

# Clinical and Microbiologic Follow-Up Evaluations After Non-Surgical Periodontal Treatment With Erbium:YAG Laser and Scaling and Root Planing

Beatriz Maria Valério Lopes,\* Leticia Helena Theodoro,† Rafaela Fernanda Melo,\* Gloria Maria de Azevedo Thompson,\* and Rosemary Adriana Chiérici Marcantonio\*

**Background:** This study compared erbium-doped: yttrium, aluminum, and garnet (Er:YAG) laser irradiation (100 mJ/pulse; 10 Hz; 12.9 J/cm<sup>2</sup>) with or without conventional scaling and root planing (SRP) to SRP only for treatment of periodontal pockets.

**Methods:** Nineteen patients with pockets from 5 to 9 mm were included. In a split-mouth design, each site was allocated to a treatment group: 1) SRPL, SRP and laser; 2) L, laser; 3) SRP, SRP only; and 4) C, no treatment. Clinical parameters of probing depth (PD), gingival recession, and clinical attachment level (CAL) were evaluated at baseline and 1, 3, 6, and 12 months after treatment. Visible plaque index, gingival bleeding index (GI), bleeding on probing (BOP), and subgingival plaque samples were also measured 12 days postoperatively, in addition to the above mentioned months. Intergroup and intragroup statistical analyses were performed ( $P < 0.05$ ).

**Results:** GI decreased for SRPL and increased for L, SRP, and C ( $P < 0.05$ ) 12 days postoperatively and decreased for SRPL and SRP ( $P < 0.05$ ) 3, 6, and 12 months after baseline; BOP and PD decreased for all treated groups ( $P < 0.01$ ) 3, 6, and 12 months after treatment. CAL gain was significant for SRPL, L, and SRP ( $P < 0.05$ ) 3, 6, and 12 months postoperatively. SRPL and L presented a significant reduction in the percentage of sites with bacteria 6 and 12 months after treatment ( $P < 0.05$ ).

**Conclusion:** Non-surgical periodontal treatment with Er:YAG laser may be an alternative treatment for reduction and control of the proliferation of microorganisms in persistent periodontitis. *J Periodontol* 2010;81:682-691.

## KEY WORDS

Clinical trial; lasers; periodontitis; scaling and root planing.

Studies suggest that subgingival scaling treatment of chronic periodontitis with manual instruments is likely to result in a modest, albeit transient, shift in composition of microbial flora.<sup>1,2</sup> This condition seems to be the result of an inflammatory response of the periodontal tissues to the continued presence of specific anaerobic microorganism species, including *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans* [Aa]); *Porphyromonas gingivalis* (Pg); and *Treponema denticola*.<sup>3</sup>

High-intensity lasers have been used to promote periodontopathogen reduction<sup>4</sup> and also for scaling and root planing (SRP).<sup>5-11</sup> The erbium-doped: yttrium, aluminum, and garnet (Er:YAG) laser has a wavelength of 2.94  $\mu\text{m}$  and it may have bactericidal effects and potential to remove bacterial endotoxins and calculus from the root surface, due to its high water absorption capacity. Therefore, it involves less thermal risk for mineralized surfaces.<sup>7-12</sup> On the other hand, when the use of Er:YAG laser in non-surgical periodontal therapy was compared to the use of mechanical instrumentation, the consequent clinical effect was similar.<sup>13-19</sup>

However, few controlled clinical studies, performed in humans, have evaluated the effects of Er:YAG laser on root surfaces for non-surgical periodontal

\* Department of Periodontology, Araraquara Dental School, UNESP-São Paulo State University, Araraquara, São Paulo, Brazil.

† Department of Periodontology, Barretos Dental School, University Center of Educational Foundation of Barretos, Barretos, São Paulo, Brazil.

therapy, especially as an adjuvant therapy to the conventional SRP.<sup>17,18</sup> In addition, a recent systematic review has demonstrated that there is a limited number of studies available that actually investigate the clinical effects of laser as an adjunct to SRP in the treatment of chronic periodontitis.<sup>20</sup> The Er:YAG laser can be applied not only as an adjunctive therapy, but also as an alternative to mechanical instruments for non-surgical periodontal therapy.<sup>21</sup>

The objective of this controlled clinical study was to evaluate the effects on periodontal tissue treated with SRP and irradiated with Er:YAG laser, or treated only with Er:YAG laser, through the use of clinical periodontal parameters and microbiologic analysis up to 12 months of follow-up.

## MATERIALS AND METHODS

### Study Sample

The present study is a continuation of a clinical study previously reported.<sup>18</sup> In this study, 21 subjects, seven men and 14 women aged 31 to 55 years (mean of 43 years) with four non-adjacent sites in different quadrants with bleeding on probing (BOP) and probing depth (PD) from 5 to 9 mm were selected. These patients sought treatment at the Periodontal Clinic of Araraquara Dental School from 2003 to 2005. Exclusion criteria were periodontal treatment within the last 12 months, systemic disease that could influence the outcome of periodontal therapy, use of antibiotics within the last 6 months, use of anti-inflammatory drugs within the last 3 months, pregnancy or use of hormone contraceptives, and smoking. During the study, two patients were excluded from the study (n = 19 patients). All of the subjects were instructed and signed an informed consent agreement approved by the Committee of Ethics in Research of the Araraquara Dental School, UNESP (CER - 95/02).

### Sample Size Calculation

The sample size was calculated considering a 5% alpha error, with 1-mm clinical significant difference between groups, and a mean  $\pm$  SD of 0.6 mm with the values of clinical attachment level (CAL). Therefore, the power of the study was calculated to be 95% with a sample size of 19 patients.

### Pre-Experimental Treatment

Six months before the treatment, all the patients were enrolled in a 15- or 30-day program to control supra-gingival plaque, in which they received instructions on oral hygiene. At this point, professional prophylaxis was also performed according to the individual needs of each patient until baseline.

### Clinical Parameters

Alginate molds of dental arches were made before the clinical evaluation to prepare acetate stents for stan-

dardizing computerized probe<sup>‡</sup> positions and manual probes during examinations.

