unaffected trees located next to unaffected trees, and ALSD-affected trees. **A,** Results for 'Sonora' at orchard A. **B,** Results for Sonora at orchard B. Orchard B was removed from production at the end of the 2007 production cycle. **C,** Results for Sonora at orchard C. **D,** Results for 'Nonpareil' at orchard C. Different letters above bars indicate significant differences. Uppercase letters refer to contrasts comparing the two unaffected tree groups, whereas lowercase letters refer to contrasts comparing ALSD-affected trees with unaffected trees.

78, $P = 0.0015$) but not in 2008 ($F = 0.56$, df = 2, 53, $P = 0.57$). Yields of ALSD-affected Sonora trees were significantly lower than yields of unaffected trees in both years of study at orchards A and C (Fig. 1A and C). However, yields of ALSD-affected Sonora trees at orchard B were not significantly lower than yields of unaffected trees in 2007, the final year of production for orchard B (Fig. 1B). Averaged over years and orchards, yields of ALSD-affected Sonora trees were 37% lower than yields of unaffected trees, similar to the 40% yield loss reported by Sisterson et al. (24) for Sonora trees from the same orchards during 2004 to 2006. Yields of unaffected Sonora trees located next to ALSD-affected trees were not significantly greater than yields of unaffected Sonora trees located next to unaffected trees in either year of study (Fig. 1A–C).

For Nonpareil, yields were not significantly affected by tree category in 2007 ($F = 2.07$, df = 2, 27, $P = 0.15$) but yields were significantly affected by tree category in 2008 ($F = 12.7$, df = 2, 27, $P < 0.0001$; Fig. 1D). Averaged over years, yields of ALSD-affected Nonpareil trees were 17% lower than unaffected trees, similar to the 19% yield loss reported for Nonpareil by Sisterson et al. (24) at the same orchard during 2004 and 2005. Yields of unaffected Nonpareil trees located next to ALSD-affected trees were significantly greater than yields of unaffected Nonpareil trees located next to unaffected trees in 2008 but not in 2007 (Fig. 1D). Averaged over years, yields of unaffected Nonpareil trees located next to ALSD-affected trees were 1.16 times greater than yields of unaffected Nonpareil trees located next to unaffected trees.

Detection of *X. fastidiosa* in samples from orchard surveys. In 2008 and 2009, samples from 345 trees were subjected to PCR-based screening for presence of *X. fastidiosa.* Four types of samples were screened: trees with scorching symptoms that had not previously been identified as infected with *X. fastidiosa* (136 trees), trees with scorching symptoms that were previously identified as infected with *X. fastidiosa* (94 trees), trees without scorching symptoms that had not previously been identified as infected with *X. fastidiosa* (109 trees), and trees without scorching symptoms that had been previously identified as infected with *X. fastidiosa* (6 trees).

For trees with scorching symptoms that were not previously identified as infected with *X. fastidiosa,* 68% of samples (15 of 22 samples) from orchard A tested positive for *X. fastidiosa*, whereas 21% of samples (24 of 114 samples) from orchard C tested positive for *X. fastidiosa.* Low detection of *X. fastidiosa* in trees with scorching symptoms at orchard C may have been due to mistaking salt injury for scorching due to ALSD (26). Specifically, Fritz and

