Slow wilt: Another form of Marchitez in oil palm associated with trypanosomatids in Peru

Alfredo Giovanni Trelles Di Lucca¹, Edwin Fernando Trinidad Chipana¹, Michael John Talledo Albújar², Waldo Dávila Peralta¹, Yasbel Catalina Montoya Piedra² & Jorge Luis Arévalo Zelada²


Author for correspondence: Edwin Fernando Trinidad Chipana, e-mail: etrinidadch@palmas.com.pe

ABSTRACT

Another form of Marchitez manifestation affecting oil palm (Elaeis guineensis) has been observed since 2002 in the Alto Huallaga area from Peru. Externally, the disease appears as chlorosis, drying and breaking of the lower leaves, rot in fruit bunches, accumulation and rot of spears. Internally, the meristem presents brown lesions with a yellowish halo around its base. A high proportion (94%) of affected plants showed trypanosomatids in their roots. After follow up asymptomatic plants for 22 months, 85% develop symptoms of slow wilt. After the appearing of the symptoms the affected plants die between 5 and 8 months. Lincus spurcus population present in affected plants was evaluated and 44% were positive for trypanosomatids. In addition, trypanosomatids were observed in wild plants such as Acalypha cuneata, Urera caracasana, Trema micrantha, Cecropia spp., present in areas with incidence of the disease. The chlorosis, the long period of incubation and the time evolution of the disease are different in comparison with sudden wilt, suggesting a new disease manifestation affecting oil palms. The presence of Lincus positive to trypanosomatids leads us to propose an association of these parasites with slow wilt as reported for sudden wilt.

Key words: Elaeis guineensis, Lincus, Phytomonas, host weeds, insect vector, “marchitez lenta”.

INTRODUCTION

The oil palm (Elaeis guineensis Jacq.) is a crop with high yield and low production costs. Palm oil is used in multiple products, from food to biodiesel (Tan et al., 2009; Yusof & Foong-Kheong, 2009). In Peru the registered cultivated area of oil palm up to October 2012 was 61,637 ha (J Figueroa, Ministerio de Agricultura of Peru, personal communication), and there is a projection to increase due to the internal demand for oils and greases and the important role as an alternative culture in the substitution of illegal crops in the Peruvian Amazon region.

Oil palm plantations are increasing around the world. In Latin America, the productivity of this crop is affected by pests and diseases, with the latter being most damaging since they are primarily controlled through the eradication of the affected palms. Among the most significant diseases are bud rot (“pudrición de cogollo”) which has been associated with Phytophthora palmivora (Torres et al., 2010); sudden wilt (“marchitez sorpresiva”), associated with trypanosomatids (Phytomonas spp.) (Dollet et al., 1977; McCoy & Martinez-Lopez, 1982; Dollet, 2001); red ring/little leaf syndrome, caused by the nematode Bursaphelenchus cocophilus (Cobb) Baujard (Griffith, 1987; Chinchilla, 1991); lethal wilt (“marchitez letal”) associated with phytoplasmas (Alvarez & Claroz, 2003); annular spot and chlorotic ring spot caused by viruses of the genera Foveavirus (Morales et al., 2002) and Potyvirus (Chinchilla, 2001; Morales, 2000) respectively; basal stem rot, caused by fungi (primarily Ganoderma sp.) (Singh, 1991; Nieto, 1994); and spear rot, of unknown origin.

Sudden wilt is a well-known disease which has been reported to affect oil palms in Latin America (Lopez et al., 1975; Van Slobbe et al., 1978; Urueta, 1985; Alvañil, 1996). In Peru, symptoms of sudden wilt were registered in 1972 in the Alto Huallaga, region San Martín (Korytkowski & Ruiz, 1979). However, since 2002 a disease manifestation different from sudden wilt has been observed in Palmas del Espino S.A. plantation (Tocache - Alto Huallaga, San Martín, Peru). The differences observed in comparison with sudden wilt suggest that it might be another form of Marchitez syndrome, called “slow wilt”.

The objective of this study was to characterize the symptoms of this new disease manifestation, assess the possible association of trypanosomatids (as reported for sudden wilt), and to establish the relationship with possible insect vectors and wild plants as possible reservoirs of these microorganisms.

MATERIALS AND METHODS

The study took place between June 2008 and July 2010 at Palmas del Espino plantation located in the Alto...
Huallaga, region of San Martin, Peru (Peruvian Amazon), 76º25’W and 08º25’S at an altitude of 500 m.

