**Comments MAPA – Brazil – 2º Draft:**

**Page 37
Paragraph # 227**

Exclude the underlined phrase.

1. Leaf-cutting ants have mechanical and chemical defenses that help them to counterbalance the effect of some control measures. Studying the adaptation mechanisms of leaf-cutting ants is recommended to improve effectiveness of strategies for their ecological management.[[1]](#footnote-1) Exocrine glands and symbiotic bacteria are the main sources of antimicrobials in leaf-cutting ants, and are used to counter biological control agents. Studying the adaptation mechanisms of leaf-cutting ants is recommended to improve effectiveness of strategies for their ecological management~~. However biological control can be effective under some conditions. In laboratory studies, the entomopathogenic~~ *~~Metarrhizium anisopliae~~* ~~can cause the decline and ultimate death of small colonies and recent research indicates that the entomopathogenic fungi~~ *~~Beauveria bassiana~~* ~~and~~ *~~Aspergillus ochraceus~~* ~~both show a high degree of control, causing 50% mortality within 4 to 5 days.[[2]](#footnote-2)~~~~,~~ ~~[[3]](#footnote-3) Effective natural products include limonoids extracted from the roots of the South Brazilian endemic plant~~ *~~Raulinoa echinata~~*~~.[[4]](#footnote-4) Further research is required to verify the effectiveness of these interventions under field conditions~~. Input from Brazil indicated that no effective biological control methods have been identified yet. Several mechanical, cultural, biological and chemical methods have been studied as early as the 50s for controlling leaf-cutting ants.

Justified by:

This information is not correct because in the original text of scientific articles” D.B. Jaccoud, W.O.H. Hughes and C.W. Jackson, The epizootiology of a Metarhizium infection in mini-nests of the leaf-cutting ant Atta sexdens rubropilosa, 1999, ENTOMOLOGIA EXPERIMENTALIS ET APPLICATA, Volume 93, Number 1, 51-61, DOI: 10.1023/A:1003830625680” is clear in the abstract, page 51 and page 59 discussion, that the decline in mini-nests was not directly due to the action of the fungus Metarhizium anisopliae [original text, in the abstract, line 14: “However, the decline of these mini-nests after day 26 was not due directly to the pathogenic action of the Metarhizium, nor to the initial ant mortality it had caused”; original text, discussion, line 43:“Secondly, the mini-nest mortality was not due to the Metarhizium infecting and killing every individual in the population. Rather, it was the result of some process set in motion by the early Metarhizium epizootic. The decline of the mini-nests was not explained by the high initial ant mortality either”].

With respect to the scientific article: “Myriam M. R. Ribeiro,1 Karina D. Amaral, Vanessa E. Seide, Bressane M. R. Souza,1 Terezinha M. C. Della Lucia, Maria Catarina M. Kasuya,2 and Danival J. de Souza, Diversity of Fungi Associated with Atta bisphaerica (Hymenoptera: Formicidae): The Activity of Aspergillus ochraceus and Beauveria bassiana, Psyche Volume 2012, 2012, Article ID 389806, 6 pages, doi:10.1155/2012/389806”, we have several comments to be made:

Firstly the experiments conducted only in the laboratory were made with isolated ant colony, which is not recommended, because the "social control" is nonexistent. This kind of conclusion is completely disconnected from reality. [see text, page 2, item 2. Material and methods, “For each concentration, 50 worker ants received 1 μL of the suspensions on the pronotum. One μL of a 0.05% Tween 80 solution was applied to the control ants. This control group was used in the comparison with the two isolates since the experiments were conducted simultaneously. After receiving the treatments, the insects were held individually in Petri dishes supplied with a honey-water solution (1:1) and sterile distilled water renewed every two days. The mortality of the worker ants was daily monitored”];

Secondly, even in conditions already described, which are experimentally incorrect because the correct would make the use of spores of the fungus colonies of at least two years old, the mortality rate is very low, only 50%.[ in the abstract, “The two species were highly virulent, achieving 50 percent worker mortality within 4-5 days”];

Thirdly, the experimental conditions the mortality should be 100%, and the whole experiment in this way, so far, showed no viability in the field, so the discussion of these fungi for use in biological control has no sense.