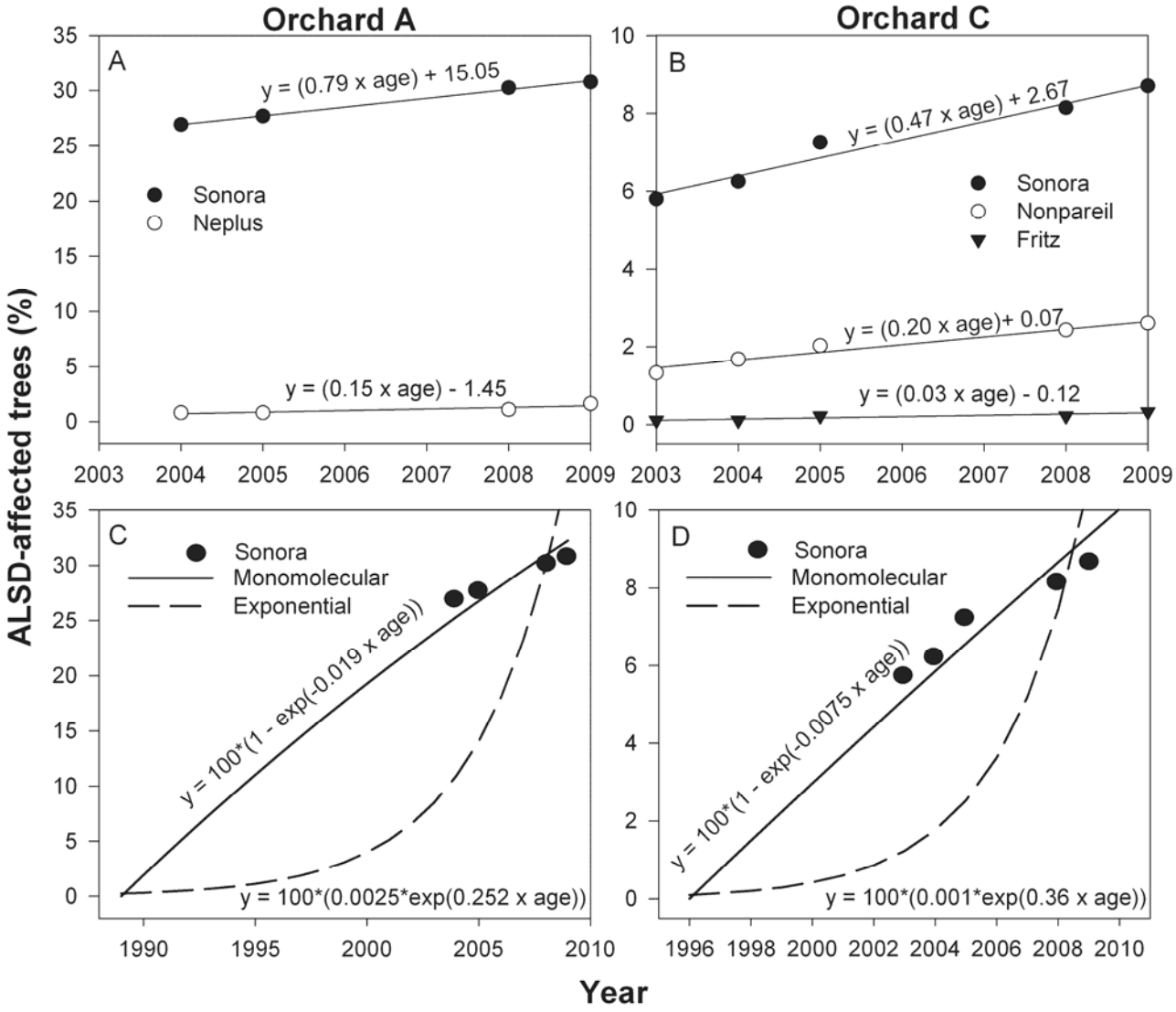

Fig. 2. Results of orchard surveys. Percentage of almond leaf scorch disease (ALSD)-affected trees at **A,** orchard A and **B,** orchard C. To evaluate the extent of primary versus secondary spread, the fit of a monomolecular and an exponential model to results for 'Sonora' at **C,** orchard A and **D,** orchard C were compared. For the curves shown, disease incidence at the time of orchard establishment (1989 for orchard A and 1996 for orchard C) was assumed to be zero for the monomolecular model and, for the exponential model, a single infected tree was assumed to be present. For calculations of percentage of ALSD-affected trees in each year, ALSD-affected trees that were removed were counted as infected in calculations for subsequent years.

