

II International Stable Fly Workshop ISFW 2019



**Brazilian Agricultural Research Corporation
Embrapa Beef Cattle
Ministry of Agriculture, Livestock, and Food Supply**

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II International Stable Fly Workshop ISFW 2019 Proceedings

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Technical Editors

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Executive Summary

The stable fly (*Stomoxys calcitrans*) is an economically important blood-sucking pest impacting animal production and welfare, as well as human beings, in countries from distinct continents. In recent decades, population explosions of this fly associated with agricultural production systems led to alarming infestations on both dairies and pastured cattle. Stable fly outbreaks are ultimately caused by the availability of suitable decaying vegetative material to support the development of massive numbers of immatures. This situation is becoming more frequent due to the industrialization of agriculture and the need for increasing food production around the world.

The high intensity and extended duration of stable fly infestations have caused serious impacts on cattle production, resulting in social and economic conflicts between livestock producers and agricultural industries; relevant commodities and agricultural systems may vary from one country to another. This cosmopolitan fly has become an internationally important emerging pest and stands out among the most important livestock pests in many regions of the world including Australia, Brazil, Costa Rica, and USA.

In addition to reducing the productivity of dairy and beef cattle, stable fly infestations also affect cattle reproduction and can ultimately result in mortality among livestock and companion animals. More efficient, cost-effective, practical and environmentally sustainable management approaches are needed for the control of this pest and prevention of outbreaks.

In Brazil, stable fly outbreaks are mainly related to the production of ethanol from sugarcane; however, the use of in natura organic fertilizers (mainly poultry litter) in fruit, horticultural, and crop production systems also plays an important role. The complexity of this situation demands both economic resources and professional expertise.

The first International Stable Fly Workshop (ISFW) held in Costa Rica in 2018 (April 16th-20h) addressed several issues related to stable fly outbreaks and led to the formation of the International Stable Fly Working Group (ISFWG). In this, the Second International Stable Fly Workshop (October 21st-25th, 2019), held in Brazil, we presented advances in research and management obtained

during past 18 months and discussed plans and collaborations for the years to come, in addition to expanding and strengthening the working group.

In response to the growing issues with stable flies, Workshop participants identified the following priorities:

- Development of stable fly management plans suitable for relevant production systems;
- Development of monitoring methods for both immature and adult stable flies which will support alert systems for decision making situations;
- Establishment of extension programs to stimulate the engagement of professionals from agricultural and veterinary fields, as well as to train crop and livestock producers;
- Development of a comprehensive and multidisciplinary stable fly research program;
- Elaboration and implementation of public policies to effectively endorse the above suggestions.

Furthermore, the ISFWG suggests that affected countries create a stable fly advisory board, which consisting of representatives from government, academia-scientific community, stakeholders representing crop and livestock producers, and affected communities.

Proceedings

Monday, Oct. 21st

The II ISFW began in the morning with an initial welcome and a brief overview by Thadeu Barros and an official opening by Ronney Mamede (Director of Embrapa Beef Cattle), with an institutional presentation. Following, David Taylor (USDA-ARS) made a presentation via video conference on the International Stable Fly Working Group, followed by the industry presentations by Maurício Saito/Guilherme Pires D'Ávila de Almeida (FAMASUL) introducing the livestock sector in Brazil and Roberto Hollanda (BIOSUL) introducing the biofuel sector in Brazil. The following presentations provided updates on the current status of the stable fly in the countries attending the event: Brazil (Thadeu Barros), Costa Rica (Erik Vargas), Uruguay (Eleonor Castro) and USA (David Taylor). The first day ended with a general discussion regarding the information provided during the day.

Ronney Mamede (Embrapa Beef Cattle). Institutional Presentations – Embrapa and Embrapa Beef Cattle. Founded on April, 1973, the Brazilian Agricultural Research Corporation – Embrapa, is under the aegis of the Brazilian Ministry of Agriculture, Livestock, and Food Supply. Since its foundation, it has taken on the challenge to develop a genuinely Brazilian model of tropical agriculture and livestock to overcome the barriers that limited the production of food, fiber, and fuel in the country. The effort has helped to change Brazil and the country presently has one of the most efficient and sustainable agricultural sectors on the planet. The company has 7 central units in its headquarters that give support to 43 research units spread all over the country, where 9,469 employees, 2,405 of them researchers, develop their activities in the most diverse themes. About 600 laboratories generate results that go beyond agricultural production: they also contribute to public policy formulation and the advancement of knowledge in other areas, like health, for instance. Embrapa has an important presence abroad. Besides maintaining two virtual laboratories (known as LABEX), in the USA and Europe, the company works in technical cooperation with 141 institutions, from 45 different countries. As a not-for-profit organization, Embrapa publicizes its results in a Social Report released on its anniversary, in April. Last year (2018) the company registered a social income of BRL\$ 43.52 billion as a

