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Pesticide Application Method and Timing Influences Contamination of Nectar in Salvia

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Summary

Exposure to pesticides is one potential factor contributing to the loss of pollinators and pollinator diversity observed over the recent past. This project evaluated the influence of pesticide application method (drench vs. spray) and timing (relative to flowering) on contamination of nectar of *Salvia* × 'Indigo Spires' (*Salvia longispicata* × *S. farinacea*). The systemic insecticide thiamethoxam (Flagship) was used as a model pesticide and applied at the lowest label-rate. The concentrations of thiamethoxam and its metabolite clothianidin in nectar were highest in drench applications, regardless of application timing, and exceeded published toxicity thresholds for native bees and/or honeybees in the case of thiamethoxam. In contrast, concentrations in nectar were below toxicity thresholds for both spray applications before and after flowering. Concentrations were lower for spray and drench applications made before flowering relative to applications made after flowers began opening.

INTRODUCTION

Pollinators are important not only for maintaining biodiversity and ecosystems, but also for the provision of food for humans, which would be greatly limited without pollination (Kearns et al., 1998). World-wide pollinator populations and diversity have declined over the past hundred years due to several potential factors, including exposure to pesticides (Biesmeijer et al., 2006). Pollinators may be exposed to pesticides by ingestion when collecting nectar and pollen from contaminated flowers, as well as from contact on contaminated flower and leaf surfaces.

Ornamental plant production and allied industries are an important economic engine. Hall et al. (2020) reported that the output for the U.S. green industry in 2018 was $159.6 billion. Ornamentals marketed as pollinator friendly are important sources of food and refuge in urbanized landscapes, as well as urban areas bordering agricultural areas (Marquardt et al., 2021) Given this critical role, minimization of unnecessary contact with pesticides is important. Lentola et al. (2017) surveyed ornamental plants at retail centers, detecting neonicotinoid insecticides in 70% of the plants. While “pollinator- friendly” plants promote and support pollinators with pollen and nectar, they may be harmful if pesticide levels are above toxicity thresholds.

Given that pesticides are necessary tools for economically producing ornamental plants, pest management practices should be optimized to reduce exposures of pollinators as much as possible, while simultaneously providing adequate control of pests. Before practices can be optimized, an understanding of the influence of pesticide application and cultural practices on contamination of floral resources is needed. Few studies have specifically focused on the relationship between pesticide management during ornamental plant production and contamination of nectar.

This research focused on the systemic neonicotinoid insecticide thiamethoxam. It is an antagonist of insect nicotinic acetylcholine receptors, and is metabolized to clothianidin, which is also highly toxic to pollinators with the same mode of action (Nauen et al., 2003). The objectives

of this project were to evaluate and quantify the influence of thiamethoxam application methods (spray vs. drench) and timing (relative to flowering) on contamination of nectar using *Salvia* × 'Indigo Spires' as a model ornamental species.

MATERIALS AND METHODS

Salvia × 'Indigo Spires' is a vigorous and non-stop bloomer, producing dark violet flowers from early summer through fall (Clebsch and Barner, 2003) (**Fig. 1A, 1B**). It is valued for its long spikes of flowers that produce large volumes of nectar and attract a wide variety of pollinators including hummingbirds, honeybees, and native bees. Plants were purchased as rooted cuttings from Hatchett Creek Farms (Gainesville, FL) and transplanted into 9.9 cm (4.5-in) pots filled with a general-purpose soilless media (Promix BX, Premier Tech Horticulture, Quebec). Plants were grown under controlled conditions in a greenhouse on the University of Florida campus for six weeks, with periodic pruning (3x) to promote branching and maintain plant size. Afterwards, plants were repotted into 11.4 L (3-gal) containers using the same media, fertilized with 15-ml (1-tbls) of Osmocote 14N-4P-14K per plant, and moved to a shade house (**Fig. 1A**). Plants were manually irrigated at least once a day.

