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Research Article

Volatile Organic Compounds from the Clone *Populus x canadensis* "Conti" Associated with *Megaplatypus mutatus* Attack

Alejandro Lucia, Paola González-Audino, and Héctor Masuh

Centro de Investigaciones de Plagas e Insecticidas (UNIDEF (MINDEF-CONICET), J. B. de La Salle 4397, Villa Martelli B1603ALO, Provincia de Buenos Aires, Argentina

Correspondence should be addressed to Paola González-Audino; pgonzalezaudino@citedef.gob.ar

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Megaplatypus mutatus (Chapuis) (Coleoptera, Platypodidae) is an ambrosia beetle native to South America. It builds internal galleries that weaken the tree trunks, causing them severe stem breakage and mortality in commercial poplar plantations. The host selection by male *M. mutatus* has previously been correlated with the increasing diameter. This work explores the possibility that differential susceptibility of individual plants to *M. mutatus* could be associated with volatiles emitted. The comparison of the VOCs profiles of attacked and nonattacked *P. x canadensis* "Conti" 12 during *M. mutatus* flying season showed both qualitative and quantitative differences. The attacked plants, but not the nonattacked ones, showed the following compounds: a long chain aldehyde, α-ylangene, δ-cadinene, α-gurjunene, and β-cubebene; on the other side, β-sesquiphellandrene and β-chamigrene were detected only in nonattacked plants. α-Copaene is a common component of all the samples analyzed, but its proportion is increased in attacked individuals. Behavioral bioassays showed that males but not females *M. mutatus* are attracted to α-copaene. The relative increase of α-copaene in attacked individuals and the positive behavioral answer of males to it suggest that this compound could play a role in the orientation of the pioneer male towards the most suitable host.

1. Introduction

Ambrosia beetles are an important insect group in forest ecosystems affecting weakened or felled trees. *Megaplatypus mutatus* (Syn. *Platypus mutatus*) (Chapuis) (Coleoptera, Platypodidae) is an ambrosia beetle native to South America. Unlike most ambrosia beetles, it attacks only living trees, penetrating the xylem of its host by boring long tunnels. The attack is initiated by pioneer males selecting a host tree to build a short nuptial gallery, from which they attract females using a sexual pheromone [1]. Following copulation, they extend their gallery in order to lodge their new brood. These galleries weaken the tree trunks, causing severe stem-breakage and mortality in commercial poplar plantations of *Populus deltoides* [2–4]. Additionally, the dark tunnels caused by the Ambrosia mycelium of the associated symbiotic fungi seriously affect the quality of the wood.

The prevalence of attacks by *M. mutatus* has been correlated with the tree diameter. Etiennot et al. [5] found that 86% of the attacked trees in a plantation had a diameter breast height (DBH) >20 cm. Also, other authors found a preference of *M. mutatus* for bigger diameters [6–9], probably because there is more room available to develop their brood [1].

Concerning the susceptibility associated with the clone, although it is well known that some clones are less susceptible than others, this differential susceptibility is more likely to be associated with the average DBH of the particular clone characteristic for its growing rate than to the clone itself [6–9]. Also, there is a strong association between the site quality and the prevalence of attacks [10]. Again, this phenomenon can be correlated with the productivity of the plantation.

With the aim of implementing an environmentally friendly management programme, a large amount of work has been done with traps baited with sexual pheromone that attract females [11–13]. However, the existence of chemical cues involved in the host selection by the male has not been explored and it gains interest in the search of synthetic attractants to be incorporated in baited traps. In this work we explore the possibility that differential susceptibility of individual plants at *M. mutatus* could be associated with VOC emitted, so we collected and analyzed VOC emitted by wood bark of the clone *P. x canadensis* "Conti" 12 attacked and nonattacked during *M. mutatus* flying season.

2. Materials and Methods

2.1. Plant Material. *Populus x canadensis* Mönch (Syn. *P. x euramericana* (Dode) Guiner) plants were selected from 10-year-old commercial poplar plantations (*Populus x euramericana* cv. "Conti 12").

The plantation had a density of 1,111 trees/ha (square of plantation 3 m × 3 m), average DBH 23,2 cm and is located at Alberti, Buenos Aires Province, Argentina (35°10′S, 60°17′W, 68 m.a.s.l.). All the selected individuals had the same age, site, clone, and history of plantation.