result of the evaluation of 165 technologies and 220 cultivars. For each BRL\$ 1.00 invested, BRL\$ 12.16 are returned to society and 69.936 new jobs were created as a result of all this effort. Technologies developed by Embrapa and its partners have helped to transform Brazilian agriculture. Today the country is a reference in science and technology for agriculture, as well as one of the largest food producers in the world, exporting to over 200 countries and feeding almost 1.5 billion people. For additional and more detailed information, please visit: <https://www.embrapa.br/international>. The Embrapa Beef Cattle was created in 1977, in Campo Grande, South Mato Grosso state, a region very favorable for the production of beef cattle. At that time, the low productivity of beef cattle did not meet domestic and foreign markets demands. To change the scenario, the only option was to invest in research. And it worked! As a result of consistent and serious work developed by Embrapa Beef Cattle, with the collaboration of public and private partners, Brazil is now (2019) the second largest producer and the largest exporter of beef in the world. The unit's headquarters is 7,613 acres and together with the "Model Farm" (3,983 acres), comprise an adequate structure for research, development and technology transfer activities. Besides the experimental fields the complex includes: 33 laboratories, 14 greenhouses and support buildings; a total of 34 thousand m² of building area. Infrastructure for research include the Multiuser Biosafety Laboratory for Livestock – BIOPEC, one of the most modern meat safety laboratory in Latin America and the Precision Livestock, Environment and Software Engineering Laboratory, where a number of IT solutions are developed in partnership with South Mato Grosso Federal University and other partners. Presently, the Unit has 232 employees, 55 of them researchers. The major areas of research are: pastures and forages; animal production and health; and production systems. During its 42 years of activities, Embrapa Beef Cattle has helped to make Brazil a world player in beef production. Among the most relevant contributions are: several tropical forages released; Embrapa-Genepus Program for genetic selection and breeding; many solutions to animal health and beef cattle production systems; and, equipment, software and Apps for different uses. For additional and more detailed information, please visit: www.embrapa.br/gado-de-corte.

David Taylor (USDA-ARS, USA). Overview and history of International Stable Fly Working Group. Although considered a primary pest of livestock, wildlife and humans for centuries, severe outbreaks of stable flies during the past 30 years brought new attention to this pest as an emerging issue in agri-

culture. Originating in the Old World, stable flies are synanthropic pests which have invaded virtually all temperate regions of the world. Highly adaptable, stable flies have exploited changing agronomic practices and climates to produce severe outbreaks with devastating impacts on livestock production and animal welfare. In response to this situation, and given the limited resources dedicated to stable fly research and management, a small group representing primarily Brazil, Costa Rica and the United States, endeavored to bring the world's stable fly expertise together to coordinate and leverage our research and management efforts. The First International Stable Fly Workshop convened in Costa Rica April 16-20, 2018. At that workshop, the International Stable Fly Working Group was established, research priorities developed, and collaborations established. The working group agreed to meet at approximately two-year intervals to discuss research progress, reevaluate research priorities, and strengthen collaborations. Hence this meeting, the Second International Stable Fly Workshop in Campo Grande, Brazil. We look forward to constructive discussions of stable fly biology, management, and research at this meeting and the reinforcement of established collaborations and development of new ones.

Maurício Saito/Guilherme Pires D'Ávila de Almeida (Famasul, Brazil).

The Famasul System presented an overview of the Brazilian and Mato Grosso do Sul state (MS) agriculture (focusing on livestock), besides the Famasul System actions regarding prevention of stable fly outbreaks. In 2018, Brazilian agricultural gross value of production (GVP) was R\$ 596.21 billion, 67.2% due to agriculture and 32.8% from livestock. In the same year, MS agriculture GVP was R\$ 31.69 billion (63.0 % agriculture and 30.0% livestock). Soybeans and corn are the main crops in both Brazil and MS. Total Brazilian cattle herd is roughly 215 million head (92% beef and 8% dairy), while MS has 21.5 million head (99% beef and 1% dairy). In both, MS and Brazil, the main cattle breed is the Nelore and the most common production system is pasture (>85%). In the last 10 years, MS has been maintaining productivity (about 33 pounds/ha/year) using less land (1.5 million hectares in 2018; <15% of 2009 area). Pasture corresponds to about 50% of the land used in MS. The main destinations of MS beef cattle exports (154 thousand tons; 87.9% in natura) are countries from Middle East, South America and East Asia. Since 2009, when stable fly (SF) outbreaks became more severe in MS (mainly in middle-southern municipalities such as Nova Andradina, Nova Alvorada do

Sul, Maracaju, Dourados and Ivinhema), the Famasul System has been working in partnership with Embrapa Beef Cattle and Biosul developing actions to prevent further outbreaks. Such actions included lectures in rural unions and public hearings. Moreover, a course “Prevention of stable fly outbreaks in the farm” was developed. FAMASUL trained 74 people in six courses given between 2016 and 2019.

Roberto Hollanda (Biosul, Brazil). Presentation on the sugar and energy sector of Mato Grosso do Sul state (MS) and its role in the fight against *Stomoxys calcitrans*, the stable fly. There are 19 sugarcane plants operating in the state, located mainly in the southeast region. Mato Grosso do Sul is the third largest producer of ethanol, about 3.2 billion liters and 1.5 million tons of sugar, and the fifth largest producer of food in the country. Here, the sugarcane bagasse is used to produce bioelectricity; MS is the second largest producer of bioelectricity in Brazil, providing approximately 2,600 GW/h, enough to supply all of the state’s homes. Currently, the sugarcane mills use about 800 thousand hectares, about 2.2% of the state area. The good news is that the sector is growing without deforestation, as fellow ranchers become increasingly efficient and use part of their land for crop cultivation, including sugarcane. In recent years, the industry left manual harvesting of sugarcane behind and today this work is done on modern machines with GPS and air conditioning. The workforce has been requalified and today the sugar-energy sector offers the best average wages in agriculture and industry. Another positive impact is local development, since the cities around sugarcane mills are experiencing growth. Within the sugarcane milling process, two residues should be considered: filter cake and vinasse, as both can boost stable fly proliferation if not properly managed. Filter cake, a compost, is returned to fields as a natural fertilizer with proper management techniques. There is also the vinasse; for each liter of ethanol, 13 liters of vinasse are produced as waste. It is a great natural fertilizer reducing our need for chemicals. Since 2016, it has been prohibited by law to burn sugar cane before harvesting. The result is that a lot of straw is left in the fields. The combination of mulch and the vinasse - again, if not properly treated - can also be a factor boosting stable fly proliferation. In this sense, there are two important aspects for the sugar-energy sector. The first one is “to deal with”, and how to deal with it is something that the sugarcane units learned from the hard work of very qualified people from Embrapa, partner researchers from the first moment. The second one is “union”. Stable

fly outbreaks are terrible and tend to irritate people, often pitting sugar cane mills and livestock producers against each other. But arguing and pointing fingers lead nowhere. It is by shaking hands and working together that this fight against the stable fly is won.