The following clinical parameters were evaluated at baseline: plaque index (PI),<sup>22</sup> gingival index (GI),<sup>22</sup> PD, BOP, CAL, and gingival recession (GR). BOP was determined by presence (+) or absence (-) of bleeding for 30 seconds after first probe insertion in the pocket. PI and GI were assessed by manual probing,<sup>§</sup> whereas PD, GR, and CAL were measured with a computerized probe. Crevicular fluid was collected for microbiologic analysis two days before examination, and PI and GI were performed at this time. All clinical examinations performed at baseline were repeated 1, 3, 6, and 12 months after treatments. PI, GI, and BOP were also evaluated 12 days after treatment. BOP was observed after absorbent paper point was removed from the sites, avoiding probing with the manual probe in this period. One calibrated masked examiner (BMVL) approved these clinical parameters and carried out SRP.

### Clinical Treatment

The four sites of each patient were randomly allocated using a computer-generated table for each group. The sequence of the procedures was randomized in a similar manner. For better standardization, site 1 was the first choice, followed by sites 2, 3, and 4, respectively. The sites were divided into groups: 1) SRPL, conventional SRP followed by Er:YAG laser irradiation for 30 seconds; 2) Er:YAG laser (L) irradiation only until the operator considered that the root surfaces were appropriately debrided; 3) SRP, conventional SRP; and 4) Control (C) (i.e., no treatment). Specific manual curets<sup>||</sup> were used for conventional SRP. Scaling with hand instruments or laser only was performed until the operator considered that the root surfaces were appropriately debrided and planed. These procedures were carried out after local infiltrative anesthesia. The 76 sites were divided equally between right and left sides. Sites were treated with Er:YAG laser (SRPL and L groups) on one side, whereas the sites on the contralateral side were treated with SRP-only or not treated (SRP and C groups). An experienced periodontal specialist (BMVL) performed the SRP and another trained operator (LHT) performed laser irradiation.

### Laser Treatment

An Er:YAG<sup>¶</sup> laser was selected with a wavelength of 2.94  $\mu$ m, 250 to 500  $\mu$ s exposure duration, repetition rate of 10 Hz using a handpiece with a special application tip (1.1  $\times$  0.5 mm) in the following parameters: energy at 100 mJ/pulse as indicated on the

‡ Florida Probe, Gainesville, FL.

§ PCP-UNC 15, Hu-Friedy, Chicago, IL.

|| Gracey Curets, Hu-Friedy.

¶ KEY Laser II, with handpiece P2056, KaVo Dental, Biberach, Germany.

display, resulting in transmitted energy of 71 mJ/pulse at the tip of the handpiece (2,056 and 574,2571; transmission factor of 71%) and fluency of 12.9 J/cm<sup>2</sup>/pulse. Sites were irradiated with coolant water. The laser optical fiber tip was conducted in apicocoronal movements, with approximately 30-degree inclination angle with respect to the root surface.<sup>18</sup> Irradiation for the SRPL group was performed immediately after scaling for 30 seconds per site. The irradiation time varied from 180 to 240 seconds (mean of 204 seconds) for the L group.

#### Plaque Sample Collection

The sample collections for microbiologic analysis were performed at baseline, 12 days, and 1, 3, 6, and 12 months after treatment. Visible supragingival plaque was removed using scalers. Selected sites were isolated from water and saliva with cotton rolls, and then gently air-dried. Subgingival plaque was collected using sterile paper points. One paper point (#30<sup>#</sup>) was inserted into the base of the pocket and held there for 30 seconds. The paper point was immediately placed in sterile Eppendorf vials containing 500  $\mu$ L of a sterilized phosphate-buffered saline solution and stored at -20°C for posterior bacterial polymerase chain reaction (PCR) analysis.

#### Microbiologic Analysis

The samples were analyzed for detection of *Aa*, *Pg*, *Prevotella intermedia* (Pi), *Tannerella forsythia* (previously *T. forsythensis* [Tf]), and *Prevotella nigrescens* (Pn) using PCR.<sup>23</sup> The number of sites positive to microbial testing for these bacteria was detected as present or not present in all experimental periods. Bacterial presence was confirmed initially using a non-specific oligonucleotide.\*\* The positive samples for non-specific reaction were then processed in PCR reaction, using the specific oligonucleotide. Bacteriologic sampling was carried out by one masked and calibrated examiner (BMVL) unaware of what treatment had been performed on each quadrant. Another masked examiner (RFM) performed microbiologic assessment and analysis.

#### Statistical Analyses

Data were submitted to statistical analyses with appropriate software.<sup>†† ††</sup> PD and CAL values were normally distributed and analyzed using the analysis of variance for repeated measurements (ANOVA) test. The multiple comparison Tukey-Kramer test was used for comparison of PD and CAL variables among groups and periods when ANOVA test presented a significant difference ( $P < 0.05$ ). GR values were not distributed normally and were analyzed using the Friedman test. The Dunn test was used for comparison of GR values among groups and periods when

the Friedman test presented a significant difference ( $P < 0.05$ ). Categorical data for PI, GI, BOP, and bacterial prevalence were submitted to the Cochran test followed by the multiple comparison McNemar Exact test. Differences were considered statistically significant when the  $P$  value was  $< 0.05$ .

#### RESULTS

In the initial distribution of the 76 sites in 76 teeth ( $n = 19$  patients), 42 were at teeth with one or two roots and 34 were at multirooted teeth.

#### Plaque Index

In comparison between baseline and 12 days after the treatments, there was a significant reduction in PI ( $P < 0.01$ ) for the SRPL (43.7% to 9.5%) and SRP (52.4% to 28.6%) groups. The comparison among groups demonstrated that PI was greater for L, SRP, and C compared to SRPL ( $P < 0.05$ ). When data were compared between baseline and 1 month after treatment, PI reduced for the SRPL (43.7% to 14.3%) and SRP (52.4% to 33.3%) groups ( $P < 0.05$ ). There was a statistically significant difference in PI when comparing SRPL and SRP ( $P < 0.05$ ), and SRPL and C ( $P < 0.05$ ). After 3 months, there was a significant reduction in PI ( $P < 0.01$ ) for the four groups compared to baseline: SRPL (43.7% to 23.8%); L (38.1% to 19.1%); SRP (52.4% to 14.3%); and C (42.9% to 23.8%). Compared to baseline and 180 days after treatments, there was a significant reduction in PI ( $P < 0.05$ ) for the SRPL (16.4%), L (22.9%), and SRP (19.7%) groups. Compared to baseline and 12 months after treatments, there was a significant reduction in PI ( $P < 0.05$ ) for the SRPL (23.2%), L (20.4%), and SRP (21.7%) groups; however, there were no differences among groups.