The selection methodology was the following: we randomly selected an attacked plant and a nearby nonattacked one with a similar diameter. We also tried to select attacked plants close to nonattacked one and vice versa. Trees were considered attacked if they had a visible pioneer calling male, characterized by the presence of a crown-like arrangement surrounding the entrance to the gallery (Figure 1). Four replicates of attacked and nonattacked trees were analyzed. Samples were collected during the flying season of *M. mutatus* (November).

2.2. Volatile Organic Compounds Emitted by Populus x euramericana cv. "Conti 12". Using a cork borer we extracted a wood bark cylinder vicinal (1.5 cm × 1 cm) to the *M. mutatus* entrance hole and placed it in a 20 mL vial standard clear glass (Scientific Specialties Service, Inc., Baltimore, MD, USA and Reno, NV, USA) with a teflon-coated cap (teflon septum with glass reinforced polypropylene resin open cap) adequately refrigerated. All the samples were collected between 10 and 12 a.m.

Once in the lab, the volatiles from the vial headspace were collected at 29 ± 2°C for 30 minutes using a solid phase microextraction fiber (SPME) covered with a 100 μm PDMS (Supelco Bellefonte, PA, USA) nonpolar phase. This coating is of general use to adsorb low molecular weight compounds. Samples were immediately analysed by GC-MS. GC-MS analyses were performed with a Shimadzu QP-5050A spectrometer in the electron impact mode, equipped with a polar fused CP wax 52CB column (30 m × 0.32 mm ID × 0.25 μm film thickness). Samples were injected in the splitless mode. Volatiles from the SPME fibres were desorbed in the injector port at 250°C during 1.5 min. The GC column was kept at 50°C for 5 min after which the temperature was programmed to increase 10°C/min up to 220°C, where it was maintained for 5 min. The carrier gas was helium with a head pressure of 30 kPa. The MS detector was set on at 70 eV.

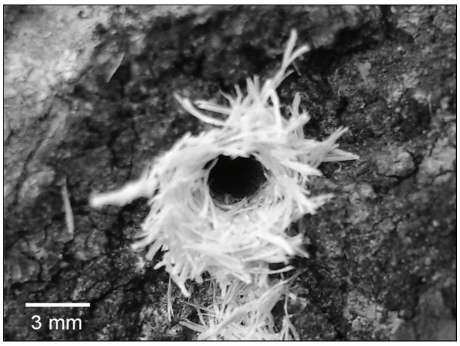


Figure 1: Crown-like arrangement in *P. canadensis* surrounding the entrance to the nuptial gallery built with the particles of boring dust (frass) produced by the male *M. mutatus* from where volatiles are emitted to attract individuals of the opposite sex.

The identities of compounds observed were assigned by comparison with spectral data of commercial libraries NIST and Wiley (tentative identifications) or with the authentic compound in the case of α-copaene.

2.3. Insects. The insects were collected shortly after their emergence (maximum 3 hours) from infested *Populus* sp. and *Quercus palustris* (Münchh) located at our institute plantation (34°33′ south, 58°30′ west). Emergence traps specifically designed for this beetle were used to avoid antagonistic interactions between emerged insects [14].

2.4. Behavioral Bioassays. Walking behavior of female *M. mutatus* was evaluated in an experimental arena with a video tracking technique [15] adapted for *M. mutatus* [16]. The floor of the test arena was covered with a round piece of Whatman No. 1 filter paper (125 mm diameter, Whatman Ltd., Maidstone, UK), and a glass cover (20 × 20 mm) was placed in the center of the paper. Next, the filter paper and glass cover were both covered with a rectangular piece of wire mesh (100 × 100 mm, 1 mm mesh size). A colorless glass ring (100 mm diameter, 50 mm high) was used to confine the insects. A new glass cover and filter paper were used in each replicate.

A closed circuit video camera providing black and white images (VC 1910, Sanyo Electrical Co., Tokyo, Japan) was suspended 22 cm over the center of the test arena. A circular fluorescent tube (22 W, OSRAM, Buenos Aires, Argentina) was placed 64 cm above the video camera.

An image analyzer (Videomex V, Columbus, OH, USA) received input from the video camera, converting the analog signal into digital data. The resolution was 256 × 192 pixels and the acquisition and processing speed was 30 frames/sec. The presence of insects in the arena was determined by visual contrast between the individuals (white) and the arena background (dark) and scored as the number of "*ON*" pixels. The area occupied by the insects was recorded by using the Multiple Zone Motion Monitor for Videomex software.

