# RELATIONSHIP BETWEEN RUST MITES *CALEPITRIMERUS VITIS* (NALEPA), BUD MITES *COLOMERUS VITIS* (PAGENSTECHER) (ACARI: ERIOPHYIDAE) AND SHORT SHOOT SYNDROME IN OREGON VINEYARDS

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**ABSTRACT** - Short Shoot Syndrome (SSS) causes severe crop losses in Oregon vineyards due to bunch necrosis during the early part of the season. Other symptoms include malformed leaves, unusually short and angled shoots, scar tissue and bronzed leaves close to harvest time. This work shows that SSS found in Oregon vineyards is closely associated with grape leaf rust mite, *Calepitrimerus vitis* (Nalepa) infestations. Very few bud mites, *Colomerus vitis* (Pagenstecher), were found in the vineyards during the current year and no relationship could be found between SSS symptoms and this species. During winter, rust mites are dormant and no evidence of direct bud damage from rust mites was found inside undeveloped buds. Tissue damage from mites was first observed between bud break and the two-leaf stage in mite infested vineyards. Rust mite colonies were found under outer bud scales and bark of canes close to the buds. Crop losses as high as 23.7% were directly linked to rust mite infestations and SSS from several vineyards sampled in Oregon.

**Key words -** Acari, Eriophyidae, *Calepitrimerus vitis* (Nalepa), *Colomerus vitis* (Pagenstecher), *Vitis vinifera,* crop loss, short shoot syndrome, vineyard, U.S.A.

## INTRODUCTION

Short Shoot Syndrome (SSS) of grape vines (*Vitis vinifera* L.) has become increasingly recognized as economically important in the Pacific Northwest of the United States. The first grower reports of SSS occurred in 2001 (Chambers, 2001) and similar symptoms were observed in Washington vineyards in 2006. Symptoms associated with SSS include shoot scarring, severely stunted growth, bunch loss and curled or malformed basal leaves (Fig. 1). However, no formal documentation is available for SSS in the Pacific Northwest. Grape vines showing SSS were found in a wide range of vineyards in the Pacific Northwest including the Milton-Freewater, Willamette, Rogue and Umpqua Valleys in Oregon and the Walla-Walla, Yakima Valley and Columbia Valley grape-growing areas in Washington State.

Symptoms of SSS are closely related to Restricted Spring Growth (RSG) symptoms described in Australian vineyards (Bernard *et al*., 2005). Growers reported complete crop losses due to the abortion of affected bunches in severe cases. In addition, crop quality was reduced due to partial necrosis of bunches resulting in unacceptably small bunches (Hluchy and Pospisil, 1992).

Several factors were attributed in Oregon and Australian vineyards as possible causes of SSS. In Australia, vine physiological factors were considered , such as chilling injury, herbicide damage, post-harvest water stress, shallow root zones, water logging during winter periods, differences in cultivar susceptibility, low carbohydrate reserves and response of young vines to over cropping and boron deficiency (Barnes, 1958; Bernard *et al*., 2005; Wilson and Hayes, 1996). Grapevine yellows phytoplasma was also implicated as a potential cause of RSG, but no positive correlation could be found between symptoms and phytoplasma positive PCR assays (Bonfiglioli *et al*., 1995, Padovan *et al.*, 1995, 1996). In Switzerland, thrips damage was associated with similar SSS symptoms

(Boller, 1984). In a five year study in Australia, the severity of symptoms showed correlations with populations of eriophyid mites, bud mite, *Colomeris vitis* (Pagenstecher, 1857) (*Col. vitis*) and grape leaf rust mite, *Calepitrimerus vitis* (Nalepa, 1905) (Acari: Eriophyidae) (*C. vitis*) (Bernard *et al.,* 2005). *Colomeris vitis* has been known to cause bud necrosis since the 1950's (Bernard *et al.,* 2005). However, no studies have been conducted to support or deny a link between SSS symptoms and eriophyid infestations in the Pacific Northwest and exhaustive mite surveys in vineyards in Oregon prior to 2000 (Prischmann, 2000) showed no eriophyid mite populations.

*Calepitrimerus vitis* is a host-specific pest of grapevines, *Vitis vinifera* L., (Jeppson *et al*., 1975; Amrine and Manson, 1996; Duso and de Lillo, 1996) occurring in many grape growing regions of the world, including the Pacific Northwest (James, 2006), Australia (James and Whitney, 1993; Carew *et al*., 2004; Bernard *et al*., 2005) and Europe (Baillod and Guignard, 1986; Kreiter and Planas, 1987; Hluchy and Pospisil, 1992; Perez-Moreno and Moraza-Zorilla, 1998; Duffner, 1999; de Lillo *et al*., 2004). Outbreaks of *C. vitis* have occurred in Europe with yield losses reported (Baillod and Guignard, 1986; Kreiter and Planas, 1987; Hluchy and Pospisil, 1992; Perez-Moreno and Moraza- Zorilla, 1998; Duffner, 1999). Large populations of *C. vitis* in Australia and Europe were associated with spring leaf and shoot distortions and retarded growth in emerging green tissue as well as late summer leaf bronzing and crop losses (Hluchy and Pospisil, 1992; Perez-Moreno and Moraza-Zorilla, 1998; Duffner, 1999; Bernard *et al*., 2005). Bud burst failure and high yield losses were correlated with *C. vitis* infestations in California (Smith and Stafford, 1948), South Africa (Dennill, 1986) and Australia (May and Webster, 1958; Frost, 1996; Whiting and Strawhorn, 1997). These symptoms are similar to SSS experienced in vineyards in the Pacific Northwest. In Australia and South Africa, a pre-bud burst spray of an acaricide (lime sulfur) was reported to reduce RSG (Dennill, 1986; Krake *et al*., 1998; Bernard *et al*., 2005).