Thadeu Barros (Embrapa, Brazil). Current status of the stable fly in Brazil. Over the last decade, population explosions of the stable fly have become an unprecedented problem affecting several Brazilian states. Stable fly outbreaks in Brazil have been mostly associated with organic residues/byproducts resulting from ethanol production in sugarcane mills. Yearly outbreaks closely related to sugarcane processing may reach alarming infestations and last several months, thus compromising livestock production in surrounding areas. Also, the use of in natura fertilizers (mostly poultry litter) in cultivated crop areas has been associated with stable fly outbreaks in several crop production areas. More recently, stable fly outbreaks apparently associated with large areas of crop and pasture straw accumulated on soil have been recorded during the rainy season on farms adopting integrated crop-livestock systems. So far, 158 municipalities from five states have been affected by stable fly outbreaks, with profound economic, social and environmental implications. The strengthening of research and extension programs focusing on pest management is urgently needed. The development of public policies, through government actions and joint programs with the involved private sectors, as well as advances on specific regulatory issues, seems to be essential to ensure the advances needed to mitigate the impact of stable fly outbreaks in the country.

Erik Vargas (INTA, Costa Rica). Current status of the stable fly in Costa Rica. Stable fly outbreaks in Costa Rica continue to be associated mainly with pineapple production and cattle residues. However, outbreaks have been reported in citrus, rice straw, oil palm, coffee, and banana residues. In August 2019, a new substrate was reported in the north region of the country: chopped crop residues of cassava. The Ministry of Agriculture issued a new Ministerial Action Plan for the Integrated Management of Vegetable and Animal Residues (July, 2018), with the detailed functions of Extension offices (MAG), Animal health service (SENASA), Phytosanitary service (SFE), Research and training institute (INTA), to address the situation of the pest. Strategies for stable fly control include: to appoint a national coordinator and define regional working groups (North, Caribbean, South and Central regions), develop a stable fly monitoring

system, increase the inspections and support of crops and cattle farms, implement sanitary orders or sanctions if farmer produces the outbreak, facilitate the formation of producer groups to attack the pest (traps, thermofogging, veterinary products), search for international support to acquire traps for the massive capture of stable flies (OIRSA financed the purchase of 600 vavoua traps and 1200 attractants of stable fly), analyze the weather conditions to stop pineapple preparation in rainy weeks of the year (not necessary in 2019), educate producers on the biology, identification and control of stable flies, and continue research on stable fly integrated management. After the first ISFW, investigations have been developed on subjects such as: harvest and chopping pineapple plants machine, traps and attractants for adult capture, parasitoids, repellents, insecticide treatment, insecticide resistance, insecticide application equipment, entomopathogenic fungi, organic matter decomposers, substrate modifiers (fungicides, bactericides, pH, others), new desiccating herbicides for pineapple plants, minimum tillage of pineapple crops and covering chopped pineapple residues (plastic, shade net).

Eleonor Castro (UDELAR, Uruguay). Actual situation of *Stomoxys calcitrans* in Uruguay. *Stomoxys calcitrans* is a livestock problem only in specific cases, associated with accumulations of organic matter (fodder), either in bales or in pastures (direct sowing). Stable fly outbreaks occurred in 2010 and 2014, for less than 2 weeks. However, some dairy farms and Lecocq Zoo Park complain about flies, so we started a study on population dynamics in the south of the country, using emergence traps and NZI traps (2018-2020). Preliminary results showed bimodal dynamics, with peaks in late spring and fall. During the period, low populations of stable flies were recorded.

David Taylor (USDA-ARS, USA). Current status of stable flies in the US. Overall, the stable fly situation in the US is much less severe than that observed in Brazil, Costa Rica, and Australia. Outbreaks of greater than 50-100 stable flies per host are rare, and usually short lived. In the US, the predominant dogma is that immature stable flies develop in vegetative materials contaminated with animal waste. Evidence of stable flies developing in agronomic residues is very limited. Hence, most observers considered stable flies to be a self-perpetuated problem. The livestock industry is responsible for the developmental substrates and also the recipients of most of the economic impacts. Given the expenses of managing livestock wastes to eliminate

stable fly development, and the marginal efficacy of such efforts when practiced on an individual premise basis, most producers are willing to accept the economic losses associated with this fly. This situation is exasperated by a paucity of knowledge relative to stable flies and other flies associated with livestock production. Few producers can differentiate stable flies from horn flies, face flies or house flies. Therefore, these four species, and any other flies that may be apparent, are lumped together into the single category of “flies.” Not surprisingly, the types of damages and levels of responses of the livestock cannot be consistently evaluated or properly attributed because they are dependent upon the respective behaviors and biology of the flies involved. Improved education of producers about fly biology, impacts on livestock, and management is a high priority. A second priority is improved characterization of the developmental substrates used by stable flies and development of methods to associate adult flies with their developmental sites. It is possible that the perception of stable flies primarily developing in materials contaminated with livestock wastes is incorrect. Cultural management methods targeting immature developmental habitats will not be successful if a significant part of the population is developing in unrecognized locations.