Characterization of external and internal symptoms of the disease

Healthy plants (n=43) from areas without incidence and 168 sick plants in different stages of this other form of Marchitez, which cannot be classified as “sudden wilt”, were followed up during four months. These plants had between 24 and 26 years old. External symptoms were established by direct observation of the coloration and aspect of the foliage, state of the petiolar bases, spears (young leaves with unopened leaflets) and fruit bunches. The state of the affected plants were established according to the degree of severity: a) initial stage (chlorosis and progressive drying of the lower leaves), b) intermediate stage (drying and drooping of leaves of the lower third) and c) advanced stage (drooping of leaves of the middle and upper thirds). Plants with slow wilt in the initial (n=37), intermediate (n=66) and advanced stages (n=65) were selected for this study, respectively. Plants in the final stage (only two or three leaves in a normal position and spears clearly observed, the rest of leaves are drooped) were not evaluated due to the advanced rot of the stems. On the other hand, the internal symptoms were established by cutting the palm at the crown of fruit bunches level and removing the petiolar bases one by one to observe the floral primordia. The crown was cut open lengthwise to observe the state of the meristem and spear base. The stem was cut lengthwise to observe its internal aspect and consistency. The number of roots was counted and their state was verified by performing excavations of 30 cm length x 30 cm width and 30 cm depth, 50 cm apart from the stem base to obtain the roots, and then the root sap was extracted and observed under a light microscope. Additionally, symptoms were observed in young plants.

Distribution of trypanosomatids in palms affected by sudden wilt and slow wilt

The sap was extracted and the presence of trypanosomatids was observed under a light microscope (1,000x) with Giemsa’s staining method. To evaluate the presence of trypanosomatids, root sap was collected from 4,077 palms affected with this other form of marchitez (according to the external symptoms, the disease observed in these plants not corresponding to sudden wilt). Root sap of 1,534 palms affected by sudden wilt was evaluated under microscopy) were followed up for five months to determine the trypanosomatid load in root sap according to the progression of the disease in its several stages. Root sap samples were collected every 15 days.

Establishment of incubation period of slow wilt disease manifestation

Root sap of 137 asymptomatic plants (positive to trypanosomatids) located in areas with high incidence of slow wilt disease were followed up for 22 months to estimate the possible period of incubation until appearing of the characteristic symptoms of the disease.

Possible insect vectors of trypanosomatids

The families of piercing-sucking insects frequently found in areas with incidence of slow wilt disease were registered. The area was located 2 km apart from areas with incidence of sudden wilt. Pit-fall traps (glassware of 7 cm diameter x 10 cm height) containing detergent solution (200 to 250 mL) were set up around 10 plants affected with initial symptoms of the disease (10 to 12 traps for each plant, placed 10 cm from steam base), while 55x40 cm yellow sticky traps were set up on the stem at 0.5, 5 and 10 m (crown of fruit bunches level) from the ground in ten plants with initial and intermediate symptoms of the disease. Other yellow sticky traps were placed on the spears of five plants, while insect glue was spread on the base of five plants, in both cases the plants were in the intermediate stage. The checking and changing of traps was performed every three days during four months. In addition, live insects were captured close to palms affected by slow wilt to evaluate the presence of trypanosomatids. Pheromone traps for the curculionidae Rhynchophorus palmarum L. and Metamasius hemipterus L. were placed close to the palms affected due to their relationship with the crop.

Trypanosomatids.mL⁻¹ = Total trypanosomatids counted in 0.2 mm² x 50,000

The results obtained were compared with 31 plants affected by sudden wilt. Likewise, 49 healthy plants were analyzed, coming from areas without incidence of either disease.

Five adult plants (24 years old) in asymptomatic stage of slow wilt disease (positive to trypanosomatids under microscopy) were used to follow up for five months to determine the trypanosomatid load in root sap according to the progression of the disease in its several stages. Root sap samples were collected every 15 days.
Insects were collected and identified from eliminated plants (n=85) with initial and intermediate stages of the disease. The head and abdomen of every individual were squashed with physiological saline solution (0.9% NaCl) to determine the presence of trypanosomatids under the light microscope. The identification of the most abundant insect specimens was performed at the Universidade Federal do Rio Grande do Sul (Porto Alegre, RS, Brazil).

**Transmission tests with insects**

Twenty healthy 27-year-old plants located in areas without incidence of slow wilt were exposed to 20 specimens of *Lincus spurcus* Rolston, collected from plants affected by slow wilt. The exposure was performed during the afternoon, on immature fruit bunches confined in anti-aphid mesh sleeves.

Similarly, 176 *L. spurcus* insects (156 adults and 20 nymphs) were exposed on a healthy 3-year-old plant, confined in a 4x4x6 m cage with aphid-proof mesh. All insects came from eliminated plants affected by slow wilt. Once the insects were collected, prior to both releases, 64 insects were randomly selected to estimate the proportion of trypanosomatid-carrying individuals by microscopic evaluation.

**Evaluation of trypanosomatids in wild plants**

This study was performed in two stages. The first one was to determine which plants other than oil palm, frequently found in areas with incidence of slow wilt, were infected by trypanosomatids. Sap extracted from the upper portion of the stem or petioles of these plants was used to determine the presence of trypanosomatids by microscopy. In the second stage, only the plant species positive to trypanosomatids in the first stage were evaluated to determine the percentage of plants positive to trypanosomatids according to the level of slow wilt incidence. The level of slow wilt incidence was categorized as: without incidence (0 positive cases in 25 ha), low (1 positive case in 25 ha), medium (2-3 positive cases in 25 ha) and high (more than 4 positive cases in 25 ha). Likewise, the root sap of 340 native palmaceae from 10 species located adjacent to the plantation was analyzed.