This study used eight replicate plants per treatment (including controls) and a 2 x 2 factorial design to evaluate the influence of application method (2 levels: drench vs. spray) and timing (2 levels: pre-bloom vs. post-bloom opening) on contamination of flower nectar. Commercially available Flagship 25WG (a water dispersible granule containing 25% thiamethoxam) was applied as a foliar spray using a hand-operated spray bottle (0.15 g/L, 155 mL per plant) or soil drench (0.30 g/L, 1 L per pot) at the lowest labeled rate for ornamentals. Applications for the "pre-bloom" treatment were made 21 days after transplanting. Two weeks later, applications for the "post-bloom" treatment were made, when half of the flowers were open. Stratified random sampling was used to select plants for each treatment.

Nectar samples were collected seven days after the last pesticide application using 50 µL glass microcapillaries (**Fig. 1C**). Nectar volume was estimated by measuring the length of liquid in the tube. Each sample was stored separately in an Eppendorf tube on ice in the field followed by storage at -80°C until analysis. Samples were diluted with H_2O:ACN (9:1), thoroughly mixed using a vortex, and centrifuged (13,000 RCF, 10 min) prior to analysis on the same day. Thiamethoxam and its' metabolite clothianidin were analyzed using a 1290 Infinity II ultra high-pressure liquid chromatograph (uHPLC, Agilent) equipped with a C18 reversed-phase column (Zorbax Eclipse C18, Rapid resolution HD, 150 × 2.1 mm, 1.8 µm) and coupled to an Agilent 6495 tandem mass spectrometer (MS/MS). The MS/MS was operated in electrospray ionization (ESI) positive mode, with nitrogen as the source and collision gas. Mobile phase solvent composition and gradient (uHPLC), and multiple reaction monitoring (MRM) transitions used for identification and quantification are shown in **Table 1**. Concentrations in the samples were determined using external calibration curves.

Statistical analyses were conducted using the software R (R Core Team, 2021). To test if method and/or timing of pesticide application influenced the concentration of thiamethoxam and/or clothianidin detected in nectar, two-way analysis of variance (ANOVA, P=0.05) was conducted after log transforming the thiamethoxam and log transforming +1 the clothianidin data to meet the assumptions of ANOVA.

RESULTS

Application method significantly influenced concentrations of thiamethoxam (P-value:<2E-16) and clothianidin (P-value:<2E-16) in nectar. When thiamethoxam was applied before flowering, concentrations in nectar were 421.6±71.6 ng/mL with drench applications (**Fig. 2A**), and 3.5±1.3 ng/mL when applied as a spray (**Fig. 2B**). Concentrations in nectar were higher in both cases when thiamethoxam was applied after flowering (**Fig. 2A,B**). In this case, thiamethoxam concentrations were 820.4±192.9 ng/mL with the drench applications and 13.7±2.6 ng/mL with

the spray applications. Clothianidin concentrations followed a similar pattern where concentrations in nectar were significantly higher when thiamethoxam was applied as a drench (**Fig. 3A, B**). However, concentrations were lower than its' precursor thiamethoxam. In this case, clothianidin concentrations in nectar were 27.2±2.9 ng/mL (drench applied before flowering), <0.5 ng/mL (spray applied before flowering), 44.8±12.1 ng/mL (drench applied after flowering), and 2.2±0.6 ng/mL (spray applied after flowering).

Timing of applications also significantly influenced concentrations of thiamethoxam (*P*-value:<2E-16) and clothianidin (*P*-value:<2E-16) in nectar. Regardless of application method (spray or drench), concentrations were higher when applications were made after flowering. For drench applications, thiamethoxam concentrations when applied after blooming (820.4±192.9 ng/mL) were almost twice the concentrations detected in nectar when the pesticide was applied before flowering (421.6±71.6 ng/mL) (**Fig. 2A**). For spray applications, thiamethoxam concentrations from applications after blooming (13.7±2.6 ng/mL) were three times the concentrations when the pesticide was applied before flowering (3.5±1.3 ng/mL) (**Fig. 2B**). For clothianidin drench applications the concentrations in nectar were 1.5x higher when thiamethoxam was applied after flowers opened (44.8±12.1 ng/mL) relative to applications made before flowering (27.2±2.9 ng/mL) (**Fig. 3A**). For spray applications, clothianidin concentrations were 4.4x higher in plants treated after flowering (2.2±0.6 ng/mL) relative to before (<0.5 ng/mL) (**Fig. 3B**).