The aim of this research was to investigate possible causes of SSS found in Oregon vineyards. It was hypothesized that SSS results from tissue damage caused by eriophyid mites.

## MATERIALS AND METHODS

Seasonal populations of *C. vitis, Col. vitis,* thrips and Willamette mite (*Eotetranychus willamettei*) (McGregor) (Acari: Tetranychidae) were monitored in grape vineyards by assessing mite incidence on dormant vine tissue (buds and bark scales surrounding buds), developing buds and shoots during early spring, and leaves during late spring through autumn in 2006 and winter 2007. Vine damage and crop losses were also estimated in each vineyard.

**Research sites -** Twelve blocks, ranging in size from 0.6 to 0.8 hectares, were selected on the basis of SSS history. Blocks in each location were separated by approximately 0.75 kilometers from each other. All vines were trained on the vertically positioned shoot system with row spacing 2 to 2.75 m and vines within the rows 1-2 m apart. Vines were cane-pruned and vine densities ranged from 480 to 1200 vines per hectare, depending on vine spacing.

Blocks were located at vineyards in the Willamette Valley, which included locations near the towns of: Sherwood (blocks 1, 2 and 3, var. Pinot Noir, 45°02'31N; 123°08'52W, alt. 105 m), Dundee (blocks 1, 2 and 3, var. Pinot Noir, 45°14'54N; 123°04'28W, alt. 150 m), Salem (blocks 1 and 2, var. Pinot Gris, Chardonnay, 45°02'31N; 123°08'52W, alt. 214 m), Corvallis (blocks 1 and 2, var. Pinot Noir and Chardonnay, 44°35'53N; 123°25'07W, alt.152 m) and Eugene (blocks 1 and 2, vars. Chardonnay and Riesling, 43°51'36N; 123°16'18W, alt. 166 m).

**Winter buds -** A systematic sampling system was used to survey entire blocks each winter on January 23, 2006 and January 9, 2007. Twenty sampling sections were evenly distributed throughout each block. A section consisted of 5 vines between two trellis poles. One dormant, 10 to 60 cm long, fruit-bearing shoot (two to three internodes) was randomly selected and cut close to its base just above the cane on each of five vines in a section. A total of 100 dormant shoots were sampled in each block. Samples were taken to the laboratory where the basal bud (proximal) on each shoot was dissected to determine tissue damage as well as thrips, *E. willametti* and eriophyid mite density. The eriophyid mite species in the dissected bud areas were distinguished based on where they were found within the buds and body shape, size and color (Duffner, 1999; Bernard *et al*., 2005). Mites within the bud area were classified as bud mites and those found on the outer bud scales as rust mites. Samples of these mites were sent to James Young (Oregon State University Insect Clinic), and Gerald Krantz, for identification and voucher specimens were deposited in the Oregon State University insect collection. Tissues collected during winter was rated as undamaged when all bud tissue lacked necrosis. Bud tissues were rated as damaged when necrosis and scarring was visible due to feeding.

In order to determine the pattern of mite infestation pattern along canes, an additional sample of 48 shoots each containing at least 12 buds was collected from a block showing SSS directly adjacent to block three in Dundee on January 9, 2007. Samples were collected from four rows and 12 evenly spaced vines in each row. Buds on each shoot were thoroughly inspected for mite presence and the number of mites recorded on each bud position. Mean number of mites per bud position was plotted.

**Double-sided sticky tape -** Double-sided sticky tape (3M Scotch Magic Tape 12 mm x 1143 cm) was placed on individual vine canes beginning March 15, 2006 in each of the 20 bays per block in each of the 12

blocks. Tape was wrapped around each cane, one at the base of a shoot close to the main trunk and another three buds away from the base of each cane totaling 40 sticky tape samples per block. Sticky tape samples were replaced every second week until the first week of July 2006. Sticky tape samples were taken to the laboratory and viewed under the microscope for the presence of mites.

**Spring developing shoots -** Developing spring shoot samples were taken from blocks on April 28, 2006 just after bud break. One developing shoot was selected randomly from each of five vines per section in each of 20 sections per block. Shoot positions ranged from the bud closest to the main trunk (1) to the bud farthest away from the main trunk (9) on fruiting canes. A total of 100 shoots were sampled per block. Sampled shoots were numbered and the GPS coordinate location was recorded. After field collection, developing shoots were immediately placed in cooled containers in order to minimize mite movement between collected shoots and were refrigerated (6°C) for periods between one and three weeks before investigation. The length of the second internode of each shoot was measured and the numbers of *C. vitis*, *Col. vitis* and thrips were recorded by searching the basal leaf and areas in close proximity. The second internode was measured as the first internode was often too short to get accurate measurements. The internode length of shoots was categorized into three groups: 'short' (1-3 mm), 'medium' (4-7 mm), and 'long' (8-20 mm). The developing shoot sample data were analyzed using one-way ANOVA, and differences of means were separated using Tukey's HSD test for unequal number of samples.

**Spatial analysis -** In order to determine the association between bud/shoot damage and mite incidence during the early part of the season, spatial patterns and associations were quantified. Data collected from the winter and spring (April 28, 2006) shoot samples were used in the program SADIE (Spatial Analysis by Distance IndicEs) and spatial correlation (Perry, 1995) was used to determine the overall index of aggregation of damage and mite incidence. The index of aggregation, $I_a$, was used to quantify aggregation patterns found in blocks collected during this stage of the season. When $I_a$ is near unity, the obser- ved counts have a spatially random arrangement (Perry, 1996; Maestre and Cortina, 2002). Values larger than unity indicate an aggregated arrangement and values smaller than unity indicate a regular arrangement. Another index given in SADIE is *v*, a dimensionless index of clustering. This index was used to measure the degree of clustering of data into areas with above-average density, or patches, or areas with below-average density, or gaps (Perry, 1995). The v indices indicate where clustering occurs, where a clustering patch is indicated by large positive values of $v_i$ (larger than 1.5) and a gap by large negative values for $v_i$ (smaller than -1.5) (Winder *et al*., 2001; Maestre and Cortina, 2002). Values of $v_i$ equal to 1 and $v_j$ equal to -1, indicate randomness (Winder *et al*., 2001; Perry and Dixon, 2002). To test for non-randomness, the mean values of the clustering indices, $v_i$ and $v_j$ were used.