**Statistic analysis**

The software R (www.r-project.org) was used for the analysis. Percents (%) of internal characteristics according to stage of the disease were compared using the chi-square test and significant differences between groups were compared using Fisher’s test for pairwise comparisons of proportion. Concentrations of trypanosomatids in sap from different parts of palm with symptoms of slow wilt and sudden wilt in different stages were compared using Kruskal-Wallis’s test and Van der Waerden’s test (only for middle stem). Trypanosomatids concentration variation on the time in root saps was also compared using Kruskal-Wallis’ test. All analysis of significance were made at the $P<0.05$ level of significance or lower.

**RESULTS**

**Symptomatology of the disease**

**External symptoms**

In the early stages of slow wilt, affected plants show chlorosis and progressive drying of the lower leaves, which begins in the end of the most distal leaflet, progressing to the middle and to the base part of the leaflet. Simultaneously, chlorosis affects other leaflets of the leave, then the chlorosis goes up according to the direction of spiraling leaves. The middle third may have a slight chlorosis, while slight chlorosis in the upper third was observed in the majority of cases (Table 1). In the intermediate stages of the disease, the adult plants show progressive dehydration of the affected lower leaves. In addition, a break at the petiole base (70 cm from its base, approximately) is observed, while the young plants do not show broken leaves. Both in adult and young plants the leaves from the middle third have mild chlorosis, while in the upper third chlorosis intensifies acquiring a lime-green hue, with shortening of leaves in some cases (Table 1). In later stages (Figure 1A), adult plants show all leaves from the lower third as dried and broken, the leaves from the middle third are also broken with drying process. The upper third leaves show more intense chlorosis and some of these leaves are broken. Mild or moderate shortening of the upper leaves was observed at low frequency (Table 1). In young plants, the chlorosis was more intense in leaves of the upper third. The leaves of the middle third were dried and some leaves from of the lower third were dried and broken approximately 1.5 m from its base.

Accumulation of spears (Figure 1B) is progressive in plants with slow wilt from initial to advanced stages (Table 1). At the level of the spears, dry rot was observed at low frequency in plants in the initial stage and more frequently in intermediate and advanced stages. In some cases a marked shortening of leaves was also observed (Figure 1C). In all stages the meristem was unaffected (Table 1).

Similar to sudden wilt, discoloration and rotting of fruit bunches is observed in slow wilt, but no rot of all bunches occurs (Figure 1D). The frequency of this occurrence increased with the progression of the disease (Table 1). Thus, in the initial stages of the disease, the fruit bunches close to maturation may be harvested, while those that are still immature have little possibility of reaching maturation.
TABLE 1 - Changes in foliage appearance / leaf appearance and other characteristics associated with the progression of symptoms of slow wilt

<table>
<thead>
<tr>
<th>Stage</th>
<th>n°</th>
<th>Foliage appearance/Leaf appearance</th>
<th>Other characteristics</th>
<th>Breaking of leaves</th>
<th>Rotting of fruit bunches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower third leaves</td>
<td>Middle third leaves</td>
<td>Upper third leaves</td>
<td>Accumulation of spears</td>
</tr>
<tr>
<td>Healthy</td>
<td>43</td>
<td>Normal green</td>
<td>Normal green</td>
<td>Normal green</td>
<td>Absent</td>
</tr>
<tr>
<td>Slow wilt - Initial stage</td>
<td>37</td>
<td>In drying out process, that start with chlorosis of distal leaflets from the oldest ones upwards, following the leaf spiral (100% of cases)</td>
<td>Normal green (60% of cases)</td>
<td>Normal green (8% of cases)</td>
<td>Slight chlorosis (40% of cases)</td>
</tr>
<tr>
<td>Slow wilt - Intermediate stage</td>
<td>66</td>
<td>In drying out process and breaking (100% of cases)</td>
<td>Green (14% of cases)</td>
<td>Slight chlorosis (96% of cases)</td>
<td>Slight chlorosis (79% of cases)</td>
</tr>
<tr>
<td>Slow wilt - Advanced stage</td>
<td>65</td>
<td>Hung and dried (100% of cases)</td>
<td>Slight or moderate chlorosis, hung and in drying process (100% of cases)</td>
<td>Slight or advanced chlorosis, hung and in drying process (82% of cases)</td>
<td>In 89% of cases</td>
</tr>
</tbody>
</table>

n°: number of trees examined.
*The percentage value refers to the proportion of plant showing these symptoms.

maturity. On the other hand, in sudden wilt rot occurs in all fruit bunches, whether they are mature or recently formed, and even inflorescences were affected. In young plants affected with slow wilt, rotten fruit bunches were not frequently observed.