DISCUSSION

Results presented herein clearly reveal that pesticide application method and timing influence contamination of nectar with thiamethoxam and clothianidin. Concentrations of thiamethoxam were 120x higher when it was applied as a drench (relative to spray application) before flower initiation, and 58x higher when applied as a drench after flowering (relative to spray applications after flowering). Relative to the timing of each application method the differences were not as

great but were significant. In this case concentrations in nectar from the drench applications were 1.9x greater when applied after flowering. Concentrations from the spray applications were 4x greater in treatments applied after flowering. These results are consistent with findings of Cowles and Eitzer (2017) who evaluated contamination of pollen and nectar in sunflower and swamp milkweed associated with spray and drench applications with the neonicotinoid insecticides dinotefuran, imidacloprid, and thiamethoxam. They reported that concentrations were generally higher with drench applications, and that concentrations of dinotefuran and thiamethoxam tended to increase as applications approached flowering. The higher concentrations detected in the drench treatments are likely related to actual amounts of active ingredient applied to each plant. Based on label recommended rates, 0.075 g of thiamethoxam was applied in the drench treatments, as compared to 0.0058 g for the spray applications. Nevertheless, while the plants in the drench treatments received 12.9x of thiamethoxam/plant compared with spray treatments, results showed that residues of thiamethoxam in nectar were 120x for drench relative to spray when applied before blooming, and 58x for drench relative to spray when applied after blooming. Clothianidin behaved similarly, though concentrations were much lower.

To assess ecological risks of both compounds, concentrations in nectar were compared with published median lethal concentrations (LC_{50}) in nectar/feeding solutions and no observable effects concentrations (NOEC) for pollinators. Thiamethoxam concentrations exceeded LC_{50} values of 227 ng/mL (European honeybee, *Apis mellifera*) and 54.3 ng/mL (native bee, *Melipona scutellaris*) with drench application treatments before and after blooming, indicating significant risks for acute toxicity (**Fig. 2A**) (Miotelo et al., 2021). The NOEC reported for thiamethoxam was 141 ng/mL for *Apis mellifera* (Overmyer et al., 2018), indicating significant toxicity would likely occur with the drench treatments. In contrast, these toxicity thresholds were not exceeded with either spray application (**Fig. 2B**). Concentrations of clothianidin in nectar for all

treatments (drench or spray, before or after flowering) were below the LC_{50} for *Apis mellifera* (81 ng/mL) (Laurino et al., 2011) (**Fig. 3**), indicating low risks of acute toxicity.

CONCLUSIONS

Application method and timing significantly impacted contamination of nectar with thiamethoxam and clothianidin, with thiamethoxam concentrations from drench treatments likely being toxic to pollinator species. Spray treatments resulted in the least contamination of nectar. Future research should evaluate other species, pesticides, and application parameters for develop best management practices (BMPs) for pollinator protection.

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Table 1. Mobile phase gradient conditions and multiple reaction monitoring (MRM) transitions (m/z) for identification and quantification of thiamethoxam and clothianidin in nectar.

Mobile Phase Gradient			
Time (min)	*A (%)	**B (%)	Flow (mL/min)
0.00	90	10	0.400
1.00	90	10	0.400
7.00	10	90	0.400
7.50	90	10	0.400

MRM Transitions for MS/MS Analysis			
Analyte	Precursor m/z	Quantifier m/z	Qualifier m/z
Thiamethoxam	292.03	211.11	181.1
Clothianidin	250	169	131.9

*Solvent A: 95% Optima LC-MS water, 5% Optima LC-MS ACN, with 0.1% Optima formic acid, 5 mM ammonium formate.