An index *X* was used to indicate spatial association of mite and thrips incidence and damage ratings (Perry, 1996). This index is the mean of individual local associations found in the vineyard block, $X_k$, first calculated in SADIE by comparing cluster indices at every sampling unit (Winder *et al*., 2001; Perry and Dixon, 2002; Perry, 1996). The significance of *X* is determined through randomizations, with values of cluster indices reassigned amongst the sample units, after allowance for small scale spatial autocorrelation in the cluster indices of either population (Winder *et al*., 2001). Large values of local association are indicated by the coincidence of a patch cluster for one set, such as pest mites, with a patch cluster for the other set, the SSS damage cluster set, or by the coincidence of two gaps. Disassociation is indicated by a patch coinciding with a gap (Perry and Dixon, 2002). A two-tailed *t*-test was used with the null hypothesis of no association against the alternatives of positive association and disassociation (Scott *et al*., 2003). This test was conducted at the 5% level, with the test statistic $P < 0.025$ indicating significant association and $P > 0.975$ indicating significant dissociation. The significant association of mite incidence and damage incidence were quantified and mapped using ArcView and with extension Spatial Analyst (ESRI Press 1999). Interpolation between data points was done using the inverse distance weighted method. This information may help determine the causal agent of SSS as well as the time when damage starts to occur if association between damage and mite incidence is evident.

**Leaves -** The central systematic sampling method was used beginning June 22, through August 16, 2006. Leaf samples were taken every two weeks at four vineyards including Dundee, Salem, Corvallis and Eugene. An average-sized leaf (10-15 cm in length) was taken from the center of the canopy of each vine, giving a total of 100 leaves per block. Twenty five leaves were collected in each row (four rows per block; 9 blocks) and pooled. Leaf samples (comprising of 25 leaves each) were taken to the laboratory and thoroughly brushed using a mite brusher (Leedom Engineering, Santa Clara, CA) onto liquid dishwashing soap-impregnated circular disks (122 mm diameter). Initially, individual leaves were inspected and mites counted prior to brushing. Mite numbers on disks were comparable to numbers found during individual leaf inspection. Disks were viewed under a microscope and numbers of mites were recorded. Repeated-measures ANOVA were used to describe *C. vitis* infestation levels per leaf through the season in blocks that showed infestation. The average mean infestation levels throughout this period of sampling were calculated for comparative purposes.

**Seasonal vine damage -** Tissue damage ratings of shoots and grape bunches were taken in conjunction with leaf samples in Dundee, Salem, Corvallis and Eugene from June 22 to August 16, 2006. Tissue damage was re-

corded every second week on five fruiting canes starting closest to the main trunk and moving farther away from the trunk, using the rating system described below. The rating system was used throughout the season in order to determine damage and crop loss as the season progressed. The rating system includes:

0 = No feeding damage; two to three normal and full-sized bunches per shoot.

1 = Some feeding damage visible on shoot, necrotic tissue/shortening; two to three normal and full-sized bunches per shoot. No economic crop loss.

2 = Necrotic tissue/shortening and medium feeding damage visible on shoot; at least one normal and full-sized bunch remaining. The remainder of bunches showed necrotic tissue and were small, containing five to ten berries each and would not be harvested at the end of the season. 50% crop loss.

3 = Necrotic tissue and shoot shortening. High feeding damage, no normal bunches remaining. The bunches showed necrotic tissue, containing only five to ten berries each which would not be harvested. 100% crop loss (Fig. 1).

4 = Only secondary shoots remain because of total necrosis of the primary shoot; no bunches and feeding damage evident. This translates to 100% crop loss.

A total of 500 fruiting canes were rated in each block. Repeated-measures ANOVA of tissue damage was conducted on data collected from Dundee (blocks 1, 2 and 3) as these were the only blocks where SSS was found.

**Crop loss assessment at harvest** - Crop losses were assessed in all 5 locations (12 blocks) one week before harvest on September 15, 2006 using the above rating system. Crop losses were recorded on five vines in each of 20 sections per block (100 vines per block), and five fruiting canes per vine starting closest to the main trunk (position 1-5) and moving farther away from the trunk (position 6-9). Preliminary data showed that no crop loss occurs on fruiting canes six to nine on all vines and these crop loss values for each fruiting cane were predicted to be 0 for the calculations. This is based on visual observations during the evaluation of crop loss where little to no damage was found on fruiting canes in positions 6-9 from the proximal side of the trunk. Crop loss per vine was then assessed by including estimated values from the first five fruiting canes and adding the zero values on the remaining four canes of each vine totaling nine values per vine. Crop loss per block was therefore estimated by averaging these 900 values per block. A comparison of crop loss and the seasonal leaf incidence of *C. vitis* was calculated by averaging the number of *C. vitis* counted from soap disks from row samples using the leaf brushing machine from June 26 through August 16, 2006.

**Virus testing** - To rule out the possibility that an RNA virus might be associated with the SSS symptoms, leaf samples from SSS symptomatic and asymptomatic plants in the Dundee, Salem and Sherwood vineyards were collected in May of 2007. Double-stranded RNA extractions were carried out on each sample to test for the presence of any RNA plant viruses using standard extraction protocols (Valverde *et al.,* 1990). Extracts were loaded into 1% agarose gels, electrophoreses stained with ethidium bromide and nucleic acid bands visualized with UV light and photographed.