Nonpareil are reported to be sensitive to salt injury, with Fritz being more susceptible than Nonpareil (12). In agreement, fewer samples with scorching symptoms from Fritz trees (only 1 of 49 samples tested positive for *X. fastidiosa*, or <2%) tested positive for *X. fastidiosa* compared with samples with scorching symptoms from Nonpareil trees (10 of 52 samples tested positive for *X. fastidiosa*, or nearly 20%) at orchard C. Relative sensitivity of Sonora to salt injury is unknown and 100% of samples (13 of 13) with scorching symptoms from Sonora trees tested positive for *X. fastidiosa* at orchard C.

For trees with scorching symptoms that were previously identified as infected with *X. fastidiosa*, 99% (93 of 94) were positive for *X. fastidiosa.* For trees without scorching symptoms that were previously identified as infected with *X. fastidiosa*, none (zero of five Sonora trees and zero of one Nonpareil trees) were positive for *X. fastidiosa* and may represent cases of winter curing (4,13). For trees without scorching symptoms that were not previously identified as infected with *X. fastidosa*, none (0 of 109) were positive for *X. fastidiosa.*

Disease progress. In general, linear models provided a good description of disease progress over the study period. Specifically, at orchard A, there was a significant linear relationship between year and disease incidence for Sonora ($F = 525$, df = 1, 2, $P = 0.0019$, $r^2 = 0.99$; Fig. 2A) although, for NePlus at orchard A, the linear relationship between survey year and disease incidence was not significant ($F = 7.71$, df = 1, 2, $P = 0.11$, $r^2 = 0.79$; Fig. 2A). At orchard C, there was a significant linear relationship between year and disease incidence for Fritz ($F = 12.85$, df = 1, 3, $P = 0.037$, $r^2 = 0.81$), Nonpareil ($F = 65.74$, df = 1, 3, $P = 0.004$, $r^2 = 0.96$), and Sonora ($F = 85.26$, df = 1, 3, $P = 0.003$, $r^2 = 0.97$; Fig. 2B).

The extent to which primary versus secondary spread of *X. fastidiosa* may have occurred was evaluated by comparing the fit of a monomolecular and an exponential model to the disease progress data for Sonora at orchards A and C. For the monomolecular model, disease incidence at the time of orchard establishment was assumed to be zero and for the exponential model a single infected tree was assumed to be present at the time of orchard establishment. Under such assumptions, the disease progress data for Sonora at orchards A and C were best described by a monomolecular model compared with an exponential model (SSE for the monomolecular model in orchard A = 0.0006, SSE for the exponential model in orchard A = 0.05; SSE for the monomolecular model in orchard C = 0.0002, SSE for the exponential model in orchard C = 0.007; Fig. 2C and D). To evaluate the robustness of this result, initial disease incidence was incrementally increased and models refitted (*results not shown*). At each orchard, values of SSE for both models decreased as initial disease incidence increased, with the monomolecular model generally providing a better fit than the exponential model. With initial disease incidence equal to approximately half of disease incidence on the first survey at each orchard, SSE values for monomolecular and exponential models were similar. This occurred because, with initial disease incidence equal to approximately half of disease incidence on the first survey at each orchard, both models essentially described a straight line over the time period between orchard establishment and the last survey.

Mortality and removal of ALSD-affected and unaffected trees. To evaluate risk of tree death due to ALSD and to assess the extent to which growers removed ALSD-affected trees, status of trees during the first survey (2003 for orchard C; 2004 for orchard A) was compared with their status in the last survey (2009). Summed across cultivars and orchards, tree death was proportionally more common for ALSD-affected trees than unaffected trees (Fisher's Exact Test, $P < 0.0001$; Table 1). In general, tree death was more common at orchard A compared with orchard C (Table 1). The relatively high mortality of unaffected trees at orchard A suggested the presence of an unidentified mortality factor at orchard A that ALSD-affected trees may have been more susceptible to than unaffected trees.

Summed across cultivars and orchards, proportionally more ALSD-affected trees were removed than unaffected trees (Fisher's Exact Test, $P < 0.001$; Table 1). At orchard A, the proportion of ALSD-affected and unaffected trees removed was similar (Table 1). In contrast, the proportion of ALSD-affected trees removed at orchard C was substantially larger than the proportion of unaffected trees removed (Table 1). Discussions with managers of orchard C indicated that trees with ALSD symptoms were specifically targeted for removal.