#### Gingival Index

At 12 days, there was an increase in GI for the L (33.3% to 52.4%), SRP (42.7% to 66.7%), and C (47.6% to 66.7%) groups ( $P < 0.05$ ), and a reduction was observed for SRPL (52.4% to 38.1%;  $P > 0.05$ ). There was a significant difference in the reduction of GI for the SRPL group (38.1%) in relation to SRP (66.7%) and C (66.7%;  $P < 0.05$ ) groups, but not in relation to L (52.4%;  $P < 0.05$ ). After 1 month, there was a reduction in GI for SRPL (52.4% to 23.8%;  $P < 0.05$ ). On the other hand, GI increased for C (47.6% to 71.4%;  $P < 0.05$ ). In comparison among groups, there was a significant reduction for SRPL (23.8%) compared to groups L, SRP, and C (42.9%, 47.6%, 71.4%;  $P < 0.05$ ). After 3 months, there was a significant reduction in GI ( $P < 0.01$ ) for SRPL (52.4% to

<sup>#</sup> DENTSPLY Maillefer, RJ, Brazil.

\*\* Invitrogen Tech-Line, São Paulo, Brazil.

<sup>††</sup> GraphPad Prism 5.0, GraphPad Software, San Diego, CA.

<sup>†††</sup> BioEstat 4.0, BioEstat Software, PA, Manaus, Brazil.

19.1%) and SRP (42.7% to 19.1%). There was a significant reduction for SRPL and SRP only when compared to group C ( $P < 0.05$ ). There was a significant reduction in GI ( $P < 0.01$ ) for SRPL (52.4% to 17.9%) and SRP (42.7% to 19.1%) 6 months after treatments. GI reduced significantly ( $P < 0.05$ ) for SRPL (52.4% to 20.1%) and SRP (42.7% to 23.5%) after 12 months.

**Bleeding on Probing**

Figure 1 shows the reduction for BOP. A reduction in BOP was observed for all treated groups during all study periods after treatments ( $P < 0.01$ ). There was a significant reduction in the control group after 3 months ( $P < 0.05$ ).

**Probing Depth**

A significant reduction in PD for the SRPL, L, and SRP groups was observed at all time points compared to baseline ( $P < 0.01$ ; Table 1), although differences were not significant among the treated groups at any time ( $P > 0.05$ ).

**Clinical Attachment Level**

The results demonstrated a significant improvement in mean CAL gain (Table 2) after 1 month only for SRP group ( $P < 0.05$ ) compared to baseline. At 3, 6, and 12 months the results presented a significant improvement in mean CAL gain ( $P < 0.05$ ) for all treated groups. A difference was not observed between groups in any periods ( $P > 0.05$ ).

**Gingival Recession**

There was a significant increase in GR for the laser-treated groups in all time periods compared to baseline ( $P < 0.01$ ; Table 3). A significant increase was observed in the SRP group only in the 6 and 12 months after treatment compared to baseline ( $P < 0.05$ ). Sig-

nificant differences among groups were not found in the different periods ( $P > 0.05$ ).

**Microbiologic Evaluation**

A reduction in *Aa*, *Pg*, *Pn*, and *Tf* ( $P < 0.05$ ) was observed in the laser-treated groups (SRPL and L) when comparing the percentage of sites with bacteria at baseline and 12 days after treatments. A reduction in *Aa* and *Tf* ( $P < 0.05$ ) was observed in SRP, but there was no significant reduction in the percentage of sites with bacterial prevalence for the control group ( $P > 0.05$ ; Fig. 2).

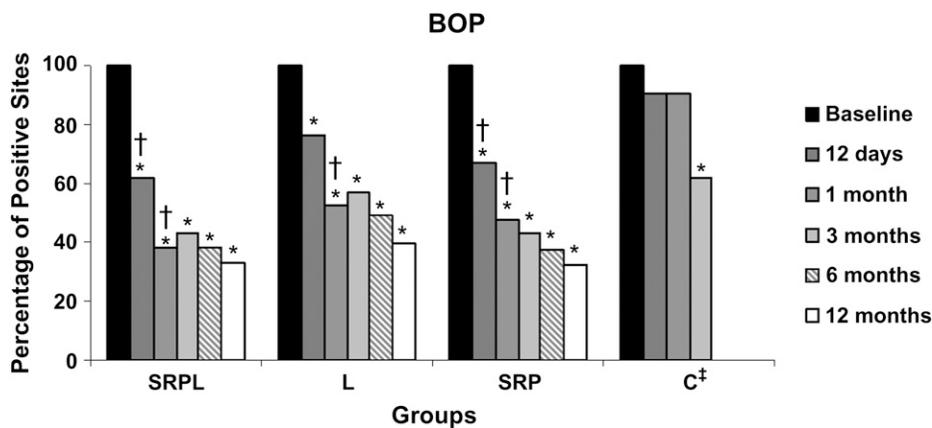
One month after the baseline, a reduction in *Pg*, *Pi*, *Pn*, and *Tf* ( $P < 0.05$ ) was observed in the SRPL group; a reduction was observed in *Aa*, *Pg*, and *Pn* ( $P < 0.05$ ) in L; a reduction was observed in *Aa* and *Tf* ( $P < 0.05$ ) in SRP; and there was no significant reduction in the percentage of sites with bacterial prevalence for the control group ( $P > 0.05$ ; Fig. 2).

After 3 months, a reduction in *Pg*, *Pi*, *Pn*, and *Tf* ( $P < 0.05$ ) was observed in the SRPL group; a reduction was observed in *Pg* ( $P < 0.05$ ) in L; and there was no significant reduction in the percentage of sites with bacterial prevalence for C and SRP (Fig. 2).

After 6 months, a reduction in *Aa*, *Pg*, *Pn*, and *Tf* ( $P < 0.05$ ) was observed in the SRPL group; a reduction was observed in *Aa* ( $P < 0.05$ ) in L; and there was no significant reduction in the percentage of sites with bacterial prevalence for SRP compared to baseline.