## RESULTS

**Winter buds** - The number of dissected buds totaled 900 and 1200 in 2006 and 2007, respectively. *Colomerus vitis* incidence was low in samples taken from all locations on January 23, 2006 and January 9, 2007. Means did not exceed one per bud (Table 1). Necrotic spotting (Bernard *et al*., 2005) was found on some of the shoot primordia where bud mites occurred. The number

Table 1. Bud mite (*Colomerus vitis*), rust mite (*Calepitrimerus vitis*), Willamette mite (*Eotetranychus willamettei*) and thrips mean population levels and proportion of damage per bud during winter bud examination conducted on January 23, 2006 and January 9, 2007 in five Oregon locations. Corvallis comprised two blocks, Dundee three, Eugene two, Salem two and Sherwood three blocks.

| | | Mean arthropod counts and proportion damaged buds | | | | | |
|---|---|---|---|---|---|---|---|
| **Location** | **Winter** | ***Col. vitis* (SE)** | ***C. vitis* (SE)** | ***E. willamett*i (SE)** | **Thrips (SE)** | **Proportion damaged buds (SE)** | ***n*** |
| Corvallis | 2006 | 0.07 (0.02) | 0 | 0 | 0 | 0.05 (0.01) | 200 |
| | 2007 | 0 | 0 | 0 | 0 | 0.02 (0.01) | 200 |
| Dundee | 2006 | 0.25 (0.06) | 0.54 (0.19) | 0.02 (0.01) | 0 | 0.04 (0.01) | 300 |
| | 2007 | 0.16 (0.06) | 15.87 (3.41) | 0 | 0.02 (0.01) | 0.05 (0.02) | 300 |
| Eugene | 2006 | 0.11 (0.05) | 0 | 0 | 0 | 0.03 (0.01) | 200 |
| | 2007 | 0 | 0 | 0 | 0 | 0 | 200 |
| Salem | 2006 | 0.24 (0.08) | 0 | 0.18 (0.06) | 0 | 0.07 (0.02) | 200 |
| | 2007 | 0 | 0 | 0 | 0 | 0 | 200 |
| Sherwood | 2006 | Not sampled | - | - | - | - | - |
| | 2007 | 0.07 (0.03) | 4.54 (1.65) | 0 | 0.01 (0.01) | 0.03 (0.01) | 300 |

Fig. 1. A branch of grape vine showing Short Shoot Syndrome (arrow) in Oregon, USA.

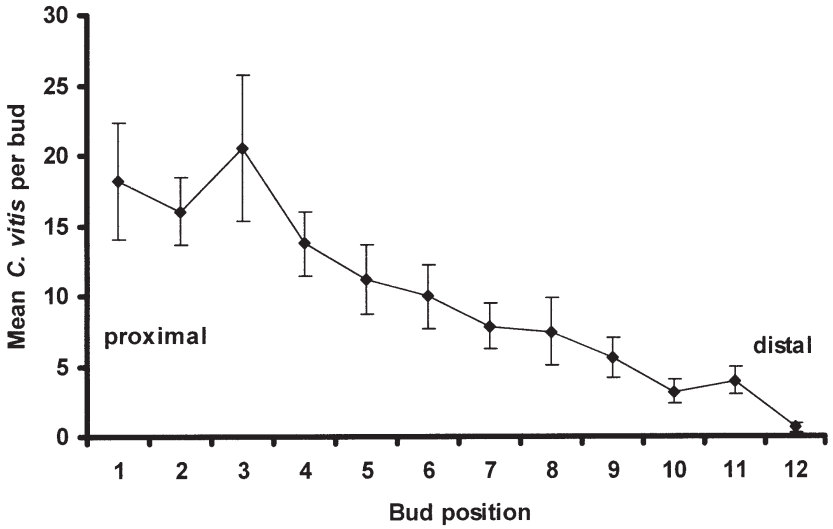


Fig. 2. Mean number of *Calepitrimerus vitis* per bud (SE) in relation to bud position. Bud one was closest to the trunk of the vine and bud twelve farthest. Forty eight buds were sampled for each bud position. Buds were sampled during the winter of 2007 in one vineyard block near Dundee, Oregon.

Table 2. Mean number of rust mites, *Calepitrimerus vitis,* found per double-sided sticky tape during seasonal sampling in Oregon during 2006. Means were calculated using values from 40 sticky tapes in each block and pooling blocks in each location. Corvallis comprised two blocks, Dundee three, Eugene two, Salem two and Sherwood three blocks.

| Location | Mean *C. vitis* (SE) counts on sticky tapes | *n* (sampling dates) |
|---|---|---|
| Corvallis | 1.25 (0.75) | 4 |
| Dundee | 2.22 (0.94) | 18 |
| Eugene | 1.00 (0.41) | 4 |
| Salem | 0.29 (0.18) | 7 |
| Sherwood | 0 | 5 |

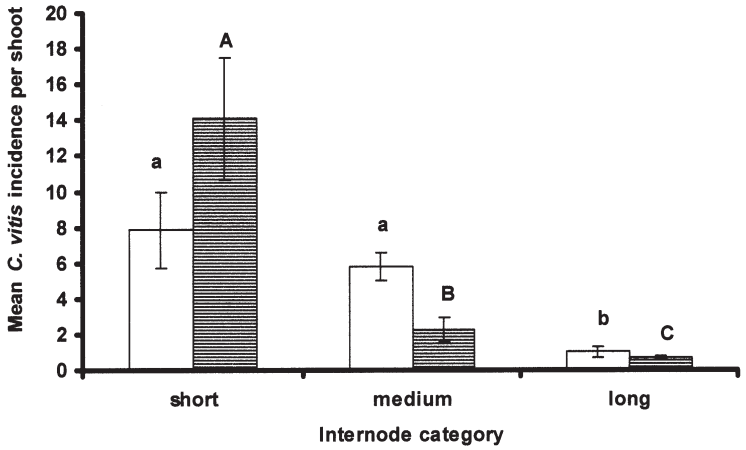


Fig. 3. Association of mean density of *Calepitrimerus vitis* per shoot and internode category (SE) on April 28, 2006 in three vineyard blocks near Dundee (a, b; clear bars) and Sherwood (A, B, C; colored bars), Oregon. Short, medium and long internodes were 1-3mm, 4-7mm and 8-12mm in long, respectively. Twenty seven, 137 and 135 internodes fell in each category, respectively in Dundee. Ninety two, 232 and 31 internodes fell into each category, respectively in Sherwood.