Tuesday, Oct. 22nd

Workshop participants left Campo Grande early for a field day at a sugarcane mill in an area affected by outbreaks, at the municipality of Nova Alvorada do Sul. At the sugarcane mill, the group was welcomed and watched a series of short presentations given by the local staff, which included a general information about the company and plant operation, agricultural management, sugarcane production, management of residues and byproducts, industrial process of ethanol production, and the safety standards for visitation. After that we visited the filter cake yard, where handling procedures were explained and the turnover of filter cake piles (to reduce humidity and preclude fly larvae development) was demonstrated. After lunch, we saw the mechanized harvesting of the sugarcane and the resulting accumulation of mulch on ground, and the process of vinasse application on a sugarcane cultivation area (applied on the sugarcane mulch). Lastly, the group went to a trapping demonstration station where we could see the field use of different traps (Alsynite, Vavoua, Nzi, blue sticky plastic). After this field visit, the workshop participants proceed to

the town of Bonito, where the scientific part of the workshop took place in the following two days.

Wednesday, Oct. 23rd

The scientific symposium of the workshop began, with a series of individual talks covering several subjects related to the stable fly problem and a discussion round about the previous presentations.

Biology, Ecology, Sampling, and Monitoring

Erik Vargas (INTA, Costa Rica). Early warning system for stable fly monitoring in Costa Rica as a strategy for decision-making management. Monitoring is an important part of the integrated pest management of stable flies. Hot spot maps are tools used to identify the density of pests in defined geographic locations and develop management strategies. In August 2018, a pilot project for stable fly monitoring was started in the North Region of Costa Rica in an area of 8.000 hectares; subsequently extended to 40.000 hectares. Maps are based upon two-weekly collections of field information about the stable fly infestation rate on cattle and farms with agricultural residues. This process was carried out by agents of the Extension Office (MAG), Animal Health Service (SENASA), Phytosanitary Service (SFE) and Research and Training Institute (INTA), with global positioning equipment (GPS or smartphones). The information was reviewed, consolidated and processed by QuantumGIS, which developed geographic projection models to estimate the areas of risk of the stable fly from the field data. The maps were analyzed by the regional work group responsible for monitoring and making decisions on the stable fly situation. Vavoua traps have been used for monitoring since August 2018 in the pilot area, but the quantity of stable flies captured was highly variable, so those data could not be used on the maps. A project was presented to the government to develop a mobile application which would allow the collection and analysis of field data for the elaboration of the maps. Drone flight tests were used to identify possible areas with agricultural residues near of livestock farms affected by the stable fly.

Taciany Ferreira (Volare, Brazil). Applicability of monitoring for control of stable fly outbreaks.

The stable fly population monitoring system is considered an important tool for successful outbreak control in sugar and alcohol producing mills in Brazil. Due to the extensive areas of vinasse application in the sugarcane fields, monitoring can operate within a radius of up to 15 km from the mill. Reflective traps (Alsynite) are used in monitoring; usually 25 to 45 traps per mill, installed up to 3 km apart. The main monitored sites are the fertigated areas, filter cake composting yard, and adjacent livestock farms. The traps are georeferenced for the elaboration of a monitoring map. Entomological material is collected weekly and sent to Volare's office for specimen counting and identification. Weekly quantitative fly catches are characterized at low, alert and high levels; then forwarded to the mill's team to direct pest prevention and control actions following technical recommendations. The applicability of the monitoring system allows mills to predict pest population increases and strategic direction of control measures, making them more efficient and sustainable. In sugarcane mills with more than two years of Volare operation, this monitoring method allowed a peaceful coexistence between the livestock and sugar-energy sectors, as well as a reduction of stable fly control costs at sugarcane mills.

Paulo Cançado (Embrapa, Brazil). Computer vision and aerial imaging for stable fly monitoring.

Monitoring populations of *S. calcitrans* is a challenge, especially in gigantic areas such as sugarcane cultivation. One of the problems presented was the huge number of flies that would need to be identified and counted if companies were required to monitor the sugarcane fields. To solve this problem, Embrapa's Beef Cattle research team, in partnership with Volare, and the Federal University of Mato Grosso do Sul, has been developing an automated system for stable fly identification and counting. This system uses mathematical algorithms and computational vision to identify and count stable flies from Alsynite traps. Counting results have not yet reached satisfactory levels for commercial use. Neural network technology will be used to improve the system in the next stage of development. Another point addressed in this talk was the use of drones to monitor large areas of vinasse application. Using drone-coupled RGB color sensors, aerial images are captured. With computer vision, these images are analyzed and the larval habitats can be identified and demarcated. This technology is still in the early stages of development and infrared and multispectral sensors should be tested. We

expect to substantially reduce expenditures on prevention and control as it will be possible to focus control actions on demarcated areas.

Thais Albuquerque (Brazil). Stable fly oviposition behavior. Stable flies are blood-feeding insects with a great negative impact on animals worldwide. Larvae develop primarily in animal manure and bacteria are essential for larval development; however, the principle of this dependence is not understood. We hypothesized that as the microbial community of animal manure changes over time, it plays an important role in stable fly fitness. Two-choice bioassays were conducted using 2 week old horse manure (control) and aging horse manure (fresh to 5 week old) to evaluate the effect of manure age on stable fly oviposition. Our data showed that fresh feces did not stimulate oviposition and that the attractiveness increased as manure aged but started to decline after 3 weeks. Bioassays assessing the effect of manure age at the time of oviposition on larval development demonstrated that 1–3 week old manure supported larval development significantly better than fresh, 4, and 5 week old manure. In addition, adult fitness (body size) was significantly higher in flies from 1- and 2-week old manure compared with that of all other treatments. Analysis of the bacterial community of aging horse manure by 454-pyrosequencing of 16S rDNA revealed a great reduction in bacterial diversity and richness from fresh to 1–5 week old manure and a major shift from strict anaerobes in fresh manure to facultative anaerobes and strict aerobes in aged manure. Overall, the microbial community of 2 and 3 week old horse manure with its dominant bacterial taxa *Rhizobium*, *Devosia*, and *Brevundimonas* stimulated stable fly oviposition the most and provided a suitable habitat for larval development. These bacteria represent the candidates for studies focused on better understanding of stable fly – microbial interactions.