After 12 months, a reduction in *Aa*, *Pg*, *Pi*, *Pn*, and *Tf* ( $P < 0.05$ ) was observed in the SRPL group; a reduction was observed in *Aa* and *Pg* ( $P < 0.05$ ) in L; and there was no significant reduction in the percentage of sites with bacterial prevalence for SRP compared to baseline (Fig. 2).

The microbiologic data (intergroup analysis) are shown in Table 4.



**Figure 1.** Distribution of proportions (%) obtained for the BOP variable for the treated groups at baseline, 12 days, and 1, 3, 6, and 12 months (periods) postoperatively. \* Significant difference compared to baseline in the same group. † Significant difference compared to C group in the same period. Cochran Q and McNemar exact tests ( $P < 0.01$ ). ‡ Group C was excluded from the study 3 months postoperatively.

**DISCUSSION**

In this study, small clinical changes were observed in PI, GI, and BOP 12 days after treatment. The SRPL group presented a significant reduction in GI compared to C and SRP.

Several changes could be observed in the different groups compared to initial data 1 month after treatment. PD reduced for SRPL and SRP groups, with differences between SRPL and C. There was a significant CAL gain only for the SRP group, but without differences among groups. The clinical results obtained 1 month after treatments are in

**Table 1.****PD Values (mm; mean  $\pm$  SD) for Groups at Baseline and 1, 3, 6, and 12 Months After Therapy (n = 19 subjects)**

Group	Baseline	1 Month	3 Months	6 Months	12 Months	P Value <sup>†</sup>
SRPL	6.48 $\pm$ 1.2	4.80 $\pm$ 1.3 <sup>A†</sup>	4.57 $\pm$ 1.5 <sup>B†</sup>	4.38 $\pm$ 1.6 <sup>†</sup>	4.29 $\pm$ 1.5 <sup>†</sup>	<i>P</i> <0.01
L	6.42 $\pm$ 1.1	5.30 $\pm$ 1.2 <sup>†</sup>	5.11 $\pm$ 1.3 <sup>C†</sup>	4.88 $\pm$ 1.3 <sup>†</sup>	4.76 $\pm$ 1.2 <sup>†</sup>	<i>P</i> <0.01
SRP	6.87 $\pm$ 1.1	5.20 $\pm$ 1.4 <sup>†</sup>	4.92 $\pm$ 1.6 <sup>D†</sup>	4.64 $\pm$ 1.5 <sup>†</sup>	4.58 $\pm$ 1.3 <sup>†</sup>	<i>P</i> <0.01
C	6.28 $\pm$ 0.9	6.30 $\pm$ 0.9 <sup>A</sup>	6.71 $\pm$ 0.8 <sup>BCD</sup>	—	—	NS
<i>P</i> value*	NS	<i>P</i> <0.01	<i>P</i> <0.01	NS	NS	—

Equal letters mean statistically significant differences among groups (column); NS = not significant; — = not applicable.

\* *P* value refers to statistically significant difference between groups in the same period (*P* <0.05); analysis of variance (ANOVA) for repeated measurements and Tukey tests.

† *P* value refers to statistically significant difference for each group when compared with baseline (*P* <0.05); analysis of variance (ANOVA) for repeated measurements and Tukey tests.

accordance with the study of Tomasi et al.,<sup>16</sup> which demonstrated a significant PD reduction and a higher CAL gain in this period compared to baseline. PD and CAL presented significant differences when comparing laser irradiation to SRP using ultrasonic instrument in this study.

Mean PD and BOP presented significant clinical and statistical improvements in the treated groups compared at baseline and 1 month after treatment. There was a significant reduction in BOP compared to baseline in previous clinical studies,<sup>13,14,17</sup> not only for the laser-treated sites, but also for the ones that were treated only with SRP as in the present study.

Three months postoperatively, there was a reduction in PD for groups SRPL, L, and SRP (*P* <0.01), and these groups presented differences compared to group C (*P* <0.01). A significant CAL gain in SRPL, L, and SRP groups (*P* <0.05) was observed, but without significant differences among groups.

The results obtained in group C are in accordance with other studies,<sup>24,25</sup> which demonstrated that an effective supragingival plaque control, in a period of 3 and 6 months after treatment, can reduce BOP in non-treated sites. In the present study, group C was excluded from the study 3 months postoperatively due to the periodontal disease advance in no treatment conditions. At 3 months after clinical analysis all sites of group C (no treatment) were treated with conventional SRP.

The percentages of PD, CAL, and BOP showed significant clinical improvements in some clinical studies with Er:YAG laser in groups treated with laser at 3 months.<sup>13,14,17</sup> Some previous studies<sup>13,17</sup> showed increases in GR for groups using lasers; however, differences from baseline were not significant. Mean GR

values increased in the sites irradiated with Er:YAG laser in the present study in contrast to these clinical studies.<sup>13,14,17</sup>

Six months after treatment, there was a reduction in PD for groups SRPL, L, and SRP, and CAL gain was observed for SRPL, L, and SRP, but without significant differences among groups. A significant GR increase (*P* <0.01) was observed for groups SRPL, L, and SRP; however, without significant differences among groups.

Few studies<sup>17,18</sup> in humans have evaluated Er:YAG laser irradiation on root surfaces combined with conventional SRP. Schwarz et al.<sup>17</sup> compared Er:YAG laser (160 mJ/pulse, 10 Hz) to SRP and laser in association with SRP (L + SRP). Clinical analyses were made before treatments, and 3 and 6 months after them. There was a reduction in CAL in the laser group, SRP, and L + SRP in this study after 3 months.<sup>17</sup> These results were statistically significant compared to initial data intragroups and intergroups. There was a reduction in mean CAL after 6 months<sup>17</sup> for the laser, SRP, and L + SRP groups (with differences between laser and SRP groups and among L + SRP and SRP groups). The result of the study of Schwarz et al.<sup>17</sup> demonstrated significant differences between SRP and SRP + L at 3 and 6 months.