of dissected buds that displayed bud mite damage was limited during both seasons, with < 1% of buds damaged during the winter period. The incidence of thrips and Willamette mite were also negligible during both winters in all locations. On January 23, 2006, we did not find evidence of *C. vitis* infestation (Table 1), but sampling on January 9, 2007 showed rust mite presence in Dundee and Sherwood. No evidence of feeding damage was found in buds where *C. vitis* occurred. Linear regression comparing bud damage to rust mite incidence from winter samples were non-significant ($y$ = 0.000793x +0.05, $F$ = 0.158; df = 1,3; $p$ = 0.717, $R^2$ = 0.05). Bud samples taken in Dundee on January 9, 2007 showed decreasing numbers of *C. vitis* in buds further away from the fruiting cane (Fig. 2).

**Double-sided sticky tape** - *Calepitrimerus vitis* was found on the double-sided tape starting March 15, 2006 in the vineyards where populations were found during the summer of 2006, but mite counts were lower on tape than those found by dissecting buds during winter (Table 2). *C. vitis* were found in three locations but data were variable and non-significant. The highest mean number of *C. vitis* was found in Dundee (2.22 ± 0.94 SE) and none in Sherwood.

**Spring developing shoots** - No evidence of *Col. vitis* or thrips was found over the sampling period. However, initial one-way ANOVA and subsequent post-hoc analysis revealed significant mean differences in internode length. Three locations had shorter internodes and two of the locations, Dundee and Sherwood, (Table 3) showed SSS. The remaining location, Corvallis, displayed short shoot length and dieback, but these symptoms were attributed to early season frost damage because of temperatures below freezing during March 10-13, 2006 (-9, -7, -10 and -9°C respectively).

Vineyards with symptoms of SSS, Dundee and Sherwood, also had detectible populations of rust mites. A test of homogeneity of variance was performed to detect differences between internode length. Sherwood internodes were significantly shorter than in Dundee (Table 3). Internode length decreased with increasing rust mite density in both of these vineyards (Fig. 3). In Dundee 'long' internodes were infested with *C. vitis* at levels of 0.71 ± 0.1 (mean ± SE), 'medium' internodes with 2.22 ± 0.68 and 'short' internodes with 14.07 ± 3.4 ($F$ = 35.236; df = 2, 296; $p$ < 0.001) . In Sherwood 'long' internodes were *C. vitis*-infested with a mean of 1.03 ± 0.3). 'Medium' internodes had a mite population mean of 5.8 ± 0.78 and 'short' internodes had 7.84 ± 2.13 ($F$ =

Table 3. Mean rust mite, *Calepitrimerus vitis,* density per shoot, mean internode lengths (mm), and Short Shoot Syndrome (SSS) symptoms found in five locations in Oregon on April 26, 2006. All values were derived by calculating the means for all blocks sampled in each location. Corvallis comprised two blocks, Dundee three, Eugene two, Salem two and Sherwood three blocks. In Corvallis internode length was short but was most likely caused by frost damage during March 10-13, 2006.

| Location | *C. vitis* (SE) | Internode length, mm (SE) | SSS | *n* |
|---|---|---|---|---|
| Corvallis | 0 | 3.68 (0.12) | No | 200 |
| Dundee | 8.92 (1.17) | 5.30 (0.13) | Yes | 300 |
| Eugene | 0 | 7.60 (0.24) | No | 200 |
| Salem | 0 | 8.77 (0.23) | No | 200 |
| Sherwood | 3.23 (1.08) | 4.70 (0.10) | Yes | 300 |

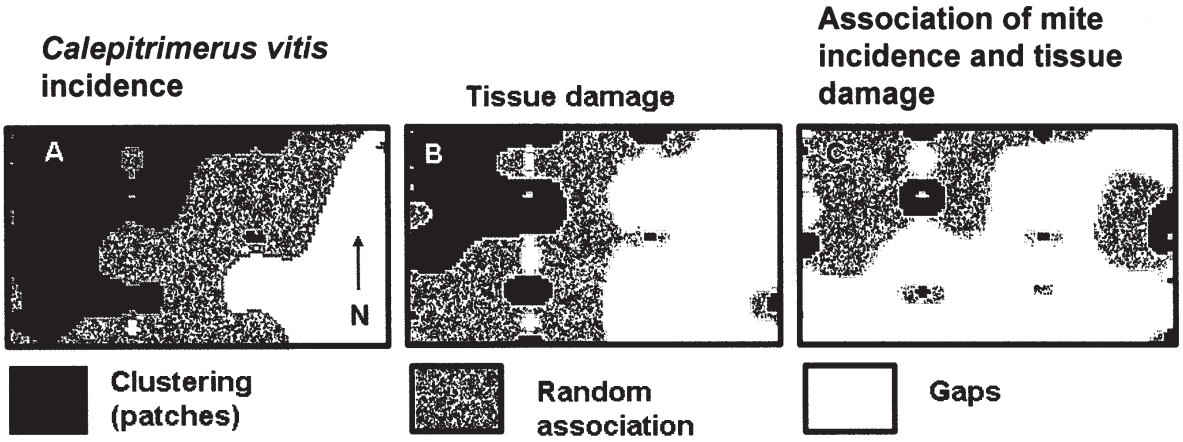


Fig. 4. Interpolated spatial clustering and association of *Calepitrimerus vitis* and tissue damage at Dundee Oregon, block 1. (A) *Calepitrimerus vitis* densities, (B) tissue damage and (C) association between *C. vitis* incidence and tissue damage. Only significant indices were mapped: $v_i > 1.5$ (Black for patches) and $v_j < -1.5$ (White for gaps); $\chi_k > 0.5$ (Black for positive association and $\chi_k < -0.5$ (White for no association). In all cases grey indicated random indices for patches and gaps or random association.