Proximity of new infections to ALSD-affected trees identified during the first survey. There were an insufficient number of new infections in NePlus at orchard A and Fritz at orchard C to warrant analysis (Fig. 2). Consequently, this analysis was limited to Sonora at orchards A and C and Nonpareil at orchard C.

The number of infections that arose within a distance of one to five trees from an ALSD-affected tree identified during the first survey was within the 95% confidence interval generated assuming that new infections were distributed randomly throughout the orchard for Sonora at orchards A and C as well as for Nonpareil at orchard C (Fig. 3). Accordingly, the distribution of infections identified after the first survey was random with respect to the spatial locations of ALSD-affected trees identified during the first survey for all cultivar–orchard combinations examined.

Large-scale evaluation of ALSD incidence. In total, 217 Sonora trees with scorching symptoms were identified in the large-scale survey, with 134 confirmed positive for *X. fastidiosa* via culturing. ALSD was widespread, with at least one infected tree in 56% (34 of 61 orchards) of the orchards surveyed (Fig. 4). Although ALSD was widespread, incidence was low (0 to 1.4%), with a mean incidence in affected orchards of 0.47% of trees.

Discussion

In perennial cropping systems, long-term data are often required to make informed management decisions. Historically, ALSD-

Table 1. Number and percentage of almond leaf scorch disease (ALSD)-affected and unaffected trees that were removed or died between the first survey (2004 for orchard A and 2003 for orchard C) and the last survey in 2009

	Number identified during first survey[a]		Number (%) removed by 2009[b]		Number (%) dead by 2009[b]	
Orchard, cultivar	**With ALSD**	**Without ALSD**	**With ALSD**	**Without ALSD**	**With ALSD**	**Without ALSD**
A						
NePlus	3	358	0 (0)	11 (3)	0 (0)	14 (4)
Sonora	103	268	12 (12)	35 (13)	**15 (15)**	**11 (4)**
Total	106	626	12 (11)	46 (7)	**15 (14)**	**25 (4)**
C						
Fritz	1	893	0 (0)	27 (3)	**1 (100)**	**1 (0.1)**
Nonpareil	24	1677	0 (0)	6 (0.4)	0 (0)	0 (0)
Sonora	52	814	**40 (77)**	**29 (4)**	0 (0)	1 (0.1)
Total	77	3384	**40 (52)**	**62 (2)**	1 (1)	2 (0.1)
A and C						
Total	183	4010	**52 (28)**	**108 (3)**	**16 (9)**	**27 (1)**

[a] Trees identified as infected after the first survey were excluded from this analysis.
[b] Pairs in bold were significantly different (Fisher's Exact Test).

affected trees were anecdotally reported to decline and die over a 3- to 8-year period (1,18,21). Monitoring of ALSD-affected trees in the current study found that 91% of ALSD-affected trees survived over a 6- to 7-year period (Table 1). Yield evaluations conducted here and by Sisterson et al. (24) found that yields of ALSD-affected trees were, on average, 20 to 40% lower than yields of unaffected trees (Fig. 1) and that relative yield loss was consistent over a 5-year period. Because the risk of tree death due to ALSD was low and infected trees produced reasonable yields, ALSD-affected trees may be more productive than replacement trees in some cases. The decision to retain ALSD-affected trees is associated with some degree of risk because infected trees could serve as a source of inocula for secondary (tree-to-tree) spread. Analysis of disease progress and spatial clustering of infections at two orchards suggests that the risk of tree-to-tree spread of *X. fastidiosa* is low (Figs. 2 and 3).