**Solvent B: 95% Optima LC-MS ACN, 5% Optima LC-MS water, with 0.1% Optima formic acid, 5mM ammonium formate.

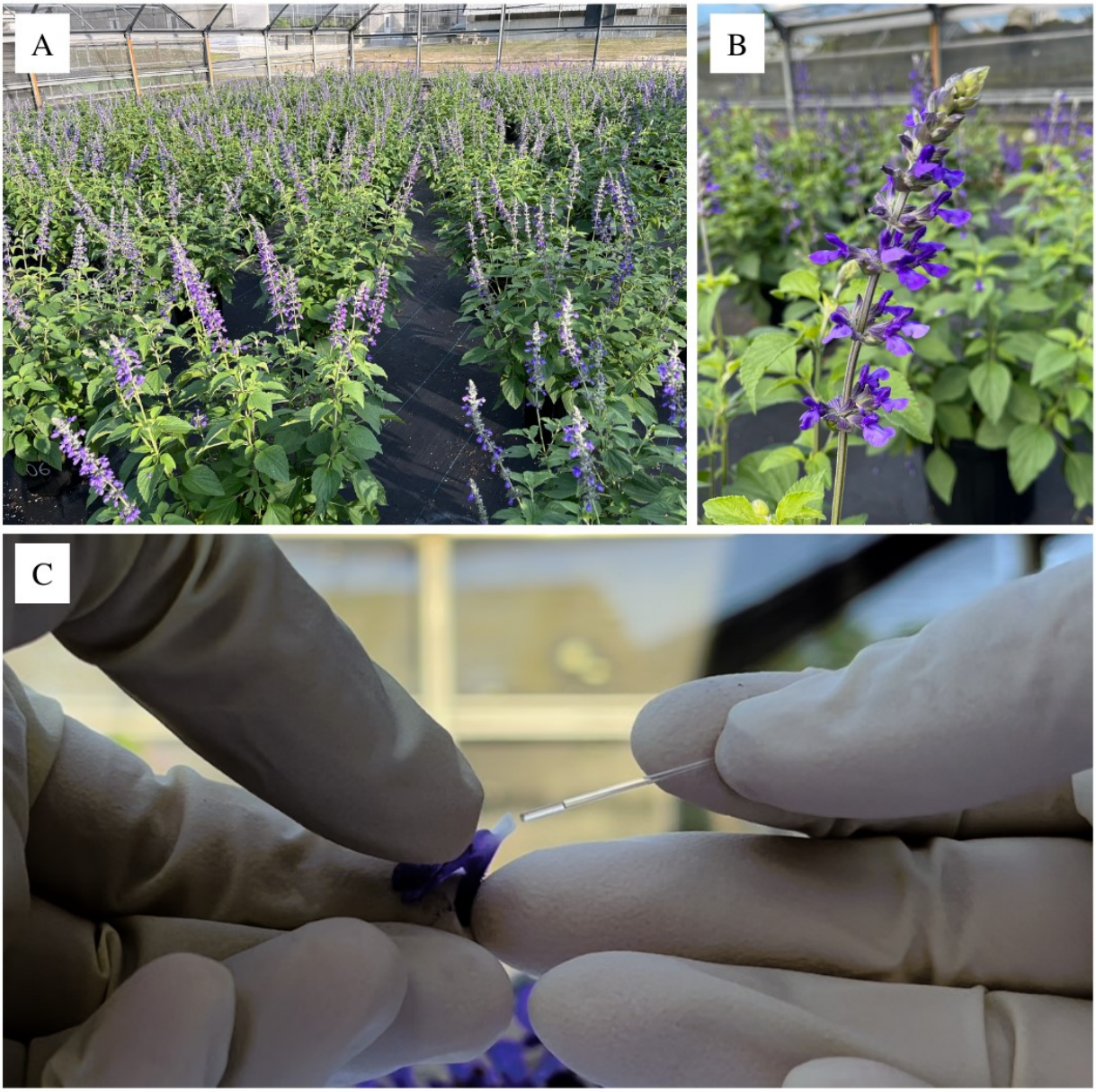

Figure 1. A) *Salv*ia × 'Indigo Spires' grown in shade house during the study. B) Representative flower spike of *Salvi*a × 'Indigo Spires'. C) Extraction of nectar from floret using a 50 µL glass microcapillary (note nectar in tip of microcapillary).