**Internal symptoms**

Rotting was observed in the root system (Figure 1E), sometimes emitting a fermenting odor. This symptom increased with the progression of the disease (Table 2).
No differences were observed in longitudinal sections of the stem between palms in initial and intermediate stages of slow wilt. Also, no differences were observed when compared with stems from healthy plants. However, some plants in the advanced stage of the disease showed indications of rotting in the lower stem. In the tissue present at the meristem base, a yellow halo was observed (Figure 1F), which intensified with the progress of the disease. This symptom was observed in all cases in the intermediate and advanced stages of the disease (Table 2).

**TABLE 2 - Internal characteristics of plants with slow wilt according to stage of the disease**

<table>
<thead>
<tr>
<th>Stage of the palm</th>
<th>Abortion of floral primordia</th>
<th>Yellow halo in the meristem base</th>
<th>Brown lesions in the meristem</th>
<th>Number of roots in 30x30x30 cm hole</th>
<th>Rotten roots (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy palms</td>
<td>7% a</td>
<td>0% a</td>
<td>0% a</td>
<td>19.2 ± 1.05 a</td>
<td>21.1 ± 2.85 a</td>
</tr>
<tr>
<td>Slow wilt - Initial</td>
<td>17% a</td>
<td>87% b</td>
<td>26% b</td>
<td>13.7 ± 1.28 b</td>
<td>14.0 ± 2.76 a</td>
</tr>
<tr>
<td>stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow wilt - Intermediate stage</td>
<td>76% b</td>
<td>100% b</td>
<td>70% c</td>
<td>15.5 ± 1.01 ab</td>
<td>35.0 ± 2.64 b</td>
</tr>
<tr>
<td>Slow wilt - Advanced</td>
<td>100% c</td>
<td>100% b</td>
<td>78% c</td>
<td>18.9 ± 1.82 a</td>
<td>35.5 ± 5.68 b</td>
</tr>
</tbody>
</table>

1Percentages based on 43 healthy plants, and 37, 66 and 65 plants observed with slow wilt in the initial, intermediate and advanced stages, respectively. Different letters (a,b,c), indicate statistical differences for the proportion of a given symptom among all stages of the disease compared in the same column, \( P<0.05 \).

2Average and standard error of 28 healthy palms, and 6, 44 and 19 palms with slow wilt in the initial, intermediate and advanced stages, respectively. Values followed by the same letter are not significantly different in the same column (Duncan’s test, \( \text{df}=3; F=2.715; P<0.05 \)).

3Average and standard error of rotten roots based on 2. Values followed by the same letter are not significantly different (Van der Waerden’s test, \( T=14.54; \text{df}=3; P<0.01 \)).
In the zone close to the meristem, brown lesions (Figure 1G) were present in the foliar primordia, a particular characteristic of slow wilt. However these lesions were not observed in all cases (Table 2). Abortion of floral primordia (Figure 1H) is another symptom in slow wilt and was observed with more frequency according to the progression of the disease (Table 2).

**Trypanosomatid - slow wilt relationship**

**Frequency, distribution and concentration of trypanosomatids**

Trypanosomatids were detected in 94% (3,837 positive plants) of root sap collected from 4,077 plants with slow wilt, while in sudden wilt 84% (1,294 from 1,534 plants) were found positive for trypanosomatids.

Trypanosomatids were found distributed throughout the plants affected by slow wilt, with variations in the frequency and concentration according to the part of the plant evaluated (Tables 3 and 4). A similar distribution was observed in palms affected by sudden wilt (Tables 3 and 4). In both diseases, the roots were the part of plant where trypanosomatids were observed at higher frequency, although in sudden wilt fruit bunch peduncles and inflorescence peduncles showed similar results. No trypanosomatids were found in healthy plants from areas with no incidence of either disease (Table 3).

When the same part of the plant was evaluated for both sudden wilt and slow wilt diseases, differences in the concentration of trypanosomatids were found (Table 4). In slow wilt, concentrations were established at $10^4$ to $10^5$ trypanosomatids/mL of sap, while for sudden wilt, between $10^6$ and $10^7$ trypanosomatids/mL of sap were found. In the asymptomatic stage of slow wilt, the highest concentration was found in the upper stem. In the initial stages, the highest concentrations were found in the upper and middle stems, in the intermediate stages the highest concentration were found in the roots and middle stem, while in the advanced stages, the highest concentration was found in the roots (Table 4). Comparatively, in the initial stages of sudden wilt, the highest concentrations of trypanosomatids were found in the fruit bunches and inflorescences peduncles, in the intermediate stages, the highest concentrations were found in the fruit bunch peduncle and roots, while in the advanced stages, the highest concentration was found in the roots (Table 4). In slow wilt, the mean concentration of trypanosomatids in roots was 102, 51 and 25 times lower compared to sudden wilt for the initial, intermediate and advanced stages, respectively.

**Slow wilt symptom manifestation and trypanosomatid concentration in the roots**

After evaluation of 719 palms in areas of high incidence of slow wilt, trypanosomatids were detected in the roots of 137 palms with no symptoms. These 137 palms were followed up during 22 months to determine the period of incubation until the appearance of symptoms. After 3, 10 and 22 months of observation, 47%, 77% and 85% of these plants developed symptoms of slow wilt, respectively. In contrast, 15% (20 plants) of these plants did not show any symptoms of slow wilt or sudden wilt. No trypanosomatids were observed in the root sap samples of these plants under microscopy at the end of this study.