The arena image was divided into a central square (4 cm^2, 5% of the total area) and a circular outer area. The center of the glass cover was located in the center of the virtual central square. A male *M. mutatus* was placed on the wire mesh and allowed to acclimatize for 5 min before starting the bioassay. During this time, the insect moved all around the arena. Insect movement was recorded for 60 min. During the first 30 min, the glass cover was clean. Then, 1.5 μL of α-copaene was placed on the cover. Temperature varied between 25 and 30°C. The first 30 min of each test was the control, and the remaining 30 min was the experimental treatment. Thus, the occupation level of the central circle during the first 30 min (control) was compared to the occupation level during second 30 min (following the introduction of the test substance). The experiment was replicated 10 times with independent males and females.

We used the central area of occupation (CAO) parameter, previously defined as the total number of "*ON*" pixels in the central circle (where the test compound is placed) during a replicate [15, 16], to quantify insect behavior. A mean CAO value was obtained for each treatment and compared to its respective control.

2.5. Chemical. (−)-α-Copaene (Technical grade > 90%, GC sum of enantiomers) was purchased from Fluka (Milwaukee, USA).

2.6. Statistical Analysis . Data from the behavioral assay were analyzed by Kruskal-Wallis Test (nonparametric ANOVA) using STATISTICA software. A mean CAO values were obtained for α-copaene and compared to its respective control. The accepted level of significance was P value < 0.01, meaning highly different from control group (Kruskal-Wallis Test).

The values of relative concentration of the compounds for each sample were transformed (log) and analyzed using one-way analysis of variance (ANOVA), and means were compared a posteriori by Tukey HSD mean multiple comparison test using STATGRAPHICS Plus Software. A value of $P < 0.01$ was considered for a significant highly difference and $P < 0.05$ for a significant difference.

3. Results

All the specimens of *Populus x canadensis* clone "Conti 12" whose volatiles where analyzed have the same age, site, clone, diameter, and history of plantation. The attacked ones had a DBH 25.5 ± 1.63 cm and the nonattacked ones 20.7 ± 1.96 cm. This means that among a similar diametrical class, the insect prefers the larger diameters (P value < 0.05).

3.1. Volatile Organic Compounds Emitted by Nonattacked Populus x canadensis Clone "Conti 12". The volatile blend emitted by the wood and bark sample of the *P. x canadensis* "Conti 12" nonattacked by *M. mutatus* was dominated by β-selinene (36.9 ± 2.6%), followed by α-selinene (27 ± 3.0%), β-chamigrene (7.1 ± 2.6%), a long chain aldehyde with $\text{Rt}_{21.82}$ (6.3 ± 1.8%), β-elemene (5.0 ± 2.6%), salicylic aldehyde (3.5 ± 2.0%), and α-copaene (1.8 ± 0.2%) (Figure 2(a)).

3.2. Volatile Organic Compounds Emitted by Attacked Populus x canadensis Clone "Conti 12". Figure 2(b) shows the typical GC trace of the volatiles emitted by the wood and bark sample of the *P. x canadensis* "Conti" 12 attacked by *M. mutatus*. In this case α-copaene was the major component (34.4 ± 23.9%), followed by a long chain aldehyde of Rt 20.57 (30.7 ± 15.8%), β-selinene (9.1 ± 3.8%), a long chain aldehyde of Rt 21.82 (8.6 ± 4.7%), α-selinene (4.4 ± 3.0%), β-cubenene (2.2 ± 0.6%), salicylic aldehyde (2.1 ± 1.8%), α-gurjunene (1.8 ± 0.5%), β-elemene (1.7 ± 0.9%), α-ylangene (1.0 ± 0.6%), and δ-cadinene (0.9 ± 0.4%).

3.3. Behavioral Response to α-Copaene. The occupation level of the central circle during the first 30 min (control) did not reveal a significant behavioral response when compared with their second 30 min (following the introduction of the test substance) (P value: 0.001).

Results were analyzed based on the central area of occupation (CAO) parameter. Significant occupation of the central area can be interpreted as an effective attraction to the source followed by an arrestment in the area [17].

CAO values of female *M. mutatus* exposed to α-copaene did not reveal a significant behavioral response (P value: 0.62) when compared with their respective controls (Figure 3). Thus, females were not attracted to the stimulus source.