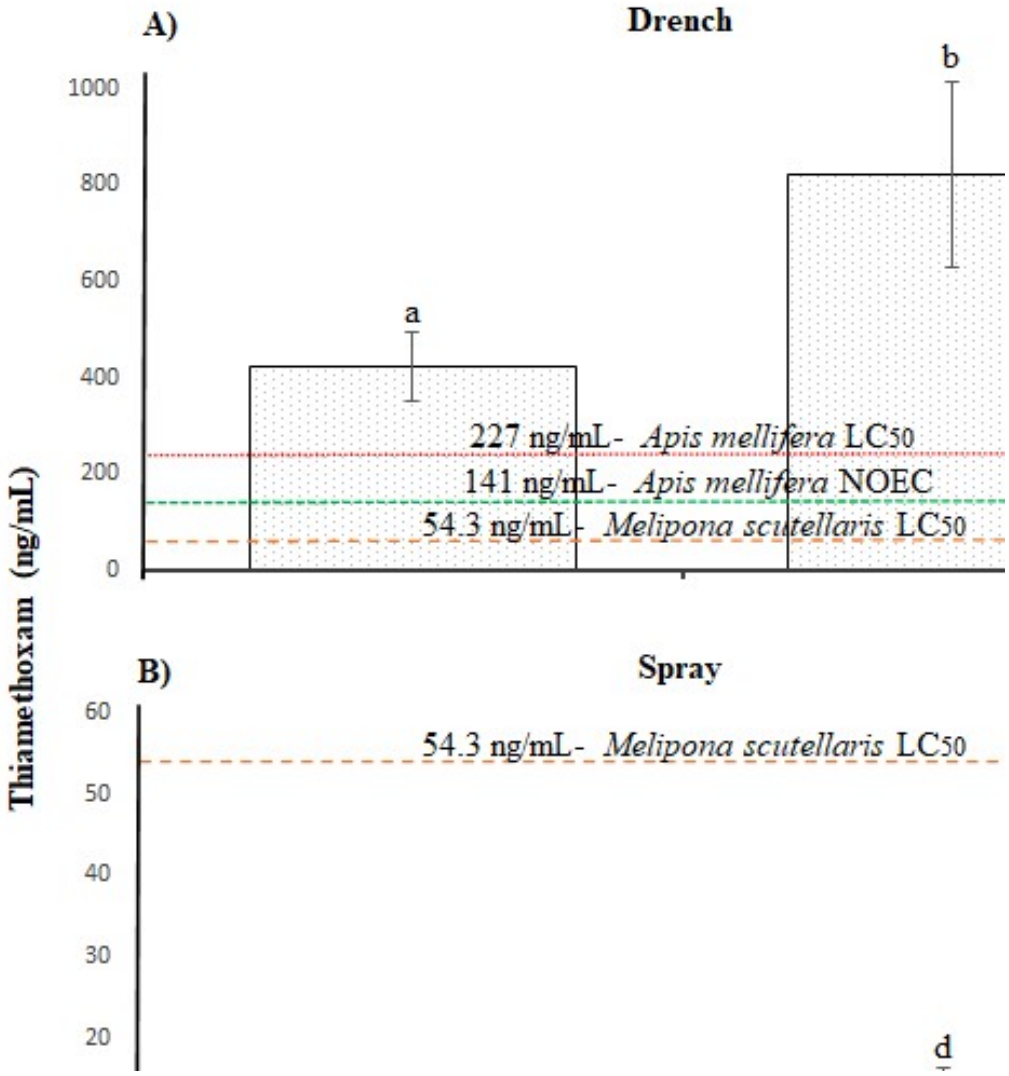

Figure 2. Thiamethoxam concentrations (± standard deviation) in nectar associated with application by A) drench applied pre- and post-bloom and B) spray applied pre- and post-bloom. Different letters indicate significant differences between timings and application methods based on 2-way ANOVA (P=0.05). Horizontal reference lines indicate median lethal concentration (LC_{50}) values for honeybees (*A. mellifera*) and native bees (*Melipona scutellaris*) (Miotelo et al., 2021) and 'no observable effects concentration' (NOEC) for honeybees (Overmeyer et al., 2018).