In the present study, reductions in PD were found in all treated groups, but there were no differences among groups at 3 and 6 months. The average reduction in PD was greater than in the studies of Schwarz et al.<sup>13,14</sup> This discrepancy can be explained by the initial differences in PD. In those studies,<sup>13,14</sup> mean initial PD, considering all groups, was around 5 mm, whereas in the present study this mean was around 6 mm. Clinical studies<sup>1,24</sup> have demonstrated that a reduction in PD and CAL gain after non-surgical and surgical periodontal treatments depends on initial

**Table 2.****CAL Values (mm; mean  $\pm$  SD) for Groups at Baseline and 1, 3, 6, and 12 Months After Therapy (n = 19 subjects)**

Group	Baseline	1 Month	3 Months	6 Months	12 Months	P Value <sup>†</sup>
SRPL	6.71 $\pm$ 1.4	6.50 $\pm$ 1.4	5.88 $\pm$ 2.1 <sup>†</sup>	5.64 $\pm$ 2.0 <sup>†</sup>	5.56 $\pm$ 1.4 <sup>†</sup>	<i>P</i> <0.05
L	6.61 $\pm$ 1.1	6.58 $\pm$ 1.3	6.33 $\pm$ 1.7 <sup>†</sup>	6.01 $\pm$ 1.3 <sup>†</sup>	5.93 $\pm$ 1.1 <sup>†</sup>	<i>P</i> <0.05
SRP	7.20 $\pm$ 1.3	6.72 $\pm$ 1.3 <sup>†</sup>	6.01 $\pm$ 1.2 <sup>†</sup>	5.85 $\pm$ 1.5 <sup>†</sup>	5.79 $\pm$ 1.3 <sup>†</sup>	<i>P</i> <0.05
C	6.80 $\pm$ 1.5	6.94 $\pm$ 1.4	6.83 $\pm$ 1.5	—	—	NS
<i>P</i> value*	NS	NS	NS	NS	NS	—

\* *P* value refers to statistically significant difference among groups in the same period; analysis of variance (ANOVA) for repeated measurements and Tukey tests.

<sup>†</sup> *P* value refers to statistically significant difference for each group compared to baseline (*P* <0.05); analysis of variance (ANOVA) for repeated measurements and Tukey tests.

NS = not significant; — = not applicable.

**Table 3.****GR Values (mm; mean  $\pm$  SD) for Groups at Baseline and 1, 3, 6, and 12 Months After Therapy (n = 19 subjects)**

Group	Baseline	1 Month	3 Months	6 Months	12 Months	P Value <sup>†</sup>
SRPL	0.24 $\pm$ 0.70	1.05 $\pm$ 1.12 <sup>†</sup>	1.10 $\pm$ 0.70 <sup>†</sup>	0.96 $\pm$ 0.86 <sup>†</sup>	0.93 $\pm$ 0.73 <sup>†</sup>	<i>P</i> <0.01
L	0.19 $\pm$ 0.40	0.86 $\pm$ 0.9 <sup>†</sup>	0.86 $\pm$ 0.33 <sup>†</sup>	0.80 $\pm$ 0.33 <sup>†</sup>	0.75 $\pm$ 0.28 <sup>†</sup>	<i>P</i> <0.01
SRP	0.33 $\pm$ 0.66	0.81 $\pm$ 1.17	0.86 $\pm$ 0.24	0.90 $\pm$ 0.49 <sup>†</sup>	0.86 $\pm$ 0.38 <sup>†</sup>	<i>P</i> <0.05
C	0.52 $\pm$ 0.98	0.62 $\pm$ 1.02	0.76 $\pm$ 0.47	—	—	NS
<i>P</i> value*	NS	NS	NS	NS	NS	—

NS = not significant; — = not applicable.

\* *P* value refers to statistically significant difference among groups in the same period; Friedman and Dunn tests.

<sup>†</sup> *P* value refers to statistically significant difference for each group compared to baseline (*P* <0.01); Friedman and Dunn tests.

PD, with a greater probability of success in PD reduction for deeper pockets, no matter what type of treatment is being used.

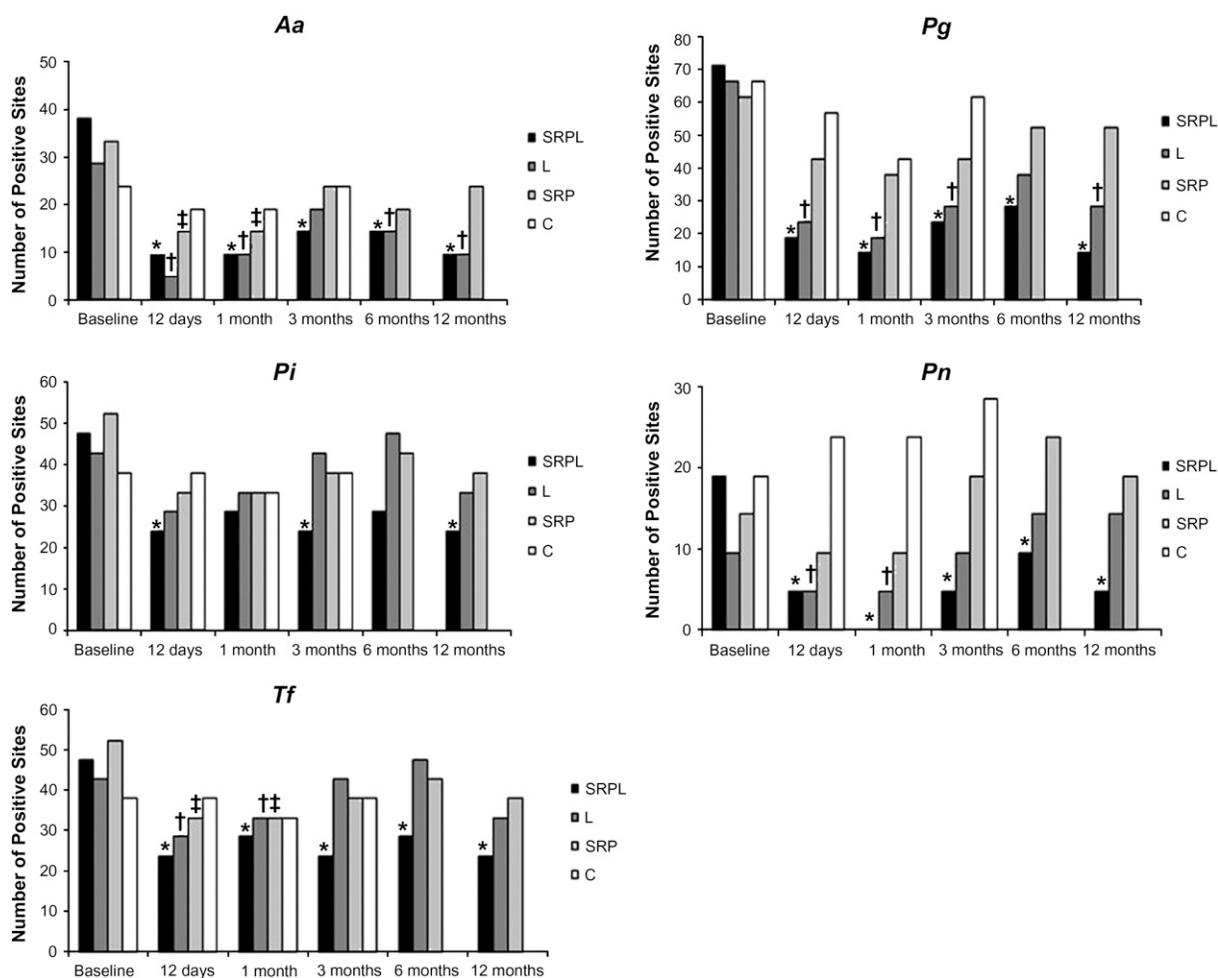
The differences found were not significant despite clinical studies<sup>13,14,17</sup> having demonstrated an increase in GR for groups treated with laser. As opposed to those findings, the present study found statistically significant differences (*P* <0.01) in GR increase for two groups treated with Er:YAG laser at all periods; however, without differences between treatments, despite CAL gain and significant reduction in PD (*P* <0.01). These results can be related to a greater contraction of gingival tissue or to edema reduction; it can also be a consequence of the increase of instrumentation in groups that used Er:YAG laser, causing possible damage on gingival tissue at the time of treatment.