CAO values of male *M. mutatus* exposed to α-copaene revealed a significant behavioral response (P value: 0.0042) (Figure 4) when compared with their respective controls. Thus, males were attracted to the stimulus source.

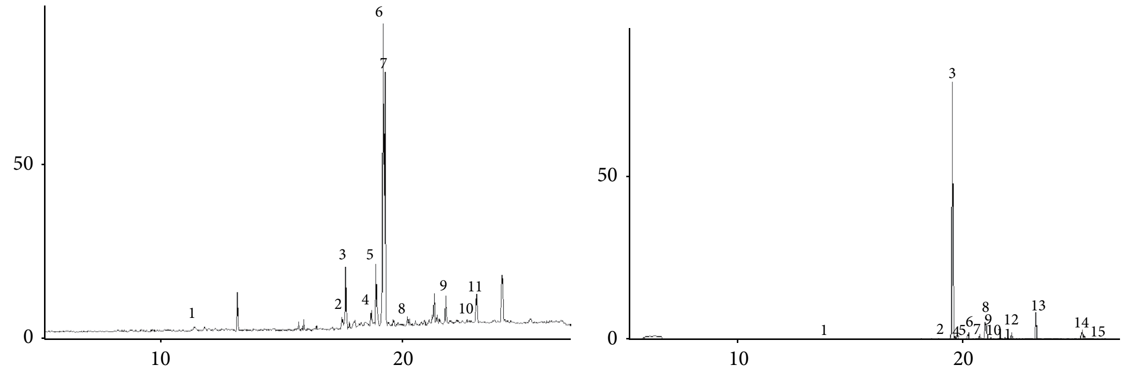
4. Discussion

The comparison of the volatile profiles of attacked and nonattacked trees showed both qualitative and quantitative differences(Figure 5). The attacked plants, but not the nonattacked ones, showed the following compounds: a long chain aldehyde of $\text{Rt}_{20.57}$, α-ylangene, δ-cadinene, α-gurjunene, and β-cubebene; on the other side, β-sesquiphellandrene and β-chamigrene were detected in nonattacked plants but not in attacked ones.

A quantitative analyses showed that α-copaene is present in 1-2% in nonattacked plants but in 34, 4% in attacked ones (P value < 0.05).

Also, the long chain aldehyde of $\text{Rt}_{21.80}$ shows the same pattern: it varies from 6.3% in nonattacked plants to 30.7% in the attacked ones (significant difference, P value < 0.05). Instead, α-selinene, β-selinene, and β-elemene decrease their relative concentrations in attacked trees with respect to nonattacked ones (P value < 0.01, P value < 0.01, and P value > 0.05, resp.).

Overall, we can conclude that although α-copaene is a common confirmed component of all the samples analyzed, its proportion is increased in attacked individuals and males



(a)

(b)

Figure 2: (a) Typical GC trace of volatile organic compounds emitted by nonattacked *Populus x canadensis* clone "Conti 12" (1: Salicylic aldehyde**, 2: α-copaene***, 3: β-elemene**, 4: N.I., 5: β-chamigrene**, 6: β-selinene**, 7: α-selinene**, 8: N.I., 9: aldehyde of $\mathrm{Rt}_{21.82}$**, 10: N.I., and 11: N.I.). **: Tentatively identified against GC-MS library, ***: identified against authentic standard, and N.I.: nonidentified. (b) Typical GC trace of volatile organic compounds emitted by attacked *Populus x canadensis* clone "Conti 12" (1: salicylic aldehyde**, 2: α-ylangene**, 3: α-copaene***, 4: β-elemene**, 5: N.I., 6: β-cubebene**, 7: α-gurjunene**, 8: β-selinene**, 9: α-selinene**, 10: δ-cadinene**, 11: N.I., 12: aldehyde of $\mathrm{Rt}_{20.57}$**, 13: aldehyde of $\mathrm{Rt}_{21.82}$**, 14: N.I., and 15: N.I.). **: Tentatively identified against library, ***: identified against authentic standard, and N.I.: nonidentified.