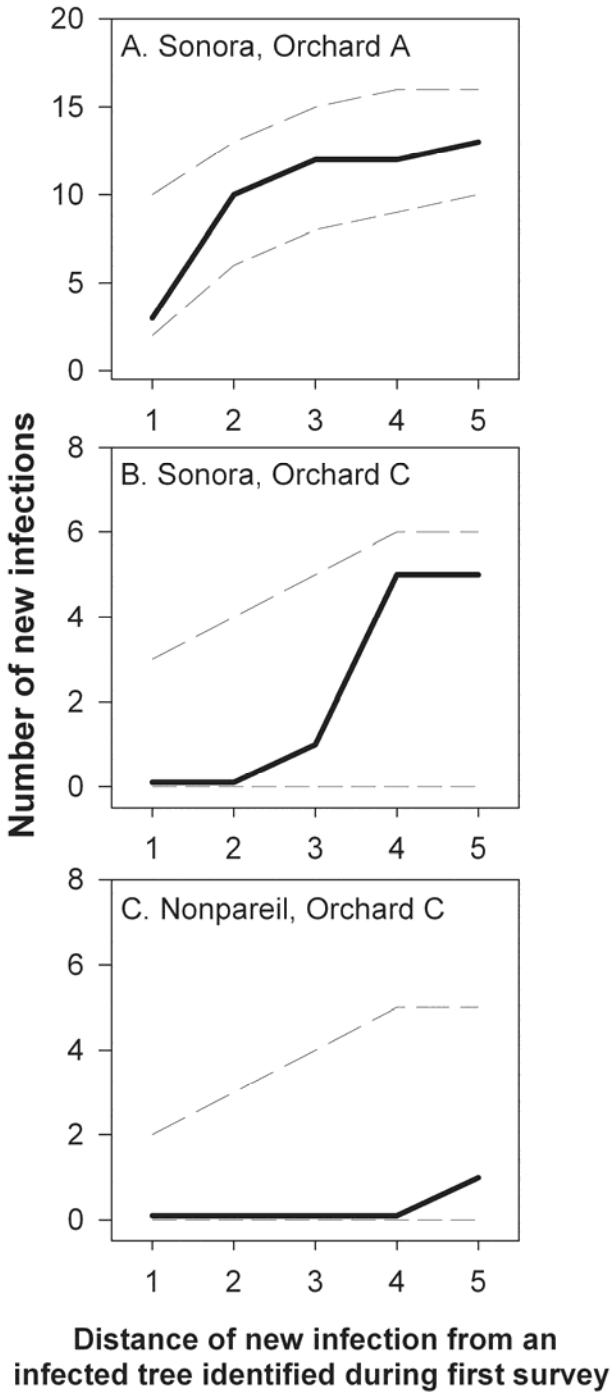

Fig. 3. Comparison of the observed (solid black line) number of new infections (infections detected after the first survey) that arose within a specified distance from an almond leaf scorch disease-affected tree that was identified during the first survey and retained throughout the study period. **A,** Results for 'Sonora' trees at orchard A. **B,** Results for Sonora trees at orchard C. **C,** Results for 'Nonpareil' trees at orchard C. Dashed lines indicate upper and lower bounds of 95% confidence intervals generated assuming that new infections were randomly distributed throughout the orchard.

There are two phases to ALSD management. The first phase focuses on preventing trees from becoming infected, whereas the second phase focuses on management decisions after trees have become infected. Orchard surveys and controlled studies indicate that there is considerable variation in susceptibility to ALSD among almond cultivars (4,9,24). As a result, risk of ALSD could be mitigated by cultivar selection. However, because ALSD incidence is typically low (Fig. 4), susceptibility to ALSD is unlikely to be a primary factor governing cultivar selection. For example, Nonpareil is the most popular almond cultivar in California due to its high market value and horticultural qualities (15) which are likely to be viewed as more important than its moderate susceptibility to ALSD. Regardless, in areas with a history of ALSD, cultivar selection is perhaps the simplest management approach.