Five asymptomatic palms were followed up during five months to observe the variation in the number of trypanosomatids per mL of root sap according to evolution of the disease (Figure 2). An increment in the number of trypanosomatids was observed since the appearance of

---

**TABLE 3** - Frequency of trypanosomatids presents in different parts of plants with different degrees of slow wilt and sudden wilt symptoms

<table>
<thead>
<tr>
<th>Part of palm tree evaluated</th>
<th>Plants with slow wilt</th>
<th>Plants with sudden wilt</th>
<th>Healthy plants</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Positive</td>
<td>N Positive</td>
<td>N Positive</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>94</td>
<td>36</td>
<td>49</td>
<td>X2 = 169.729; df = 2; P&lt;0.001</td>
</tr>
<tr>
<td>Lower stem (0.6 m)</td>
<td>89</td>
<td>24</td>
<td>49</td>
<td>X2 = 65.023; df = 2; P&lt;0.001</td>
</tr>
<tr>
<td>Middle stem (7.9 m)</td>
<td>71</td>
<td>22</td>
<td>49</td>
<td>X2 = 99.354; df = 2; P&lt;0.001</td>
</tr>
<tr>
<td>Upper stem (15.9 m)</td>
<td>63</td>
<td>21</td>
<td>49</td>
<td>X2 = 64.574; df = 2; P&lt;0.001</td>
</tr>
<tr>
<td>Meristem base</td>
<td>87</td>
<td>24</td>
<td>49</td>
<td>X2 = 35.849; df = 2; P&lt;0.001</td>
</tr>
<tr>
<td>Meristem</td>
<td>63</td>
<td>18</td>
<td>49</td>
<td>Fisher's exact tests. P&lt;0.259</td>
</tr>
<tr>
<td>Fruit bunch peduncle</td>
<td>44</td>
<td>6</td>
<td>49</td>
<td>Fisher's exact tests. P&lt;0.001</td>
</tr>
<tr>
<td>Fruit spike peduncle</td>
<td>15</td>
<td>2</td>
<td>49</td>
<td>Fisher's exact tests. P&lt;0.05</td>
</tr>
<tr>
<td>Inflorescence peduncle</td>
<td>52</td>
<td>2</td>
<td>47</td>
<td>Fisher's exact tests. P&lt;0.01</td>
</tr>
<tr>
<td>Base of floral primordia</td>
<td>78</td>
<td>22</td>
<td>49</td>
<td>X2 = 49.894; df = 2; P&lt;0.001</td>
</tr>
<tr>
<td>(spathes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf base 1</td>
<td>59</td>
<td>21</td>
<td>49</td>
<td>Fisher's exact tests. P&lt;0.001</td>
</tr>
<tr>
<td>Spear base (leaf-1)</td>
<td>57</td>
<td>16</td>
<td>49</td>
<td>Fisher's exact tests. P&lt;0.001</td>
</tr>
</tbody>
</table>

1Percentages indicate the presence of trypanosomatids in different part of palms for plants with slow wilt, sudden wilt and health are compared (same row). Percentages with the same letter (a, b, c), are not significantly different, P>0.05
symptoms until the intermediate stage of the disease. A drop in the concentration of trypanosomatids was observed at the advanced stage in comparison with the intermediate stage (Figure 2). Concentration variations were significantly different (Kruskal-Wallis's test; H=24.3; fd=3; P<0.001).

Families of insects found in palms affected by slow wilt

In the pit-fall traps, 526 insects specimens were captured belonging to the families Elateridae (87; 17%), Scarabaeidae (97; 18%), Carabidae (138; 26%), Formicidae (117; 22%), Blatellidae (10; 2%), Blaberidae (36; 7%), and Acrididae (41; 8%). No piercing-sucking insects were found. A very scarce number of insects from the families Cicadellidae, Membracidae and Sarcophagidae were captured by the sticky traps installed on the spears. The sticky traps installed on the stem at 0.5 m captured insects from the families Cicadellidae (952), Cercopidae (164), Membracidae (13), Cixiidae (12),...
Slow wilt: Another form of Marchitez in oil palm associated with trypanosomatids in Peru

Coreidae (17), Reduvidae (6) and Pentatomidae (34). The sticky traps installed on the stem at 5.0 m captured insects from the families Cicadellidae (368), Cercopidae (43), Membracidae (10) Coreidae (6) and Pentatomidae (8), and the sticky traps installed at the crown of fruit bunches level (10 m) captured insects from the families Cicadellidae (776), Cercopidae (43) and Membracidae (63). These insects were not observed under microscopy because all of them were dead insects.