The modified bacterial composition after scaling represents the base for periodontal healing expressed as PD reduction and CAL gain.<sup>26</sup> Studies have demon-

strated that when periodontitis progresses, despite treatment, high levels of *Aa*,<sup>27</sup> *Pg*,<sup>28</sup> *Pi*,<sup>3,28,29</sup> and *Tf*,<sup>3,30</sup> are found on subgingival plaque. The present study shows significant reduction in the number of sites with bacteria; moreover, this situation tended to increase 3 and 6 months after treatments.

In relation to the microbiologic findings in the initial periods of the present study, SRPL and L groups presented less prevalence of sites with *Aa* compared to SRP at 12 days. The SRPL presented less prevalence of *Pg* and *Pn* compared to SRP. These findings corroborate in part with the study of Tomasi et al.,<sup>16</sup> which demonstrated a significant reduction in *Tf*, *Pg*, *Pi*, and *Pn* in evaluation 2 days after laser irradiation, but without differences between SRP and ultrasonic scaling.

In this study, there were differences in *Pg* and *Pn* among SRP compared to SRPL and L, whereas in the study by Tomasi et al.<sup>16</sup> there was a significant reduction in *Tf*, *Pg*, *Pi*, and *Pn* in evaluation 1 month after



**Figure 2.**

Percentage of sites positive to microbial testing for Aa, Pg, Pi, Pn, and Tf categorized by treatment at different periods. Symbols (\* for SRPL, † for L, and ‡ for SRP) on columns indicate a significant difference with baseline in same group; Cochran Q and McNemar exact tests ( $P < 0.05$ ).

irradiation; however, without differences between groups.

A reevaluation of PI, GI, and BOP, and prevalence of Aa, Pg, Pi, Pn, and Tf was conducted at 12 and 30 days to verify a possible immediate effect of SRPL and L treatments compared to SRP only and to the control group. An early detection of the clinical improvements after debridement with SRP and Er:YAG laser can be associated with a combination of mechanical disorganization of dental biofilm, caused by mechanical therapy associated with irradiation effect on the soft tissue of the periodontal pocket, and with a reduction in the inflammatory process as a consequence of the reduction in viable bacteria, due to the thermal effect of the pockets irradiation using Er:YAG.<sup>4</sup>

The results in the present study corroborate with other studies<sup>15,16</sup> that observed a significant reduction in bacteria after periodontal treatment until 3 months after baseline. The tendency presented in

our study, as in Derdilopoulou et al.,<sup>15</sup> is one of an increase in sites with bacteria present in the period between 3 and 6 months, but without significant differences between these values. According to Haffajee and Socransky,<sup>31</sup> the gingival inflammatory presence in one site considerably affects the composition of its microflora. Aa, Pg, Pi, and Tf were significantly elevated in sites that presented bleeding on probe, an index used as a clinical indicator of periodontal inflammation. Haffajee and Socransky<sup>31</sup> believe that the specimens that presented elevated levels of inflamed sites could have benefited from the inflammation. This is likely to have happened because they were closer to the gingival crevicular fluid, and also because this fluid could be enriched with products from the tissue degradation.<sup>32,33</sup>

At 6 months, the SRPL group showed less prevalence of Pg and Pn compared to SRP. SRPL showed less prevalence in sites with all the analyzed bacteria at 12 months compared to baseline. In comparison

**Table 4.****Percentage of Sites Positive to Microbial Testing for Specific Bacteria Categorized by Treatment at Different Periods**