2.57; df = 2, 392; $p = 0.078$) (Fig. 3). These differences in internode length and mite numbers were significant. However, in the Sherwood vineyard, differences in mite incidence in the medium and short shoot categories were not significant.

**Spatial analysis** - In order for spatial analysis to make biological sense, it is important that an adequate proportion of the sampling units (about 30% when a large number of sampling units, such as 100, are used) should have positive or non-zero values. The only dataset that met these requirements was obtained from block 1 at Dundee on April 28, 2006. The remainder of the blocks had too few vines with mites and SSS and did not meet these requirements and were therefore not used in this analysis. In all cases, only values of $v_i$ larger than 1.5 (significant patches) and values of $v_j$ smaller than -1.5 (significant gaps) (Perry and Dixon, 2002) and values of $X_k > 0.5$ (significant association) or < -0.5 (significant disassociation) (Veldtman and McGeoch, 2004) were used to create the spatial maps. Rust mite incidence and SSS had a spatially aggregated pattern in block 1 at Dundee (Table 4). All infestations were clustered into both patches and gaps. Short shoot symptoms were strongly associated with mite incidence (X = 0.7889, $p <$ 0.001) (Table 3; Fig. 4A-C).

**Leaves** - Three of the nine blocks (all at the Dundee location) sampled over approximately a two month period (between January 22 and August 16, 2006) showed rust mite incidence on leaves. Two-weekly averages of rust mite incidence were higher in block 1 compared to blocks 2 and 3, where data was combined as similar rust mite infestation levels were found (Fig. 5A). Block 1 at Dundee displayed rust mite levels above 30 per leaf ($n$ = 100 leaves). These levels increased above 50 per leaf but *C. vitis* numbers decreased after July 6, most likely due to an application of sulfur. However, these levels increased again to above 150 per leaf by August 16. In blocks 2 and 3, rust mite infestations were below 2 per leaf throughout the growing season. Seasonal *C. vitis* density on leaves collected in the Dundee vineyard was 59.02 ± 20.35, 26.53 ± 10.5 and 8.3 ± 4.79 in blocks 1, 2 and 3, respectively (Table 5). Seasonal leaf sampling was not conducted at Sherwood thus the seasonal *C. vitis* leaf incidence could not be calculated there.

Table 4. Spatial pattern of rust mite, *Calepitrimerus vitis,* incidence and Short Shoot Syndrome (SSS) severity, and spatial association between bunch and stem infestation in block 1, Dundee, Oregon evaluated on April 28, 2006. $I_a$ = index of aggregation, $P_a$ = probability level; $v_i$ = mean cluster index for patches, $P_i$ = probability level; $v_j$ = mean cluster index for gaps, $P_j$ = probability level; $X$ = index of overall spatial association, $P$ = probability level.

| | Spatial association indexes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $I_a$ | $P_a$ | $v_i$ | $P_i$ | $v_j$ | $P_j$ | $X$ | $P$ |
| Mite density | 1.734 | 0.0256 | 1.691 | 0.0128 | -1.791 | <0.001 | | |
| SSS severity | 1.941 | 0.0128 | 1.949 | <0.001 | -2.015 | <0.001 | | |
| Mite density vs. SSS severity | | | | | | | 0.7889 | <0.001 |