Management and Control

Francisco Gonzalez (Chentica, Costa Rica). Development and registration of a commercial attractant for *Stomoxys calcitrans*. In Costa Rica, the stable fly, *Stomoxys calcitrans* develops on pineapple plant debris and pastures in which coffee-derived waste water is applied, among other different oviposition substrates. So far, farmers have mainly used white sticky bags to try to mass trap incoming flies by means of interception and visual attraction.

This method involves changing the bags every 1-3 days, which makes this solution extremely expensive and represents a dependency on plastics and glue and affects negatively non-target species. Therefore, during the last 2 years, we aimed to find the compounds driving the attraction of flies towards the oviposition substrates. Although several molecules have been proposed in addition to the ones known as produced by the feeding hosts no specific compound has shown an important effect. For example, the compound Styrene, found in volatile collections from 3 different substrates preferred by *Stomoxys*, was not effective alone or combined with other compounds in the attraction of the fly. In an attempt to emulate the rotting smell of plant-refuse and waste water we tested in pineapple and coffee fields the attraction of a fermenting mixture of protein origin in combination with the compound 1-octen-3-ol. The results showed the synergism between the two different stimuli and showed increased efficacy when used in Vavoua traps in comparison to white sticky traps in values of 6X, 16X and 41X in one coffee and two pineapple farm experiments respectively. The current situation of crisis, added to the great potential of the attractant, allowed us to advocate for the registration of the product. However, in Costa Rica, attractants used for pest control have been traditionally registered through the Plant Health Service, and considering that *Stomoxys* is a pest of livestock, the registration needed was not with the Plant Health Service, but rather with the Animal Health Service. Nevertheless, the Animal Health Service did not have the legal instrument to register this type of product, reason why, the Ministry of Health, considering the current crisis and the socioeconomical risk that stable fly represents granted us the registration, which allowed the Animal Health Service to authorize the commercialization of the attractant for use against *Stomoxys calcitrans*. Although this technique allows for mass trapping of stable flies, several new research avenues have opened and more pending questions will be resolved to understand the biology and behavior of this pest.

Thadeu Barros (Embrapa, Brazil). Insecticide-treated targets for stable fly control in outbreak areas. Insecticide-treated targets have been considered a promising tool for use in integrated stable fly management strategies. Although their empirical use has been already adopted in many sugarcane mills and cattle farms/ranches affected by stable fly outbreaks in Brazil, advances are needed to increase the efficiency of this technology. This goal is being pursued by a project underway at Embrapa. Studies have been per-

formed to chemically identify volatile compounds present in substrates from sugarcane processing (such as vinasse, straw + vinasse, vinasse sludge, and filter cake) known to be attractive to stable flies. So far, a total of 99 compounds, organic acids, alcohols, aldehydes, ketones, esters, hydrocarbons, and sulfur compounds, have been identified and are being tested to identify potential attractants for use in treated-targets as well as in other traps. Current bioassays with insecticide-impregnated fabrics demanded prior methodological adjustments for selection of insecticide candidates for field tests. Because of resistance, pyrethroid insecticides did not perform well in *in vitro* bioassays, so, organophosphates and phenylpirazoles were tested on treated-targets kept under field conditions. In an initial field trial, fipronil 1% was highly effective for up to 12 weeks, while chlorpyrifos 1% provided lower efficacy for a shorter period. Questions regarding the fabric itself (type, color attractiveness, durability, etc.) are pending. The preliminary results obtained so far are promising and should provide significant advances in the use of this strategy.

Samir Kassab (Legado, Brazil). Monitoring, prevention and control of stable flies in areas of concentrated vinasse application. Stable fly (*Stomoxys calcitrans*) can oviposit and develop in various byproducts from the sugar and alcohol sector, including sugarcane straw associated with vinasse and filter cake. Vinasse is commonly spray-applied to fields, which may restrict application to small areas due to the high cost of infrastructure necessary for such operation. Concentrating the vinasse is a possibility; besides reducing the volume of the product, concentrated vinasse can be applied in additional locations. Initially, it was believed that vinasse concentration would be of interest for stable fly control due to the lower volume applied in the field. However, stable flies continued to exploit the concentrated vinasse, even with the reduced volume. Thus, we suggest that plant residues be removed from sites where the trucks are loaded with vinasse in order to reduce the possibility of the insect development in this environment. After the application of vinasse in sugarcane plots, it is extremely important that the areas are cultivated and/or scarified in order to expose the straw present in the soil and promote drying of the material, avoiding the proliferation of insects. Thus, with relatively simple processes, oviposition and stable fly development can be prevented in plots intended for the application of concentrated vinasse.