During elimination of palms affected with slow wilt (n=85), L. spurcus (Figure 3A and 3B) was the most frequent specie in plants with this disease (84; 99%). In one plant, Lincus sp. was found. High number of L. spurcus (between 1 and 349 individuals/plant) were located in the upper organs (fruit bunches, inflorescences, and leaves from the upper third). Trypanosomatids were found in the head and abdomen of 44% (224 of 504) of the Lincus specimens evaluated (female and male adults and nymphal stages III, IV and V) collected from plants with slow wilt. A similar proportion of Lincus carrying trypanosomatids (44%; 163 of 372) was found in plants affected with sudden wilt. No trypanosomatids were observed in 398 Lincus specimens collected from healthy plants from areas without incidence of either disease.

No trypanosomatids were observed in other insects collected in areas with high incidence of slow wilt. The insects found in those areas belongs to the families Pentatomidae different from Lincus (162), Coreidae (81), Cercopidae (6), Membracidae (75), Cicadellidae (48), Cixiidae (30), Curculionidae (Rhynchophorus palmarum, 168; Metamasius hemipterus, 225) and Elateridae (39).

**Transmission tests with L. spurcus carrying trypanosomatids**

The average proportion of L. spurcus population positive to trypanosomatids used for the transmission test with adult plants (27 years old) was 51%, and for the young plants (3 years old) 37%. After 25 months of incubation for the adult plant and 19 months for the young plants no trypanosomatids were found in the root sap from either adult and young palms evaluated.

**Wild plant hosts of trypanosomatids**

Trypanosomatids were detected in four species of weeds frequently found in areas with incidence of slow wilt: Acalypha cuneata Poepp. (Euphorbiaceae), Urera caracasana (Jacq.) Gaud. ex Griseb (Urticaceae), Trema micrantha (L.) Blume (Ulmaceae) and Cecropia spp. (Cecropiaceae). The four weeds evaluated showed an increment in the proportion of trypanosomatids according to the degree of slow wilt incidence in the areas where they were collected (without incidence, low incidence and medium incidence): Cecropia spp., 12%, 25%, and 57%; A. cuneata, 0%, 15% and 38%;
U. caracasana, 0%, 41% and 67%; T. micrantha, 33%, 79% and 86%, respectively. In all cases the proportion of weeds positive for trypanosomatids located in areas with high incidence of slow wilt was lower than in areas with medium incidence of the disease.

No trypanosomatids were detected in the following wild palms: Socratea exorrhiza Mart. H. Wendl. (n=23), Scheelea sp. (n=41), Oenocarpus bataua (Mart.) Burret (n=2), O. multicaulis Spruce (n=11), Cocos nueifera L. (n=16), Mauritia flexuosa L.f. (n=78), Bactris gasipaes HBK (n=43), Astro Caryum sp. (n=24), Phytelephas macrocarpa Ruiz & Pav. (n=7), Euterpe precatoria Mart. (n=95).

DISCUSSION

Difference between symptoms of slow wilt and sudden wilt

Our observations suggest that another form of “Marchitez” disease affects oil palm plants in the Peruvian Amazon, whose symptoms manifestation are different to the very well-known sudden wilt. The symptom manifestation of this syndrome corresponds to a lethal progressive wilt with a slow evolution period, the reason why we propose to denominate this other form of Marchitez as “slow wilt” (“marchitez lenta”).

A particular characteristic of slow wilt is the period of evolution since the appearing of the symptoms until death of the plant, which is between 5 to 8 months, compared with 2 to 3 weeks in sudden wilt in young plants (3 or 4 years old) (Dollet, 1984). A similar situation was reported by Parthasarathy et al. (1978), where adult coconut trees with hartrot showed a slower symptom evolution than young trees, with 12 to 16 weeks and 4 to 6 weeks respectively. However, in this study we observed that old and young palms may have a rapid (sudden wilt) or low evolution (slow wilt) of symptoms, therefore we consider that palm age might be not a factor for the disease expression. In addition, all genetic material in Palmas del Espino plantations showed both forms of Marchitez, which suggests that genetics does not influence the outcome of the disease. Both types of Marchitez were observed to be present in well and poorly drained soils.

Likewise, the chlorosis and drying of leaves in the initial stages of slow wilt are present in an isolated manner, while in sudden wilt such symptoms are widespread among all the lower leaves, which turn reddish brown in color (Reyes, 1985).

The breaking of the leaves in slow wilt in the intermediate stage is due to the dehydration of the petiolar bases in adult plants and is not present in young plants, possibly due to the smaller size and lower weight of the leaves. This symptom is not present in adult and young plants with sudden wilt, possibly due to the fast dehydration observed (Sánchez, cited by Uruea, 1985). The accumulation of spears is a symptom which evidences the deficient flow of liquids inside the plant (Mejía, 2000). This symptom was observed in slow wilt since the intermediate stage. On the other hand, this symptom is not observed in sudden wilt, possibly due to the rapid progress of this disease, where the spears rotted and broke in all cases, emitting a fetid odor resulting from the bud decomposition (Alvaniil, 1996). The meristem in sudden wilt was affected, enabling the spears to be easily pulled out by hand, which does not happen in cases of slow wilt.