With respect to Article :“ Maique W. Biavatti\*, I; Rosângela Westerlon I; Paulo C. Vieira; M. Fátima G. F. da Silva; João B. Fernandes; M. Fernanda G. V. Peñaflor; Odair C. Bueno; Javier Ellena, Leaf-cutting ants toxicity of limonexic acid and degraded limonoids from Raulinoa echinata. X-ray structure of epoxy-fraxinellone, Journal of the Brazilian Chemical Society Print version ISSN 0103-5053. Chem. Soc. vol.16 no.6b São Paulo Nov./Dec. 2005”, in fact this study was not designed to test the efficacy of substances for control of ants. The work has as main objective to present the isolation and identification of limonoids by the technique of x-ray diffraction. A survey of toxic activity on isolated workers (see page 1445, item “Atta sexdens bioassay”: Each treatment consisted of 50 ants distributed in 5 Petri dishes. The leaf-cutting ants were isolated from their nest and fed with an artificial diet. The ants from the treatment received a diet added with epoxy-fraxinellone or limonexic acid, at a concentration of 200 μg. The ants from the control treatment were fed with a diet without this compound is not feasible, because this technique is used for screening when you have dozens, hundreds of substances to be tried. We cannot infer on the efficiency of cutting ant control because after screening several additional experiments are needed to obtain any conclusion about control.

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.55, 62.

**Page 37**

**Paragraph # 232**

Suggestion of three paragraphs to be include at the document:

1. Mechanical control of leaf-cutting ants consist in excavating their nests for queen ant removal. Such a technique is no longer recommended for leaf-cutting colonies that are more than 4 months old, this is when the queen will be lodged at depths exceeding 1 meter, thus rendering the technique unviable due to the great effort required. In practice, mechanical control will be unviable in areas used for commercial plantations, in reforestation projects and grazing systems.
2. Cultural control has a conventional soil preparation by plowing and harrowing could mean the mortality of newly formed Atta nests, However, with the practice of minimum cultivation adopted in several cultivars and reforestation projects, such control has been abandoned. For adult Atta nests, the result could even be harmful, as soil mechanization could partially upset the anthill causing it to become temporarily inactive and giving the false impression of having been controlled.
3. Natural biological control, through predators: parasitoids and pathogenic microorganisms (fungi, bacteria and viruses), is of importance in regulating leaf-cutting ants but not to control in commercial plantations. Spiders, acarida, predating ants and beetles should also be mentioned. However, leaf-cutting ants has a complex biology, with a small number of progenies per female, such factors representing hindrances to control which causes low or none efficiency under field conditions.

**See references:** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.51, 68.

**Page 37**

**Paragraph # 234, 235**

Exclude the underlined paragraphs.

~~234 However biological control can be effective. The entomopathogenic Metarrhizium anisopliae can cause the decline and ultimate death of small colonies[[5]](#footnote-5) and recent research indicates that the entomopathogenic fungi Beauveria bassiana and Aspergillus ochraceus both show a high degree of control, causing 50% mortality within 4-5 days.~~

~~235 Effective natural products include limonoids extracted from the roots of the South Brazilian endemic plant Raulinoa echinata.~~