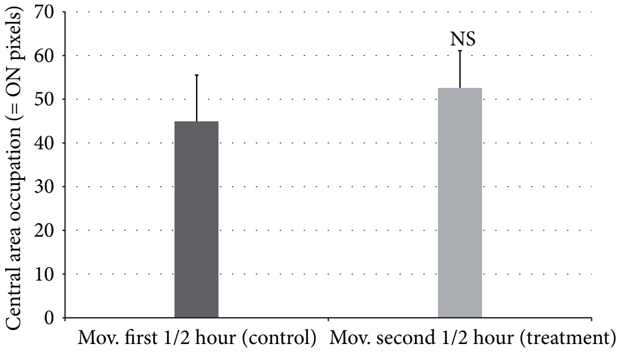


Figure 3: Response of female *Megaplatypus mutatus* measured as the central area occupation (=*on* pixels) for α-copaene compared to its respective control. Each bar represents the mean of 10 independent replicates ± SE. NS: not significant differences between treatment and control group (Kruskal-Wallis Test, $P > 0.01$).

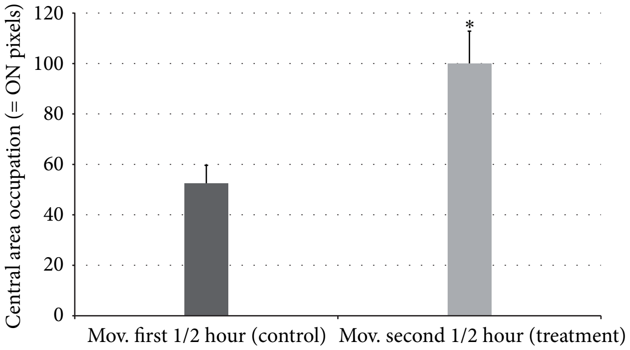


Figure 4: Response of male *Megaplatypus mutatus* measured as the central area occupation (=*on* pixels) for α-copaene compared to its respective control. Each bar represents the mean of 10 independent replicates ± SE. *: significant differences between treatment and control group (Kruskal-Wallis Test, $P < 0.01$).

M. mutatus are attracted to it at short range but females are not.

The relative increase of α-copaene in attacked individuals and the positive behavioral answer of males to it suggest that this compound could play a role in the orientation of the pioneer male towards the most suitable host.

α-Copaene and its stereoisomer α-ylangene are active kairomones of *Archangelica officinalis* essential oil; however, their proportion goes from 0.5 to 1% and pure α-copaene is quite more active. The Angelica essential oil has been used in baited traps to catch fruit flies in Florida [18]. Also, extracts of *Litchi chinensis, Ficus retusa*, and *Ficus benjamina* were active for males of the same species being this response attributed to the presence of α-copaene [19].

Our result is interesting for our goal of finding natural attractants to be set up in baited traps in the field. Attraction of bark beetles to pheromone baited traps is increased by the addition of host volatiles as monoterpenes to pheromone baits [20, 21] and commercial lures based on the combination of synthetic attractants are available. In this sense, the introduction of α-copaene to pheromone baited traps could be a promising tool that optimizes adult trapping, leading to improve monitoring and control systems in infested plantations.

5. Conclusions

The volatile profiles of attacked and nonattacked trees showed both qualitative and quantitative differences.

α-Copaene is a common confirmed component of all the samples analyzed, but its proportion is increased in attacked individuals.