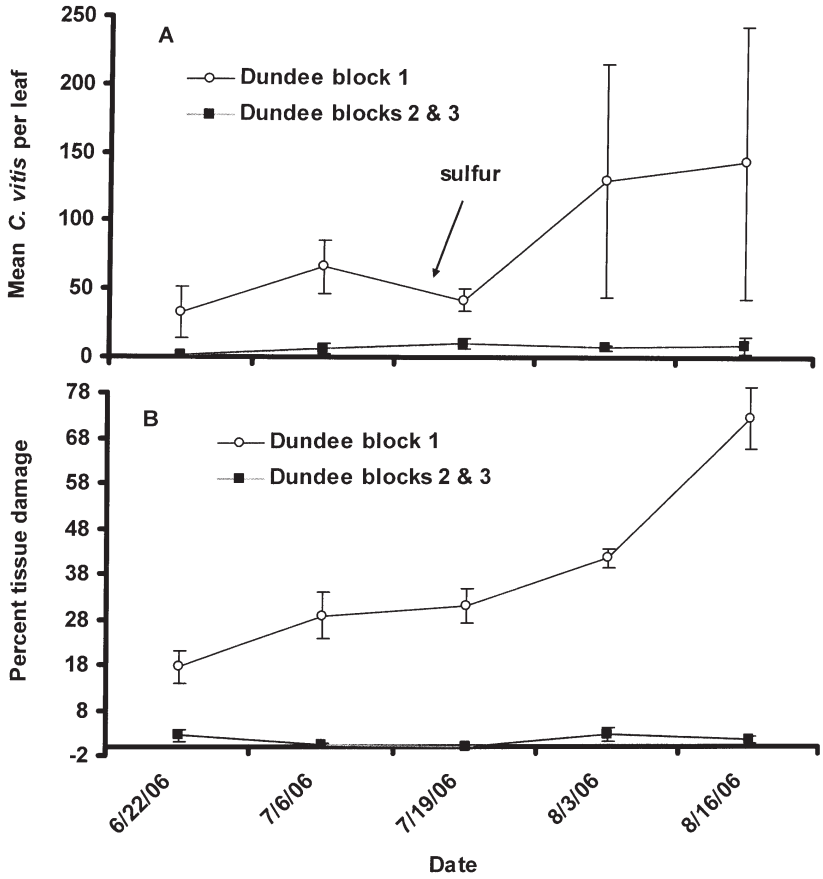


Fig. 5. (A) Mean number of *Calepitrimerus vitis* per leaf ($n$ = 100) in block 1 and blocks 2 and 3 (combined) in Dundee, Oregon during 2006. The arrow indicates a sulfur application. (B) Tissue damage rating of shoots and grape bunches in block 1 and blocks 2 and 3 combined in Dundee, Oregon during 2006. Shoot tissue damage was recorded on five fruiting canes starting closest to the main trunk and moving farther away from the trunk, using a percent damage rating system.

Table 5. Percent crop loss in five Oregon vineyards during 2006. Crop losses were recorded on five vines per bay in 20 bays per block (100 vines per block), and five fruiting canes per vine starting closest to the main trunk and moving farther away from the trunk. On fruiting canes six to nine on all vines crop loss values was assumed to be 0. Crop loss per vine was determined by including estimated values from the first five fruiting canes and adding the zero values on the remaining four canes on each vine (nine values total per vine). Crop loss per block was estimated by averaging these values (900 cane values per block). No seasonal mite sampling indicated by a dash (-).

| Location | Block | Estimated crop loss (%) | Seasonal mites per leaf (SE) |
|---|---|---|---|
| Corvallis | 1 | Crop loss unrelated to rust mite | - |
| | 2 | incidence | - |
| Dundee | 1 | 23.72 | 59.02 (20.35) |
| | 2 | 7.17 | 26.53 (10.5) |
| | 3 | 1.54 | 8.30 (4.79) |
| Eugene | 1 | 0 | - |
| | 2 | 0.01 | - |
| Salem | 1 | 0.01 | 0 |
| | 2 | 0.28 | 0 |
| Sherwood | 1 | 0 | - |
| | 2 | 0.63 | - |
| | 3 | 2.04 | - |

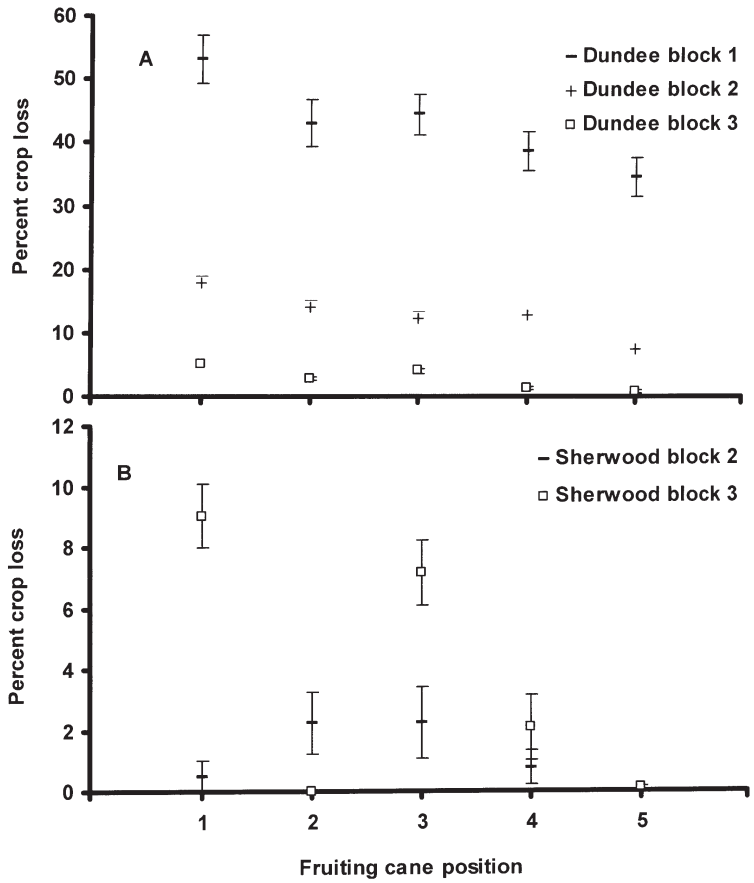


Fig. 6. Mean percent crop loss on fruiting cane position (100 vines/fruiting cane) in vineyard blocks in (A) Dundee and (B) Sherwood, Oregon during 2006. Cane one was situated closest and five farthest from the trunk of the vine.

**Seasonal vine damage -** In Dundee, vine damage over the season was significantly higher in block 1 and lower in blocks 2 and 3 and displayed a mean tissue damage of 38.1% and 1.3% respectively ($t = 11.12$, $df = 58$, $p < 0.0001$) (Fig. 5B). During the early part of the season (before June 22) vine tissue damage levels (five shoots per vine) were 19% in block 1 and gradually increased towards the latter part of the season (August 16) to 80%. Damage symptoms found on vine tissue were much lower in blocks 2 and 3 and remained below 2% over the entire season (August 16, Fig. 4B). The remainder of blocks displayed an absence of SSS symptoms.

**Crop loss assessment at harvest** - Crop loss at harvest was recorded in all three blocks at Dundee and blocks 2 and 3 of Sherwood. The remaining locations displayed little to no crop losses (Table 5). In general, the mean crop loss values in affected blocks based on location of shoots on vines showed a decrease in crop loss in shoots positioned further away from the main vine trunk (Fig. 6A, B). The greatest estimated crop loss was found in Dundee block 1 (mean loss of 23.7%), and in blocks 2 and 3 with mean losses of 7.2 and 1.5%, respectively. In Sherwood, blocks 2 and 3, crop losses were 0.5 and 2.0%, respectively (Table 5, Fig. 6B).