The rotting of roots in slow wilt increased progressively until reaching an average of 35% of roots affected in the advanced stage. In sudden wilt, rot affects nearly 100% of roots (Reyes, 1985), with a characteristic fetid odor not perceived in slow wilt. The formation of a yellowish halo in the tissue of the meristem base is a symptom detected from initial stages, which intensifies with the development of slow wilt. Apparently, this is typical in disturbances that cause symptoms of wilt in the oil palm, such as lethal wilt (Sánchez et al., 2003), basal stem rot due to Ganoderma and sudden wilt. In the case of the latter disease, this symptom occurs with a pronounced orange color in the advanced stage (our own observations). The lesions observed close to the meristem are characteristic of slow wilt and have not been reported for any other disease in oil palm.

Trypanosomatid – slow wilt relationship

Trypanosomatids have an association with slow wilt because 94% of palms affected by this disease were positive to these microorganisms. High frequencies of trypanosomatids were found also in palms affected by sudden wilt (84%), a disease which has a proven relationship with these organisms (Alvaniil, 1996). A similar distribution of trypanosomatids in different parts of the plant was observed in slow wilt and sudden wilt, suggesting a similar behavior of these protozoa in both diseases. The absence of trypanosomatids in healthy plants from areas without incidence of either disease supports the association between these organisms and slow wilt in Peruvian oil palm plantations.

The high number of trypanosomatids found in the upper stem of the oil palm in asymptomatic stages of slow wilt (4.9х10^4 trypanosomatids/mL), compared to other plant organs, suggests that the infection occurs initially in the crown and/or spears of palm. Something similar happened in sudden wilt (Rairán et al., 2000), where the highest concentrations of these parasites were found in the inflorescence and fruit bunch peduncles in the initial stages of the disease.

With the progression of slow wilt, the concentration of trypanosomatids decreased in the upper stem and increase in the roots, apparently because the trypanosomatids descend through the stem, a pattern also observed by Rairán et al. (2000) for sudden wilt. The high number of trypanosomatids observed in sudden wilt in comparison with slow wilt suggests differences in each disease manifestation. However, the presence of different trypanosomatids species...
or genotypes should not be discarded. Some studies have been done on the genetic difference of plant trypanosomatids (Sturm et al., 2007). DNA minicircles have been used for establishing groups among phloematic and latticiferous trypanosomatids such as fruit and seed trypanosomatids. Protein studies have showed similarities among each trypanosomatid group (phloematic, latticiferous, and those from fruit and seeds) (Muller et al., 1994). However, no molecular studies have been performed for the genetic characterization of the trypanosomatid, and further studies are needed to clarify this point.

Some palms (15%) positive to trypanosomatids did not develop symptoms of disease after 22 months of follow up. This fact may indicate that the incubation period will cover a broad and variable time range longer than 22 months, since other previous stages within the development of disease must also be considered (inoculation, incubation and establishment of infection by the pathogen) (Agrios, 2005). The variations in time probably depend on the stress level to which the plants are subjected (nutrition, water and edaphic conditions, etc.) (Hartley, 1977; Agrios, 2005). In cases of sudden wilt of the oil palm and hartrot of the coconut palm, the incubation period is estimated at 4 to 6 months (Genty, 1985; Louise et al., 1986; Perthuis et al., 1986). The long incubation period until development of symptoms makes slow wilt a potential risk for oil palm plantations compared to sudden wilt, because there is also the possibility that these plants may act as sources of dissemination during this period.

The increase in the concentration of trypanosomatids in the roots, based on the progression of the symptoms of slow wilt up through the intermediate stage, is similar to the process described for sudden wilt and hartrot, where the number and propagation of trypanosomatids inside the sieve tube elements of the phloem increase proportionally with the development of the disease (Agrios, 2005). Likewise, the decrease in the concentration of trypanosomatids as a consequence of tissue deterioration in the advanced stages of slow wilt was also mentioned by Alvañil (1996) and Rairán et al. (2000) for the case of sudden wilt, which would support the obligate parasitic nature of trypanosomatids.

According to Villegas (1999), the probable action mechanism of trypanosomatids in sudden wilt is the occlusion of the sieve tube elements of the phloem, which would affect the transport of photoassimilates. However, the low number of trypanosomatids observed in slow wilt suggests a distinct action mechanism.

### Possible insect vectors of trypanosomatids

Among the species identified, *L. spurcus* seems to be the most closely related with slow wilt due to its presence in palms affected by this disease. The fact that high populations of *Lincus* were found in the upper organs of the affected palms (crown of fruit bunches, inflorescences, upper third leaves) is related to the high concentrations of trypanosomatids existing in the asymptomatic stages of slow wilt. Insects of this same genus have also been associated with the transmission of sudden wilt of oil palm (Desmier de Chenon, 1984; Perthuis et al., 1986; Vickerman & Dollet, 1992; Alvañil, 1993; Dollet et al., 1993) and hartrot of coconut palm (Louise et al., 1986; Dollet et al., 1993; Sgrillo et al., 2005) in other countries of Latin America.