Taciany Ferreira (Volare, Brazil). Management of stable flies in sugarcane mills and livestock areas. Outbreaks of the stable fly, *Stomoxys calcitrans*, remain a problem and cause direct and indirect damage to the livestock and sugarcane sectors. The economic importance for the livestock sector is mainly related to the reduction of herd weight gain and milk production. For the mills the damage involves the negative exposure of the company's brand, the lawsuits of the ranchers, as well as the high cost to control the stable fly. The main breeding sites for the stable fly at the sugarcane mills are: areas of vinasse application, vinasse open channels, vinasse tanks and filter cake composting yards. The operational quality and timing of mitigation measures are critical to pest control, so mill employees should be properly trained in assertiveness in directing recommended actions. In addition, Volare's activities with the mills involve technical visits and orientation talks to ranchers regarding the importance of sanitary practices and the use of adult control traps (bicolor insecticide-impregnated targets and blue entomological glue). For prevention and control of stable fly outbreaks, effective management of the organic byproducts generated by the sugarcane mills and sanitary measures in adjacent livestock establishments are required.

Thursday, Oct. 24th

Avelino Bittencourt (UFRRJ, Brazil). Biological control of stable flies with fungi and nematodes. The beginning of the talk approached the use of fungi, which, in my opinion, should be used on eggs and larvae. Presented data showed that mortality should be observed throughout the whole cycle (not at each stage); when applied to eggs, the effect of the fungus will be observed on adult emergence. Among fungi, *Metarhizium anisopliae* and *Lecanicillium lecanii* showed the best results, reaching 96% mortality in some cases. The results with *Beauveria bassiana* were poor, either with eggs (20-22%) or larvae (34-43%); the low larvae mortality is due to the antifungal effect of the bacteria *Stenotrophomonas maltophilia*, which was isolated from the bacterial mucus and showed an inhibitory effect on the development of the fungus in vitro. Regarding the entomopathogenic nematodes (EPNs), their mechanism of action was presented, in which they invade the stable fly larvae and kill them causing sepsis, consuming the nutrients inside the larvae, and causing membrane rupture when leaving. He showed his initial studies using

the EPNs *Heterorhabditis bacteriophora* (HP88) and *H. baujardi* (LPP7). In water, the former showed a high mortality (> 90%) but mortality due to the latter was low (33%). After the first ISFW, studies were conducted associating EPNs with vinasse, filter cake, ash and sugarcane bagasse. When EPNs were applied to the vinasse at 35°C, larvae mortality was above 90%, but it decreased to 60% at 40°C. In the filter cake, the EPNs caused 70-100% mortality, however, larvae mortality depended on the EPN load and a higher number of EPNs (400 EPNs/larva) reduced larval mortality, probably due to competition for larvae nutrients. In ashes, larval mortality increased with the EPN load (50-200 EPN/larva). When vinasse and chopped sugarcane were associated, larvae mortality did not exceed 70%, possibly due to the large amount of substrate (sugarcane) to be transposed by the EPNs to reach the stable fly larvae. It is important to stress that biological control agents can act alone or associated with insecticides, attacking resistant populations and assisting in the control of pests. We still have studies to conduct in the laboratory before move on to the field, but I see some possibilities of EPN use associated with vinasse in fertigation and spraying with water on the edges of filter cake piles, since in these places the humidity and temperature are more favorable.

Resistance, Molecular Biology, Socioeconomic, and Regulatory Issues

Andrea Egito (Embrapa, Brazil). Detection of *kdr-his* mutation in stable fly populations in midwestern Brazil. The stable fly is currently one of the ectoparasites with the greatest economic impact on the livestock production chain. In order to reduce the damage caused by the stable fly, sanitary management measures are implemented associated with chemical control based on the use of insecticides. However, the continued use of insecticides has led to the emergence of resistant individuals. In the case of insecticides from the pyrethroid class, this resistance has been associated with a *kdr* (knockdown resistance) in the voltage-sensitive sodium channel gene (*Vssc*), where the fly's nervous system becomes insensitive to the action of those insecticides. This is the first report of the existence of the mutation *kdr-his* in Brazil. Stable fly samples were obtained from two colonies (CNPGC - resistant colony and Kerrville -susceptible, a negative control) and two wild

populations from sugarcane mills. The presence of the *kdr-his* mutation was detected in 23 of 117 flies by sequencing a 300 bp region of the *Vssc* gene, amplified with primers designed from the reference sequence (HQ010283). Of these, 83% were heterozygous and 17% homozygous. In the insects collected in the field, 50% had at least one *kdr-his* allele, while only 10% of the colony had it. Differentiation tests based on the FST index showed that the CNPGC colony is genetically different from field populations, which are statistically similar. The relatively high resistance levels observed in previous insecticide bioassays and the low frequency of the *kdr* mutation in the CNPGC colony suggested that there may be other resistance mechanisms involved. The highest frequency of the *kdr-his* allele in the field when compared to the CNPGC colony may be due to a higher field selection pressure, imposed by the use of insecticides or due to some fitness cost, caused by the presence of the mutation in the absence of insecticide. It was also possible in this work to prospect three new SNPs whose possible association with pyrethroid resistance should be evaluated in future studies.

Lenita Santos (Embrapa, Brazil). Stable fly bacteriome in populations from livestock and sugarcane mills. Adult stable flies (*Stomoxys calcitrans*) are serious hematophagous pests of pasture and feedlot cattle and larvae occupy and develop in almost any type of decomposing vegetable matter, often contaminated with animal wastes. Thus, stable flies are consistently exposed to microbes. It is known that bacteria are essential for larval stable fly survival and development, but little is known about the innate microbial communities of adult stable flies in different habitats. Using metagenomics analysis it is possible to identify the fly microorganisms, even if they are not cultivable. Understanding the innate microbial community associated with adult stable flies and relating it to their hosts and environment may assist in preventing pathogen transmission.