Justified by: The three studies presented by **(D.B. Jaccoud, W.O.H. Hughes and C.W. Jackson, The epizootiology of a Metarhizium infection in mini-nests of the leaf-cutting ant Atta sexdens rubropilosa, 1999, ENTOMOLOGIA EXPERIMENTALIS ET APPLICATA, Volume 93, Number 1, 51-61, DOI: 10.1023/A:1003830625680. )** (Myriam M. R. Ribeiro, Karina D. Amaral, Vanessa E. Seide, Bressane M. R. Souza, Terezinha M. C. Della Lucia, Maria Catarina M. Kasuya, and Danival J. de Souza, Diversity of Fungi Associated with Atta bisphaerica (Hymenoptera: Formicidae): The Activity of Aspergillus ochraceus and Beauveria bassiana, Psyche Volume 2012, 2012, Article ID 389806, 6 pages, doi:10.1155/2012/389806**. Maique W. Biavatti, I; Rosângela WesterlonI; Paulo C. Vieira; M. Fátima G. F. da Silva; João B. Fernandes; M. Fernanda G. V. Peñaflor; Odair C. Bueno; Javier Ellena, Leaf-cutting ants toxicity of limonexic acid and degraded limonoids from Raulinoa echinata. X-ray structure of epoxy-fraxinellone, Journal of the Brazilian Chemical SocietyPrint version ISSN 0103-5053. Chem. Soc. vol.16 no.6b São Paulo Nov./Dec. 2005**, those studies do not conclude the information made in this guide. See paragraph 227, besides it was not referenced that these studies are in laboratory conditions and there is a need to research in field to conclude the effectiveness of these products.

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.55, 62.

**Page 39**

**Paragraph # 226**

Suggestion to change paragraph:

226. Fenoxycarb, pyriproxyfen, diflubenzuron, teflubenzuron, silaneafone, thidiazuron, tefluron, prodrone and methoprene had been tested for leaf-cutting ants, but they were not effective.

Change paragraph to: *Fenoxycarb, pyriproxyfen, diflubenzuron, teflubenzuron, silaneafone, thidiazuron, tefluron, prodrone, abamectin, methoprene, Hydramethylnon, boric acid, some insecticides from the group of neonicotinoids, pyrethroids, Spinosyns, etc., had been tested for leaf-cutting ants, but they were not effective.*

Justified by: update from the Report sent by Brazil and presented at COP-7 (UNEP-POPS-COP.7-INF-11.English.pdf) p. 22.

**Page 39**

**Paragraph # 240**

Exclude the underlined paragraph:

~~240. The non-conventional are Entomopathogenic fungi are a) organic bait consisting of rice grains with Beauveria sp. b) Diatomaceous earth mixed with waste water in the principal entry of each anthill c) Vegetal substances with insecticide effect: extract of eucalyptus, castor-oil plant and “Palo Amargo” (“Bitter stick”, Aeschirium crenata Vell. Simaroubaceae) d) Natural enemies: parasitoids known as “Moscas descapitadotas” (“Decapitating flies”, Diptera: Phoridae) (and e) organic baits based on yeast and rice. All these alternatives have been tested in Argentina with promising results.~~

Justified by: These products do not have efficiency and technical feasibility according to references below.

*Organic baits based on rice and beer yeast*

Contrary to what was asserted here, the rice and beer yeast baits were not the best treatment, as may be ascertained from BORGETTO (2009) 's text. This same author concludes that sufluramida was the most effective treatment. Such results were are confirmed by COLL (2003). He textually concludes “para el control de hormigas cortadoras pertencentes al gênero Acromyrmex, el inseticida (sulfluramida) fue más eficiente” = for controlling the leaf-cutting ants of the Acromyrmex kind, the insecticide (sulfluramida) was more effective. (page 40). As to the rice, CARRERE (2006) concluded that the rice merely prevented the ants' continuing to cut the plants, and had not the effect desired of killing the fungus garden.

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.47, 48.

*Diatom earth*

On the Argentine document, the publication date is incorrect, it is not 2012 but rather 2010. First, the research performed in RIOS DE SALUSO (2010): “Manejo de las hormigas cortadoras de hoja em los principales sistemas agropecuários y forestales de la província de Entre Ríos” (Handling of leaf-cutting ants in the main forest, agriculture and cattle-raising systems of the Entre Rios province), points out and concludes that sulfluramidand fipronil, in toxic bait, were the most effective products for controlling Acromyrmex lundi in agricultural systems. It the small discussion of its abstract, it points out that the treatment with diatom earth and fipronil on complete atomization ranked third in effectiveness. We emphasize that there were only four treatments and the witness.