Groups and Bacteria	Baseline	12 Days	1 Month	3 Months	6 Months	12 Months
<b>SRPL</b>						
<i>Aggregatibacter actinomycetemcomitans</i>	38.1	9.5 <sup>B</sup>	9.5	14.3	14.3	9.5 <sup>Q</sup>
<i>Porphyromonas gingivalis</i>	71.4	19 <sup>C</sup>	14.3 <sup>G</sup>	23.8	28.6 <sup>O</sup>	14.3 <sup>RS</sup>
<i>Prevotella intermedia</i>	47.6	23.8	28.6	23.8	28.6	23.8
<i>Prevotella nigrescens</i>	19 <sup>A</sup>	4.8 <sup>D</sup>	0 <sup>HI</sup>	4.8 <sup>LM</sup>	9.5 <sup>P</sup>	4.8 <sup>TU</sup>
<i>Tannerella forsythia</i>	76.2	14.3	19	28.6	33.3	33.3
<b>L</b>						
<i>Aggregatibacter actinomycetemcomitans</i>	28.6	4.8 <sup>BE</sup>	9.5	19.0	14.3	9.5 <sup>V</sup>
<i>Porphyromonas gingivalis</i>	66.7	23.8	19 <sup>J</sup>	28.6	38.1	28.6 <sup>RX</sup>
<i>Prevotella intermedia</i>	42.9	28.6	33.3	42.9	47.6	33.3
<i>Prevotella nigrescens</i>	9.5 <sup>A</sup>	4.8 <sup>F</sup>	4.8 <sup>HK</sup>	9.5 <sup>LN</sup>	14.3	14.3 <sup>T</sup>
<i>Tannerella forsythia</i>	66.7	19.1	28.6	38.1	42.9	47.6
<b>SRP</b>						
<i>Aggregatibacter actinomycetemcomitans</i>	33.3	14.3 <sup>E</sup>	14.3	23.8	19	23.8 <sup>QV</sup>
<i>Porphyromonas gingivalis</i>	61.9	42.9 <sup>C</sup>	38.1 <sup>GJ</sup>	42.9	52.4 <sup>O</sup>	52.4 <sup>SX</sup>
<i>Prevotella intermedia</i>	52.4	33.3	33.3	38.1	42.9	38.1
<i>Prevotella nigrescens</i>	14.3	9.5 <sup>DF</sup>	9.5 <sup>IK</sup>	19 <sup>MN</sup>	23.8 <sup>P</sup>	19 <sup>U</sup>
<i>Tannerella forsythia</i>	71.4	23.8	33.3	42.9	52.4	57.1
<b>C</b>						
<i>Aggregatibacter actinomycetemcomitans</i>	23.8 <sup>†</sup>	19 <sup>†</sup>	19 <sup>†</sup>	23.8 <sup>†</sup>	—	—
<i>Porphyromonas gingivalis</i>	66.7 <sup>†</sup>	57.1 <sup>†</sup>	42.9 <sup>†</sup>	61.9 <sup>†</sup>	—	—
<i>Prevotella intermedia</i>	38.1	38.1	33.3	38.1	—	—
<i>Prevotella nigrescens</i>	19 <sup>†</sup>	23.8 <sup>†</sup>	23.8 <sup>†</sup>	28.6 <sup>†</sup>	—	—
<i>Tannerella forsythia</i>	66.7 <sup>†</sup>	61.9 <sup>†</sup>	61.9 <sup>†</sup>	57.1 <sup>†</sup>	—	—
P value*	P <0.05	P <0.05	P <0.05	P <0.05	P <0.05	P <0.05

\* P value refers to statistically significant difference among groups; Cochran Q and McNemar exact tests.

† Statistically significant difference compared to all treated groups in the same period.

Groups with equal letters mean statistically significant difference (column); — = not applicable.

between groups, SRPL showed less prevalence in sites with *Aa*, *Pg*, and *Pn* compared to SRP, and group L showed less prevalence of *Aa* and *Pg* compared to SRP at 12 months.

Thermal and photodisruptive laser effects result in the elimination of periodontopathogenic bacteria. The laser would promote bacterial reduction, such as in the root of the soft tissue of the pocket, leaving the environment of periodontal pocket decontaminated.<sup>5,8</sup> Furthermore, this laser has been assumed not only to eliminate bacteria, but also to inactivate bacterial toxins diffused in the root cementum without producing a smear layer.<sup>11</sup>

In the present study, energy density for pulse was 12.9 J/cm<sup>2</sup> with variable exposition time between groups SRPL and L. Theodoro et al.<sup>34</sup>, using the same parameters of irradiation in vitro, showed adhesion of blood elements on the irradiated root surfaces. Although they have used higher irradiation parameters (160 mJ/pulse) than the ones presented in this study (100 mJ/pulse) for scaling, Schwarz et al.<sup>13,14,17</sup> did

not verify significant differences regarding PD and CAL between treatments with L and SRP + L. The angular position of 30 degrees between the outlet tip and the root surface used in this methodology was based on the study of Folwaczny et al.<sup>35</sup>

The clinical results obtained in the present study are, in a certain way, similar to the findings in other clinical studies.<sup>13-16,19</sup> The results demonstrated that the Er:YAG laser monotherapy was effective in non-surgical periodontal treatment, but it did not present clinical benefits in the treatment of periodontal pockets compared to SRP procedures or in association with SRP. Microbiologic evaluation showed that SRP reduced the prevalence of sites with all the bacterial analyzed in this study when associated with laser irradiation.

## CONCLUSIONS

The present results indicate that non-surgical periodontal treatments with Er:YAG laser monotherapy and SRP with Er:YAG laser irradiation are effective;

however, clinical benefits were not observed compared to conventional SRP procedures. Microbiologic findings obtained in the present study suggest that non-surgical periodontal treatment with Er:YAG laser may be an alternative treatment for reduction and control of the proliferation of microorganisms in persistent periodontitis.

### ACKNOWLEDGMENTS

This study is attributed to Department of Periodontology, Araraquara Dental School, UNESP-São Paulo State University, Araraquara, São Paulo, Brazil; and supported by a grant from CAPES (Coordination for the Improvement of Higher Education Personnel), Brasília, DF, Brazil. The authors report no conflicts of interest related to this study.