Luciana Pasternak/Anderson Saravia (INIA, Uruguay). Use of traps for the control of stable fly in Uruguay. The presence of stable fly, associated with inadequate waste management, has been a problem for some dairy farms in Uruguay. A good mechanical control for the stable fly has been the use of NZI traps. However, importing these traps becomes unviable for the farmers due to their high cost and waiting periods. Thus, it is important to develop nationally produced traps that allow easy access at low cost maintaining the

efficiency. With this objective a project for developing traps is underway. This is part of a larger project that seeks to determine effective mechanisms to prevent the spread of diseases transmitted iatrogenically and by vectors, in which horseflies and stable flies are important due to their biting habits. In the INIA these traps are being developing, considering local availability of the materials. Once the traps are developed, the efficacy will be compared with the original NZI bought in USA. Having the produced traps proved, this will be largely produced in Uruguay and distributed to the farmers. These traps are expected to restrain spreading fly-related problems and to prevent possible outbreaks in the country.

Paulo Cançado (Embrapa, Brazil). Economic and social impact of stable flies in Brazil. Previous studies on the economic impact of stable flies in Brazil did not take into account the outbreaks associated with sugarcane mills. A preliminary approach on socioeconomic impacts of the stable fly in Brazil was presented. First, social impacts that cannot be quantified were presented, since there are no official data about them. These results included people who lost their job in the countryside because stable fly outbreaks and had to move to the cities, the migration of the livestock activity to non-affected regions, and the cultural impact suffered by families that have worked with livestock for centuries. Also, there are reports of idleness and depression among ranchers in regions affected by outbreaks. Some secondary economic losses, despite they have not yet been accounted due to insufficient data, have been reported. In this context, abortions, dystocic deliveries, increased mortality in cattle, as well as legs and tail fractures can be highlighted. A preliminary estimative of economic losses due to stable fly outbreaks was based on the available information on reduction in milk and meat productivity. Potential loss estimations were based on data from the IBGE (Brazilian Institute of Geography and Statistics) regarding the herd size existing in each affected municipality in Brazil. The reduction in milk production was estimated in 40% and estimated weigh gain loss was 32 pounds per animal during outbreaks. We consider that sugarcane mills spend an average of US\$ 40,000 to prevent and control stable fly outbreaks for every million tons of sugarcane processed. Thus, it has been estimated that, only during the outbreak periods, economic losses due to the stable fly in Brazil is about US\$ 108 million yearly. If we include losses not related to outbreaks in sugarcane mills, the potential impact caused by stable fly infestations was estimated over US\$ 443 million per year.

Alessandro Minho (Embrapa, Brazil). Regulatory issues regarding stable fly outbreaks in Brazil - São Paulo State Regulation. States can make supplements to the Brazilian legislation, as long as they are stricter than the federal law. The practice of sugarcane pre-harvest burning has been restricted in the country and prophylactic burning can be used only in extreme cases. In a new outbreak report, the inspectors should: check for the presence of a stable fly infestation; identify the causes and places of the problem; recommend the necessary measures, such as: monitor fly population (alsynite traps) and larval development (emergence traps); suspend or reduce the fertigation in places soaked by rain or vinasse; perform scarification of straw before fertigation; eliminate or clean open vinasse distribution channels; turnover all filter cake piles at least twice a week, and adjust the equipment to ensure that all the filter cake material is turned over from ground level. After 30 days, if the outbreak has not been contained, the perpetrator will be fined.

II ISFW Conclusions

In response to the presented situation and considering the current available information, as well as the conclusions from the first workshop, the II ISFW considered essential to advance in the topics below:

- Development of stable fly management plans suitable for each particular production system involved, containing specific recommended practices for mitigating the development of stable flies in agronomic and livestock associated residues/byproducts, thus preventing eventual population explosions;
- Development of specific monitoring methods for both immatures and adult stable flies, which, ultimately, will support alert systems and decision making tools;
- Establishment of an extension program to stimulate greater engagement of professionals from both the agricultural and veterinary fields, as well as to train crop and livestock producers regarding preventive and corrective management practices to be adopted throughout the year by involved production systems;

- Development of a comprehensive research program on topics including monitoring methods, bioecology, toxicology and insecticide resistance, chemical and biological control, “attract-and-kill” approaches (traps, targets, repellents, and attractants), socioeconomic impact and development of economic thresholds;
- Elaboration and implementation of public policies, enabling prompt action by inspectors and requiring immediate corrective actions, as well as appropriate sanctions in case of negligence and/or recurrence, and supporting the above suggestions.
- Creation of a stable fly advisory board, which may consist of representatives from the government, academia-scientific community, stakeholders representing crop and livestock producers, and affected communities.

With this morning round of presentations and discussions we concluded the scientific part of the workshop.

After that, we went to the “Balneário Refúgio da Barra”, where the group had lunch and spent the afternoon. After previous days of an intense exchange of information and experiences in a formal atmosphere, this afternoon provided a valuable time for a more informal interactivity, stimulating conversation and strengthening connections among participants.

Friday, Oct. 25th

Return trip to Campo Grande with stop at Gruta do Lago Azul (Blue Lake Cave) for visiting the post-card place of Bonito.