We consider that a product, ranking third for controlling *Acromyrmex lundi* nests that have only one fungus chamber and are located in the top 30 cm of the ground (GRANGI et al , 2000), is not encouraging. Would it then be encouraging for controlling Atta nests with up to 8,000 chambers (MOREIRA et al, 2004a; MOREIRA et al 2004b).

Diatom earth was already tried in Brazil, as candidate for controlling *Atta sexdens rubropilosa* nests, and the result was the following: "The product based on silica dioxide (Diatom earth), in both dosages tested, has shown inefficient for controlling Atta sexdens rubropilosa settlements, under operating conditions" (VeracelTM - Technical Report).

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.48.

*Natural enemies: parasites known as phoridae (Diptera, Phoridae)*

The work deals with life history (biology) and percentage of parasitism. The work discussion involves potential candidates for a future biological control program. In our opinion, this type of control might not obtain successful control. We must consider that the species studied (4 species) are "candidates for integrating the assemblage of biocontrolagents against Atta vollenweideri”; as written in the article published on the periodical “Journal Economic Entomology 104 (1): 32-40.2011

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.68-69.

*Entomopathogenic fungi*

Until this time, the use of entomopathogenic fungus for controlling leaf-cutting ants has produced no encouraging result, even for the Acromyrmex species having smaller nests. The difficulties in being successful on using entomopathogenic fungi, under field conditions, might be related to the defense strategies of the leaf-cutting ants' settlements, along with its mutualist fungus and the associated microbiota, against parasites and pathogens, of morphological, mechanical and biochemical nature (BOARETTO & FORTI, 1997). According to KERMARREC et al., (1986), the extraordinary resistance of the leaf-cutting ants' mutualist fungi to the epizootic and epiphytic diseases is due to many factors related to the nests' internal hygienics. Attempts for using baits with Beauveria bassiana and Metharrizium anisopliae, under field conditions, with Acromyrmex nests, was not encouraging, since, in this case, the worker ants disinfected, pruned and isolated the fungus culture and, in more severe instances, the ants abandoned the nest.

Certainly, biological control, including with entomopathogenic fungi, is a promising research area, however, it is currently clear the need for basic biological knowledge, in order that control strategies for leaf-cutting ants might actually be applied.

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.53-59.

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**Paragraph # 246**

246. Pesticide resistance is a genetically based phenomenon. Resistance can occur when a pest population is exposed to a pesticide and not all insects are killed. Those individuals that survive frequently have done so because they are genetically predisposed to be resistant to the pesticide.

Change paragraph to: Pesticide resistance is a genetically based phenomenon. Resistance can occur when a pest population is exposed to a pesticide and not all insects are killed. Those individuals that survive frequently have done so because they are genetically predisposed to be resistant to the pesticide. The resistance to insecticides does not apply to leaf-cutting ants. In this case, the target of insecticides are the workers who are sterile, thus not allowing the selection of resistant individuals.

Justified by: Fallow attached to this document, the report with references.

**Page 39**

**Paragraph # 244**

244. Fenoxycarb, pyriproxyfen, diflubenzuron, teflubenzuron, silaneafone, thidiazuron, tefluron, prodrone and methoprene had been tested for leaf-cutting ants, but they were not effective.

Change para to: *Fenoxycarb, pyriproxyfen, diflubenzuron, teflubenzuron, silaneafone, thidiazuron, tefluron, prodrone, abamectin, methoprene, Hydramethylnon, boric acid, some insecticides from the group of neonicotinoids, pyrethroids, Spinosyns, etc., had been tested for leaf-cutting ants, but they were not effective.*

Justified by: update from the Report sent by Brazil and presented at COP-7 (UNEP-POPS-COP.7-INF-11.English.pdf) p. 22.

**Page 89**

**Appendix # 2**

Exclude: Hydramethylnon

Justified by: The active ingredient Hydramethylnon is registered and commercialized in the USA against fire ants (*Solenopsis* spp.). Also in the USA, it is registered to *Atta* Texas, for which it has been ineffective. In accordance to the Forest Service Texas, with the bait with the active ingredient hydrametlylnon presents efficiency of only 30% and there is a need of more than one application, especially for large colonies. Another disadvantage is that this insect bait should not be stored for long periods of time, due to its relatively short life time.