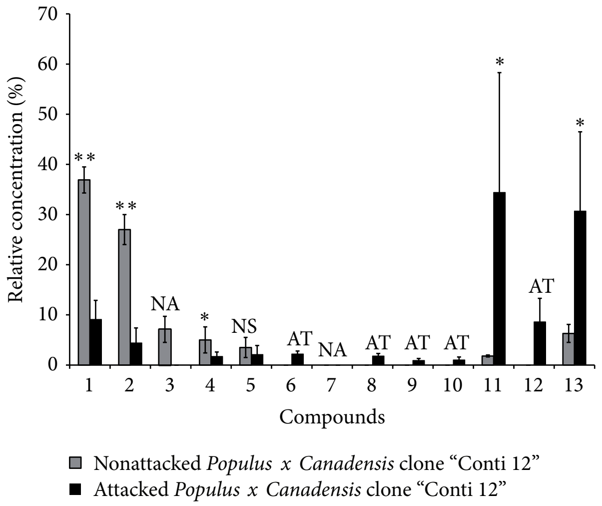


FIGURE 5: Volatile organic compounds emitted by attacked and nonattacked *Populus x canadensis* clone "Conti 12." The numbers represent the major compounds, 1: β-selinene, 2: α-selinene, 3: β-chamigrene, 4: β-elemene, 5: salicylic aldehyde, 6: β-cubebene, 7: β-sesquiphellandrene, 8: α-gurjunene, 9: δ-cadinene, 10: α-ylangene, 11: α-copaene, 12: aldehyde of $Rt_{20.57}$, and 13: aldehyde of $Rt_{21.82}$. The area normalization performed only on identified compounds and the values are the mean of four replicates ± SD. NS: not significant differences between relative concentration in attacked and non-attacked plants ($P > 0.05$). * and **: relative concentration in the attacked plants is significantly different ($P < 0.05$) or highly different ($P < 0.01$) respectively, from non-attacked plants (ANOVA-Tukey HSD mean multiple comparison test). AT: compounds only present in attacked plants and NA: only present in nonattacked plants.

In behavioral bioassays, males *M. mutatus* are attracted at short range to α-copaene, while females are not.

Introduction of α-copaene to pheromone baited traps could optimize adult trapping.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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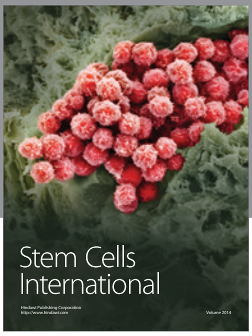
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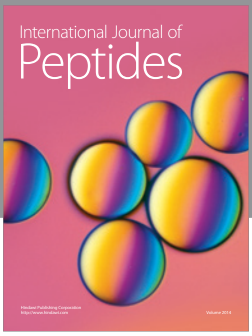
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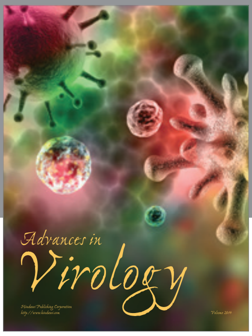
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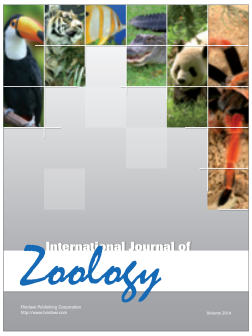
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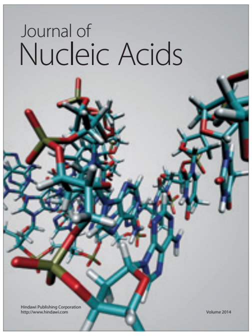
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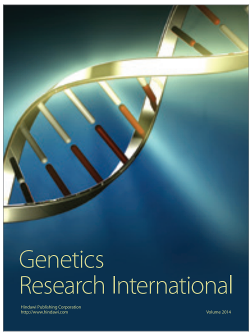
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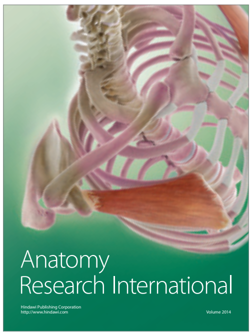
Anatomy
Research International


International Journal of
Microbiology


Biochemistry
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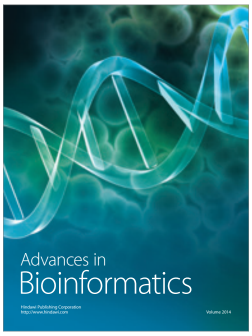
Advances in
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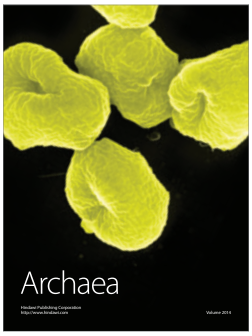
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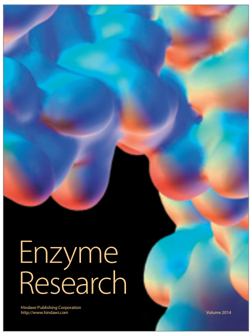
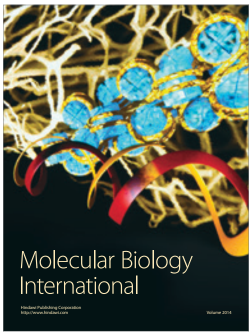

Enzyme
Research


International Journal of
Evolutionary Biology


Molecular Biology
International


Journal of
Marine Biology