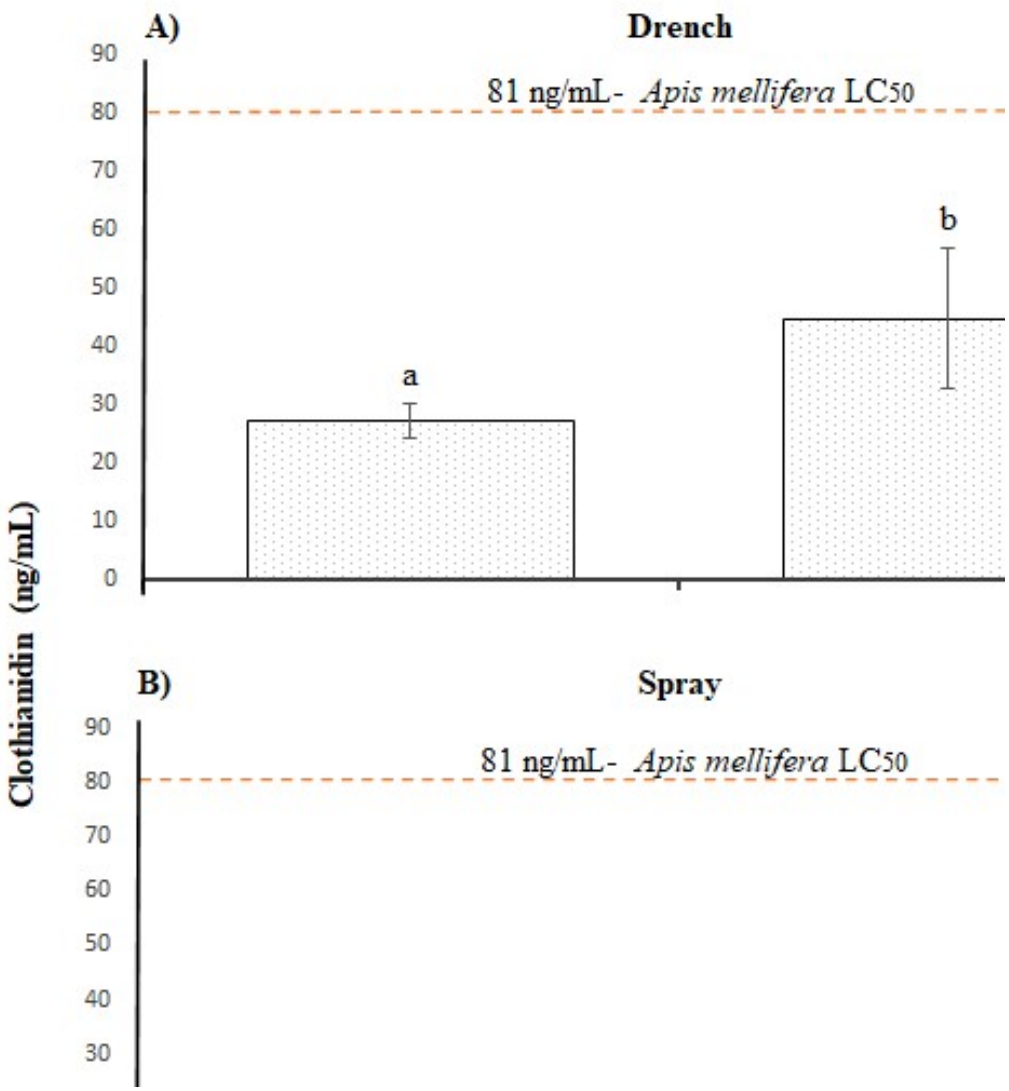

Figure 3. Clothianidin concentrations (± standard deviation) in nectar associated with application of thiamethoxam by A) drench applied pre- and post-bloom and B) spray applied pre- and post-bloom. Different letters indicate significant differences between timings and application methods based on 2-way ANOVA (P=0.05). Horizontal reference line indicates median lethal concentration (LC_{50}) values for honeybees (*A. mellifera*) (Laurino et al., 2011).