### REFERENCES

- Cobb CM. Non-surgical pocket therapy: Mechanical. *Ann Periodontol* 1996;1:443-490.
- Sherman PR, Hutchens LH, Jewson LG, Moriarty JM, Greco GW, McFall WT. The effectiveness of subgingival scaling and root planing. I. Clinical detection of residual calculus. *J Periodontol* 1990;61:3-8.
- Haffajee AD, Cugini MA, Dibart S, Smith C, Kent RL, Jr., Socransky SS. The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *J Clin Periodontol* 1997;24:324-334.
- Ando Y, Aoki A, Watanabe H, Ishikawa I. Bactericidal effect of erbium YAG laser on periodontopathic bacteria. *Lasers Surg Med* 1996;19:190-200.
- Aoki A, Ando A, Watanabe H, Ishikawa I. In vitro studies on laser scaling of subgingival calculus with an Er:YAG laser. *J Periodontol* 1994;65:1097-1106.
- Israel M, Cobb CM, Rossmann JA, Spencer P. The effects of CO<sub>2</sub>, Nd:YAG and Er:YAG lasers with and without surface coolant on tooth root surfaces: An in vitro study. *J Clin Periodontol* 1997;24:595-602.
- Folwaczny M, Mehl A, Haffner C, Benz C, Hickel R. Root substance removal with Er:YAG laser radiation at different parameters using a new delivery system. *J Periodontol* 2000;71:147-155.
- Aoki A, Miura M, Akiyama F, et al. In vitro evaluation of Er:YAG laser scaling of subgingival calculus in comparison with ultrasonic scaling. *J Periodontol Res* 2000;35:266-276.
- Theodoro LH, Haypek P, Bachmann L, et al. Effect of Er:YAG and diode laser irradiation on the root surface: Morphological and thermal analyses. *J Periodontol* 2003;74:838-843.
- Theodoro LH, Garcia VG, Haypek P, Zezell DM, Eduardo CP. Morphologic analysis, by means of scanning electron microscopy, of the effect of Er:YAG laser on root surfaces submitted to scaling and root planing. *Pesqui Odontol Bras* 2002;16:308-312.
- Ishikawa I, Aoki A, Takasaki AA. Potential applications of Erbium:YAG laser in periodontics. *J Periodontol Res* 2004;39:275-285.
- Schwarz F, Aoki A, Becker J, Sculean A. Laser application in non-surgical periodontal therapy: A systematic review. *J Clin Periodontol* 2008;35(Suppl.8):29-44.
- Schwarz F, Sculean A, Georg T, Reich E. Periodontal treatment with an Er:YAG laser compared to scaling and root planing: A controlled clinical study. *J Periodontol* 2001;72:361-367.
- Schwarz F, Sculean A, Berakdar M, Georg T, Reich E, Becker J. Periodontal treatment with an Er:YAG laser or scaling and root planing: A 2-year follow-up split-mouth study. *J Periodontol* 2003;74:590-596.
- Derdilopoulou FV, Nonhoff J, Neumann K, Kielbassa AM. Microbiological findings after periodontal therapy using curettes, Er:YAG laser, sonic, and ultrasonic scalers. *J Clin Periodontol* 2007;34:588-598.
- Tomasi C, Schander K, Dahlén G, Wennström JL. Short-term clinical and microbiologic effects of pocket debridement with an Er:YAG laser during periodontal maintenance. *J Periodontol* 2006;77:111-118.
- Schwarz F, Sculean A, Berakdar M, Georg T, Reich E, Becker J. Clinical evaluation of an Er:YAG laser combined with scaling and root planing for non-surgical periodontal treatment: A controlled, prospective clinical study. *J Clin Periodontol* 2003;30:26-34.
- Lopes BM, Marcantonio RA, Thompson GM, Neves LH, Theodoro LH. Short-term clinical and immunological effects of scaling and root planing with Er:YAG laser in chronic periodontitis. *J Periodontol* 2008;79:1158-1167.
- Crespi R, Cappare P, Toscanelli I, Gherlone E, Romanos GE. Effects of Er:YAG laser compared to ultrasonic scaler in periodontal treatment: A 2-year follow-up split-mouth clinical study. *J Periodontol* 2007;78:1195-1200.
- Karlsson MR, Diogo Löfgren CI, Jansson HM. The effect of laser therapy as an adjunct to non-surgical periodontal treatment in subjects with chronic periodontitis: A systematic review. *J Periodontol* 2008;79:2021-2028.
- Ishikawa I, Aoki A, Takasaki AA, Mizutani K, Sasaki KM, Izumi Y. Application of lasers in periodontics: True innovation or myth? *Periodontol 2000* 2009;50:90-126.
- Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975;25:229-235.
- Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol* 1996;11:266-273.
- Badersten A, Nilveus R, Egelberg J. Effect of non-surgical periodontal therapy. I. Moderately advanced periodontitis. *J Clin Periodontol* 1981;8:57-72.
- Gomes SC, Piccinin FB, Susin C, Oppermann RV, Marcantonio RA. Effect of supragingival plaque control in smokers and never-smokers: 6-month evaluation of patients with periodontitis. *J Periodontol* 2007;78:1515-1521.
- Socransky SS, Haffajee AD, Cugini MA. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;25:134-144.
- Van Winkelhoff AJ, Van der Velden U, De Graaff J. Microbial succession in recolonizing deep periodontal pockets after a single course of supra- and subgingival debridement. *J Clin Periodontol* 1988;15:116-122.
- Chaves ES, Jeffcoat MK, Ryerson CC, Snyder B. Persistent bacterial colonization of *Porphyromonas*

- gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans* in periodontitis and its association with alveolar bone loss after 6 months of therapy. *J Clin Periodontol* 2000;27:897-903.
29. Mombelli A, Schmidt B, Rutar A, Lang NP. Persistent patterns of *Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens* and *Actinobacillus actinomycetemcomitans* after mechanical therapy of periodontal disease. *J Periodontol* 2000;71:14-21.
  30. Magnusson I, Lindhe J, Yoneyama T, Liljenberg B. Recolonization of a subgingival microbiota following scaling in deep pockets. *J Clin Periodontol* 1984;11:193-207.
  31. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol* 2000 1994;5:78-111.
  32. Ramfjord SP, Caffesse RG, Morrison EC. Four modalities of periodontal treatment compared over 5 years. *J Clin Periodontol* 1987;14:445-452.
  33. Kaldahl WB, Kalwarf KL, Patil KD, Molvar MP, Dyer JK. Long-term evaluation of periodontal therapy: I. Response to 4 therapeutic modalities. *J Periodontol* 1996;67:93-102.
  34. Theodoro LH, Sampaio JE, Haypek P, Bachmann L, Zezell DM, Garcia VG. Effect of Er:YAG and diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces. *J Periodontol Res* 2006;41:381-390.
  35. Folwaczny M, Thiele L, Mehl A, Hickel R. The effect of working tip angulation on root substance removal using Er:YAG laser radiation: An in vitro study. *J Clin Periodontol* 2001;28:220-226.
- Correspondence: Professor Rosemary Adriana Chiérice Marcantonio, Department of Periodontology, Araraquara Dental School, UNESP-São Paulo State University, Rua Humaitá 1680, Araraquara, São Paulo, Brazil. 14801-903. Fax: 55-16-3301-6369; e-mail [adriana@foar.unesp.br](mailto:adriana@foar.unesp.br) and [letheodoro@uol.com.br](mailto:letheodoro@uol.com.br).
- Submitted May 27, 2009; accepted for publication December 28, 2009.