Studies were conducted in Brazil to evaluate the potential of such active ingredient in the control of leaf-cutting ants of *Atta* genus. The results were even more inconsistent, despite the use of high concentrations of this active ingredient in the formulation and higher dosages. Hydramethylnon also has shown not to be efficient to control other ants, just as the leaf-cutting ants case. The lack of further research using hydramethylnon in the control of leaf-cutting ants may be to the fact that such active ingredient present fast degradation in the presence of light, and this property is considered undesirable for insect baits for leaf-cutting ants used in tropical and subtropical conditions.

Spite of being an insecticide known for more than 30 years, it was neither registered nor has been currently used in Brazil for the control of leaf-cutting ants, certainly because of the proven inefficiency.

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.40-41.

**Page 90**

**Appendix # 2**

Exclude: Non-chemical

Justified by: The three studies presented by **(D.B. Jaccoud, W.O.H. Hughes and C.W. Jackson, The epizootiology of a Metarhizium infection in mini-nests of the leaf-cutting ant Atta sexdens rubropilosa, 1999, ENTOMOLOGIA EXPERIMENTALIS ET APPLICATA, Volume 93, Number 1, 51-61, DOI: 10.1023/A:1003830625680. )** (Myriam M. R. Ribeiro, Karina D. Amaral, Vanessa E. Seide, Bressane M. R. Souza, Terezinha M. C. Della Lucia, Maria Catarina M. Kasuya, and Danival J. de Souza, Diversity of Fungi Associated with Atta bisphaerica (Hymenoptera: Formicidae): The Activity of Aspergillus ochraceus and Beauveria bassiana, Psyche Volume 2012, 2012, Article ID 389806, 6 pages, doi:10.1155/2012/389806**. Maique W. Biavatti, I; Rosângela WesterlonI; Paulo C. Vieira; M. Fátima G. F. da Silva; João B. Fernandes; M. Fernanda G. V. Peñaflor; Odair C. Bueno; Javier Ellena, Leaf-cutting ants toxicity of limonexic acid and degraded limonoids from Raulinoa echinata. X-ray structure of epoxy-fraxinellone, Journal of the Brazilian Chemical SocietyPrint version ISSN 0103-5053. Chem. Soc. vol.16 no.6b São Paulo Nov./Dec. 2005**, those studies do not conclude the information made in this guide. See paragraph 227, besides it was not referenced that these studies are in laboratory conditions and there is a need to research in field to conclude the effectiveness of these products.

**See references** BRITTO, J. S.; FORTI, L. C.; OLIVEIRA, M. A.; ZANETTI, R.; WILCKEN, C. F.; ZANUNCIO, J. C.; LOECK, A. E.; CALDATO, N.; NAGAMOTO, N. S.; LEMES, P. G. and CAMARGO, R. S., 2016. *Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants Atta and Acromyrmex*, **International Journal of Research in Environmental Studies (2016) 3(2): pp.11-92.** p.55,62.

**Page 101**

**Appendix # 2**

Exclude: Hydramethylnon for leaf-cutting ants

**Justified by: see Appendix 2, page 89.**

**Page 102**

**Appendix # 2**

Change: Fenitrothion as insect bait for Thermonebulizable solutions (thermal fogging)

Change: Deltamethrin as insect bait for Dried Powder Formulations

Justified by: to be consistent with the Appendix 2.

**Appendix # 3**

Exclude the others pesticides because it does not concern about leaf-cutting ants. Leaving only Fipronil, Deltamethrin and Fenitrothion to be the same as Appendix 2.

1. [↑](#footnote-ref-1)
2. [↑](#footnote-ref-2)
3. [↑](#footnote-ref-3)
4. [↑](#footnote-ref-4)
5. [↑](#footnote-ref-5)