**Virus testing** - All virus tests were negative suggesting that there is not an RNA plant virus associated with the SSS. DsRNA analysis was used for virus testing since this should detect a very broad range of plant viruses. Since SSS has not been associated with any viruses previously it was thought that a broad virus test would be more useful than screening only for viruses known to infect grapevines.

## DISCUSSION

This paper describes the syndrome known to grape growers in Oregon as Short Shoot Syndrome (SSS). By correct description and diagnosis, Bernard *et al*. (2005) were able to link *C. vitis* and *Col. vitis* to Restricted Shoot Growth symptoms. Similar symptoms referred to as Short Shoot Syndrome are for the first time linked to *C. vitis* incidence in the sampled Oregon vineyards and confirm the relationship between SSS and *C. vitis* incidence first reported in Australian vineyards. Damage caused by feeding of *C. vitis* on young developing tissue appears to be responsible for SSS and its season-long consequences in Oregon vineyards. The general trend during 2006 was that vineyard blocks with high mite infestations displayed

high crop-losses at harvest. These mites migrate to suitable over wintering sites such as the outer bud scales and under the bark in close proximity to buds (Duffner, 1999). Developmental studies show that these mites remain dormant in these areas without feeding or causing damage (Duffner, 1999). This hypothesis was supported by sampling and dissections of buds during the winter in 2006 and 2007 in Oregon showing no damage to bud tissue. To date, very few *Col. vitis* have been found in bud tissues during winter in Oregon vineyards, thus it appears that this species plays little, if any role in SSS.

Sampling using double sided sticky tape was found not to provide significant information for rust mite monitoring. The latter part of the 2005 and 2006 winter was rainy, which made tape placement impractical due to moisture and interfered with the stickiness of tape, causing discoloration of the glue which obscured vision and monitoring. Therefore, the number of *C. vitis* found on double-sided sticky tape was not representative of incidence in vineyards. This method worked well in Australia (Bernard *et al*., 2005), but environmental conditions may have been more amenable to this method there.

Initial SSS symptoms first occurred in early spring, thus most damage likely occurred after vines break dormancy. Spatial analysis during spring in Dundee confirms the relationship between vine damage and mite incidence and is supported by data presented by Bernard *et al*. (2005). This association remained consistent using subsequent spatial analyses during the remainder of the season. Generally the spatial analysis showed that high rust mite incidence during spring caused early season damage and ultimately results in severe crop losses at harvest. In-block *C. vitis* populations displayed clumped infestation patterns often associated with arthropod infestations (Reusink and Kogan, 1982). *Calepitrimerus vitis* infestations in winter, early spring and during summer resulted in late season leaf bronzing in the upper canopy of vines and were associated with reduced grape yield.

High yield loss was noted (Perez Marin, 1992) and berry loss and bunch damage (Baillod and Guignard, 1986) due to mites, but no data were presented. In severe cases in Oregon, harvest was prevented because of the severity of SSS and the lack of quality grape bunches. A significant association was found between yield reduction and *C. vitis* levels, ca. 220 per leaf at the 2 to 3 leaf stage (Hellmann, 2003), and ca. 170 per leaf at véraison (Hluchy and Pospisil, 1992). Generally, the density of *C. vitis* per shoot during the early season in Oregon was much lower than populations reported in Australian vineyards. Cool springs typically found in Oregon vineyards may result in slow tissue growth and relatively longer periods of exposure to *C. vitis* populations and may contribute to the severity of crop losses found in Oregon vineyards.

This raises the question why *C. vitis* outbreaks occurred on an industry-wide level in Oregon, and whether pesticide regimes used by many producers in the Pacific Northwest are detrimental to natural enemies, such as predatory mites. Phytoseiids are known to control mite populations on grapevines in Australia (James and Whitney, 1991, 1993; James *et al*., 1995). James *et al.* (2002) reported that complexes of natural enemies broader than phytoseiid mites may be important for sustainable mite management in PNW viticulture. Early establishment of specialist and generalist mite predators is crucial to development of eriophyid and tetranychid mite management in vineyards. In Oregon, many growers currently use sulfur at 10 to 14 day intervals up to two weeks before harvest in order to control *C. vitis* and powdery mildew (*Erysiphe necator*). The cryptic behavior of *C. vitis* during the late dormant period makes them less susceptible to these sprays. Developing integrated pest management (IPM) approaches for these pests is further complicated because management of powdery mildew diseases on grapevine, as well as eriophyid mites (Bernard *et al*., 2005), relies heavily on sulfur because of its low cost, high efficacy, and low potential for resistance development. On grapevine, sulfur applications may provide temporary suppression of eriophyid mites (Dennill, 1986) and *Tetranychus urticae* Koch (Croft, 1990; Koleva *et al*., 1996; James., 2002; James *et al*. 2002; Auger *et al*., 2003), but negatively affect predatory mite populations (Bernard *et al.,* 2004). Spider mites and certain natural enemies are suppressed by sulfur, but spider mite populations tend to rebound when sulfur applications cease (McMurtry *et al*., 1970; Hluchy, 1993; Thomson *et al.*, 2000, James and Coyle, 2001; James *et al*., 2002; Prischmann and James, 2005; Prischmann *et al*., 2006).

Pest management strategies that reduce fungicide and acaricide applications are essential to maintain and improve grower profitability, sustainability, and ensure environmental stewardship. A focused research effort to develop integrated control practices for eriophyid mites, powdery mildew, and conservation biological control of spider mites is essential. More research is needed in Oregon to determine the exact impact of *C. vitis* on vine yield and economic threshold levels. Work is currently being done in order to better understand and prevent SSS in PNW vineyards and includes preservation biocontrol and optimal timing of pesticide treatments.

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