A survey of 61 orchards found that ALSD was widespread but, as stated above, incidence was typically low (Fig. 4). In agreement, Daane et al. (6) detected at least one ALSD-affected tree in 9 of 10 orchards surveyed, with an average incidence of 0.45%. Although incidence of ALSD across orchards is typically low, ALSD incidence in individual orchards can be high. For example, Cao et al. (4) reported >15% incidence in Peerless and Nonpareil in a single orchard in the Sacramento Valley. Similarly, incidence of ALSD in orchards used in this study was relatively high (Fig. 2). Correlative studies that provide clear linkages between risk factors and elevated incidence of ALSD are lacking, although risk of ALSD is presumed to increase with proximity to permanent pastures and weedy alfalfa fields that may harbor green sharpshooters and *X. fastidiosa* (6,19,25). Observationally, orchard A bordered a permanent pasture that harbored a large green sharpshooter population (6), orchard B was adjacent to a weedy alfalfa field that harbored green sharpshooters and *X. fastidiosa* (25), and orchard C was in proximity to weedy alfalfa fields that harbored green sharpshooters (25).

Prior to any decision to retain or remove ALSD-affected trees, infected trees must first be accurately identified. Scorched leaves are the primary symptom associated with ALSD but infection by *X. fastidiosa* is not the only factor that can cause scorching symptoms on almond trees. Teviotdale and Connell (26) warned of mistaking scorching from salt injury for ALSD. Results from our surveys reinforce this observation. At orchard C, 79% of trees with a scorching symptom were negative for *X. fastidiosa,* with a large

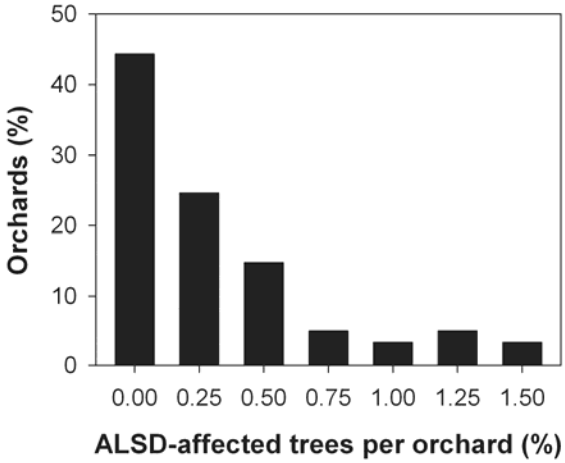

Fig. 4. Histogram of the percentage of almond leaf scorch disease-affected 'Sonora' trees detected at 61 orchards in Kern County, CA.

proportion of those samples taken from Fritz, which is reported to be sensitive to salt injury (12). The scorching symptoms observed on Fritz trees were not visually consistent with ALSD but diagnostic testing was required to confirm this suspicion. Accordingly, before management action is taken, confirmation of infection with *X. fastidiosa* is critical.

Once an ALSD-affected tree is identified, growers must decide to retain or remove the infected tree. Because almond orchards are typically kept in production for approximately 25 years (7), removed trees could be replanted. An alternative to replanting is top-working (regrafting), where only the scion is replaced. Currently, there is a lack of information to determine whether top-working is a viable strategy. However, top-working has an advantage over replanting in that top-worked trees return to normal production faster than replants (3). Furthermore, recent research suggests that peach and peach-almond rootstocks commonly used by almond growers may be resistant to *X. fastidiosa* (14), alleviating any epidemiological reason for removing the root system. Additional research is needed to verify that removing infected scions and regrafting does not result in transfer of *X. fastidiosa* from resistant rootstocks to new scions.

Assuming that ALSD-affected trees are replanted, yields of replanted trees are expected to be low over the first 3 to 5 years (3,7). ALSD-affected trees produced, on average, 60 to 80% the yield of unaffected trees over a 5-year period; therefore, a key factor to consider when deciding to retain or remove ALSD-affected trees is orchard age (24). In older orchards, there may be insufficient time for replants to mature to the point where they produce yields equivalent to or greater than those of ALSD-affected trees. Furthermore, growth of replants in older orchards may be hampered by replant disorder (2) and shading. In contrast, in young orchards, there is ample time for replanted trees to mature before the orchard is removed from production. Furthermore, confidence in extrapolating results of this study beyond the 5- to 7-year study period declines with each additional year. Accordingly, the longer the period between infection and orchard termination, the greater the period for potential decline of ALSD-affected trees. Collectively, the results suggest that replanting is likely to be beneficial in young orchards but not in old orchards.