The transmission tests performed in old and young plants to confirm the role of *L. spurcus* in the epidemiology of slow wilt have not shown positive results until the end of this study. Insects were observed alive until one month on the fruit bunches confined in old palms and until four months of follow up on the petiolar base of upper third leaves in the young palm. However, the number of insects placed on old palms was lower than in young palm, but acclimations were not possible because no insects were observed after four months; unfortunately no replications were performed. Both situations could affect the success of transmission tests.

Dollet (1993) mentioned a successful transmission test with a low number of insects (between 2 and 40), but in young coconut trees (12 to 16 months old). Old oil palms could need a greater number of insects for development of slow wilt. In studies performed by Desmier de Chenon (1984), healthy oil palm plants (4 and 5 years old) reproduced the symptoms 2.5 to 3.5 months after placement of infected insects of *Lincus* spp. In Ecuador, Perthuis et al. (1986) were able to reproduce the symptoms of sudden wilt within six months of the exposure of 199 *Lincus* insects in a 4-year-old oil palm. Likewise, Louise et al. (1986) reproduced the symptoms of hartrot in coconut palms four months after exposure to high quantities of *L. croupius* (2,873 nymphs and 832 adults). Because of the possible long incubation period of slow wilt, a longer follow up time could be needed to confirm the transmission tests.

### Wild plant hosts of trypanosomatids

*A. cuneata, U. caracasana, T. micrantha* and *Cecropia* spp. have been identified as host of trypanosomatids in this work. The role of certain weeds as reservoirs of plant trypanosomatid pathogens has been mentioned by other authors (Solarte, 1995; Alvañil, 1996). It is important to note that the first sick plants with slow wilt were observed in areas near the edge of the plantation, where there is a greater presence of these weeds. Despite having determined a significant relationship between the presence of weeds carrying trypanosomatids and the levels of incidence of slow wilt, it is still not possible to define these weeds as hosts of the trypanosomatids associated with the disease, given that it is necessary to confirm that the trypanosomatid found in the weeds is the same as which causes the disease in oil palm.

In Colombia and other countries, plants from the same families have been cited as carriers of trypanosomatids (Alvañil, 1996). Likewise, Dollet, cited by Alvañil (1996), considered that weeds that do not
contain latex, such as *Urea*, *Cecropia* and *Heliconia*, are possible carriers of trypanosomatids associated with diseases in the oil palm. However, Sturm et al. (2007) reported differences between groups of trypanosomatids of palm trees and trypanosomatids of other plants by analysis of DNA of mitochondrial minicircles conserved regions, so those microorganisms from other plants are not similar to causal agent of sudden wilt. Sturm et al. (2007) also mentioned *Euphorbia hirta* as a plant capable of carrying trypanosomatids belonging to both groups, so it is possible that a weed species could be host of trypanosomatids belong to both groups. Furthermore, *Cecropia* spp. has been observed frequently in areas with high incidence of hartrot in coconut palm, and *Edessa loxaltili* is a common vector of this plant (Kastelein, 1985). However, *Lincus* spp. occasionally comes down from the coconut crown to the soil, feeds from *Cecropia* spp., transmitting or acquiring trypanosomatids, and then returns to the crown. Thus, *Lincus* can be a casual vector of trypanosomatids from weeds to coconut palms. It is therefore premature to discard a relationship between weeds and slow wilt, and it is necessary to clearly establish the role of weeds from the Tocache area as reservoirs of trypanosomatids associated with slow wilt in oil palm by molecular analysis.

Although no trypanosomatids were detected in the wild palmaceae close to Palmas del Espino plantation, it is important to analyze their role as a food source for *Lincus spurcus*, since adults and nymphs of this pentatomid have been found on plants of *Scheelea* sp. and *Bactris gasipaes*. This finding, as well as the reports of Couturier & Kahn (1989, 1992) and Llosa et al. (1990), show the possibility of the participation of certain palmaceae in the epidemiology of both slow wilt and sudden wilt.

Slow wilt is another form of Marchitez with a different symptomatology in comparison with sudden wilt, a widely known disease of oil palm in Latin America and the world. Although we do not have conclusive results for the transmission tests, the evidence found in this study suggests an association of trypanosomatids with the presence of slow wilt. As reported similarly for sudden wilt, *L. spurcus* is the insect most closely associated with the transmission of this another form of Marchitez. Molecular analysis must be used to elucidate whether trypanosomatids of wild plants such as *A. cuneata*, *U. caracasana*, *T. micrantha* and *Cecropia* spp. are the same found in plants with slow wilt and to explain the relation between weed abundance and high incidence slow wilt areas. Subsequent works are aimed to corroborate the disease-trypanosomatid-weed-vector relationship.

**ACKNOWLEDGEMENTS**

The authors would like to thank the management of Palmas del Espino S.A., as well as Alfredo Chigné, Ernesto Candiotti and the whole staff of the Plant Health Department for their collaboration during this investigation. Special thanks to Dr. Jocélia Grazia and Dr. Luiz Alexandre Campos of the Universidade Federal do Rio Grande do Sul for their support in the identification of the *Lincus* specimens.

**REFERENCES**


Slow wilt: Another form of Marchitez in oil palm associated with trypanosomatids in Peru