The yield benefits associated with retaining ALSD-affected trees must be balanced against the risk of ALSD-affected trees serving as a source of inocula for secondary (tree-to-tree) spread. Ultimately, the only way to guarantee that an ALSD-affected tree will not serve as a source of inocula is to remove it. Nonetheless, evidence from these orchard surveys suggests that risk of secondary spread is low. Specifically, the relationship between disease incidence and survey year was linear over the period for which data were collected (Fig. 2A and B), indicative of a simple interest disease (27). Furthermore, under the assumption of low disease incidence at the time of orchard establishment, disease progress for Sonora was better described by a monomolecular model than an exponential model (Fig. 2C and D), supporting the notion of a lack of secondary spread (16). Finally, the possibility of secondary spread was also assessed by evaluating the clustering of new infections near ALSD-affected trees identified during the first survey that were retained throughout the study period. Results indicated that the spatial distribution of infections detected after the first survey was random with respect to the location of infections identified during the first survey (Fig. 3). Accordingly, retaining ALSD-affected trees does not appear to increase the probability that neighboring trees would become infected. Regardless, if growers retain ALSD-affected trees, neighboring trees should be monitored for signs of secondary spread.

For analyses comparing the fit of a monomolecular and an exponential model to disease progress data for Sonora at orchards A and C (Fig. 2C and D), disease incidence at time of orchard establishment was assumed to be low. Because values for initial disease incidence are unknown, it is important to consider whether an assumption of low initial disease incidence is reasonable. Trees used to establish orchards are purchased from nurseries. Scions that have been grafted onto rootstocks are exposed to field conditions for less than 1 year before sale. Accordingly, nursery trees are exposed to, at most, 1 year of primary spread before sale. Using the annual rate of increase in the percentage of infected Sonora trees at orchard A as an upper limit (Fig. 2A), the percentage of infected Sonora trees received from nurseries could be expected to be 0 to 0.79%. Over this range, the relative fit of the monomolecular model was always better than that of the exponential model at both orchards. What are the consequences to the interpretation of model comparisons if initial disease incidence was not low? With relatively high initial disease incidence (half that of disease incidence on the first survey at each orchard), the monomolecular and exponential models were relatively linear over the time period between orchard establishment and the last survey, making it impossible to determine which model fits better. In this case, the simplest model to describe the data would be a straight line.

In addition to evaluating effects of ALSD on yield, the hypothesis that yields of unaffected trees that are located next to ALSD-affected trees compensate for yield loss due to ALSD was evaluated. Mean yields of unaffected trees next to ALSD-affected trees were often numerically greater than yields of unaffected trees next to other unaffected trees; however, this difference was generally not significant (Fig. 1). Although this lends some degree of support to the concept of yield compensation, such minor gains in yield are unlikely to compensate for losses due to ALSD.

The goal of this project was to provide the information needed to evaluate whether ALSD-affected trees should be retained or removed from orchards. Results indicated that the risk of tree death over a 6- to 7-year period was low and that ALSD-affected trees produce reasonable yields over a period of 5 years (Table 1; Fig. 1). The risk of secondary spread appears to be low (Figs. 2 and 3); therefore, retaining ALSD-affected trees may be economically beneficial in older orchards (24). Because *X. fastidiosa* causes disease in a wide array of plants, it is important to consider to what extent observations in almond are relevant to other diseases caused by *X. fastidiosa*. For example, Pierce's disease of grapevine is also caused by *X. fastidiosa*. Because Pierce's disease is considered lethal (8), there is little economic incentive to retain Pierce's disease-affected vines, regardless of their role as a source of inocula.

Acknowledgments

Partial funding was provided by the Almond Board of California. We thank T. de la Torre, D. Dwyer, E. Felts, F. Higuera, J. Magana, M. Schreiber, and S. Uchima for help with field and laboratory work; and R. Krugner, D. Stenger, and C. Wistrom for comments on an earlier draft of the manuscript.

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