Participants and Biographies

Alessandro Pelegrine Minho. Graduate in Veterinary Medicine (1996), Master in Animal Health (2002), PhD in Sciences (2006), and Postdoctoral (2008). Researcher of the Brazilian Agricultural Research Corporation (Embrapa), Animal Health Area, in the Southern Livestock Unit (2011 - 2017) and the Southeast Livestock Unit (2018 - current). Research interests in

endo and ectoparasite control, alternative control and parasite resistance. alessandro.minho@embrapa.br

Anderson Saravia de Melo, Bachelor's in clinical laboratory and Master's student in Animal Production at the Veterinary Faculty of the University of the Republic of Uruguay (UDELAR). I worked as a scientific advisor for Siemens healthcare diagnostics and I am currently working as a Laboratory Assistant in the Parasitology Department at the Animal Health Platform (PSA) of the National Institute from Agricultural Research (INIA) since 2015. I am currently participating in research projects from different areas, such as: *Fasciola hepatica*, gastrointestinal nematodes, and dipterans (*Stomoxys calcitrans*, *Haematobia irritans* and *Cochliomyia hominivorax*). asaravia@inia.org.uy

Andrea Alves do Egito, PhD. Veterinary molecular geneticist of the Brazilian Agricultural Research Corporation (Embrapa), from 1989 until August 2010 worked at the National Center for Research on Genetic Resources and Biotechnology - CENARGEN and is currently at Embrapa Beef Cattle in Campo Grande, MS. She is currently accredited at the Federal University of Mato Grosso do Sul Foundation (UFMS), as a Professor at the Graduate Programs of Veterinary Sciences and Animal Science. Has experience in Molecular Genetics applied to Animal Breeding and Animal Health, acting on the following subjects: molecular markers, candidate genes, genomics, insecticide resistance genes, population structure, genetic diversity and DNA banks. andrea.egito@embrapa.br

Avelino José Bittencourt, PhD. Veterinary with Masters and PhD in Veterinary Parasitology at UFRRJ. Full Professor of Clinical Propaedeutic at the Veterinary Institute of UFRRJ. Studying the stable fly since 1993, before starting PhD (1994). His studies have addressed the stable fly seasonality in cattle and horses, body distribution, host behavioral changes, epidemiology on farms by assessing farmers' perceptions of the stable fly problem as well as other parasites. He oriented theses and dissertations, addressing the bacterial microbiota in flies and cows affected by mastitis; histopathology of skin lesions and hematological changes in artificially infested rabbits. He carried out applied biological studies, evaluating the development of stable fly stages in substrates from sugarcane mills (vinasse, filter cake, bagasse and ashes). Lately, along with his undergraduate, masters and doctoral students,

he has conducted biological control studies using fungi and entomopathogenic nematodes. *bittenc@ufrj.br* / *avelino.bittencourt@gmail.com*

David Taylor, PhD, is a Research Entomologist with USDA-ARS Agroecosystem Management Research Unit in Lincoln, Nebraska and has over 35 years of experience in Veterinary Entomology. Dr. Taylor received his BS and PhD degrees from the University of Notre Dame in 1977 and 1982 respectively. Dr. Taylor began his professional career with ARS in 1983 working on screwworm genetics and mass rearing technologies at the Screwworm Research Laboratory in Tuxtla Gutiérrez, Chiapas, Mexico. In 1991 Dr. Taylor moved to the USDA-ARS Midwest Livestock Insect Research Laboratory in Lincoln, Nebraska and in 1994 began a research program on flies affecting livestock production with an emphasis on stable flies which he continues today. He has worked on biological control, genetics, ecology and management of stable flies and other muscoid flies associated with livestock. During a 40+ year research career, he has published 75 journal articles and 4 book chapters. [https://works.bepress.com/david_taylor1/]. *dave.taylor@usda.gov*

Eleonor Castro Janer, PhD. Veterinary, professor since 1984 in the Department of Veterinary Parasitology, School of Veterinary, University of the Republic (UDELAR). Major areas of interest: epidemiology, control, IPM and resistance to insecticides in livestock diseases. *elinorcastro59@gmail.com*

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Eronides Marques de Souza, MSc. Bachelor in Veterinary Medicine at Universidade Federal de Mato Grosso do Sul (UFMS) in 1997; Master's degree in Animal Science (concentration area in Immunoparasitology) from the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP-Ilha Solteira) in 2001. Doctorate in progress in Veterinary Science, UFMS, under Drs. Flávio Ribeiro de Araújo, Lenita Ramires dos Santos, and Paulo Henrique Duarte Cançado. Major areas of interest: biology and ecology of stable flies, animal health. *eronidesmarques@hotmail.com*

Francisco J. Gonzalez Fuentes, PhD. Chemical Ecologist from Costa Rica. Since 2008 has worked on different aspects of Integrated Pest Management, from behaviour to the chemical and molecular mechanisms mediating multitrophic interactions. He carried out his Master's degree in Wageningen University and Research Centre, The Netherlands, and his doctorate studies at the Swedish University of Agricultural Sciences, Sweden. Since 2017 he is the Director of Research & Development of the company ChemTica Internacional S.A. dedicated to provide semiochemicals for pest control in more than 50 countries around the world. Currently, he coordinates and executes more than 15 research projects in countries such as Costa Rica, Colombia, Ecuador, Tunisia and the United Arab Emirates, in pests such as weevils, moths and of course stable fly. *francisco_gonzalez@chemtica.com*

Lenita Santos, PhD. Immunologist of the Brazilian Agricultural Research Corporation (Embrapa) since 2010, currently at Embrapa Beef Cattle. Has experience with molecular biology techniques (cloning, expression, purification of proteins) and metagenomics analysis. Major areas of interest: biology, animal health, vaccines and diagnostic tests. *lenita.santosros@embrapa.br*

Luciana Grigolo Pasternak, Veterinary, Master's student in parasitology at the Animal Health Platform of the National Institute for Agricultural Research and the University of the Republic (Udelar) since 2019. Graduated from Laureate International Universities (Uniritter) in 2018. Major areas of interest: animal health, parasitology, ectoparasites, dipterans, mechanical control of dipterans (traps). *lpasternak@inia.org.uy*

Olman Salas Avila, Degree in agricultural sciences with the academic degree of Agricultural Engineer from EARTH University. Agriculture superintendent at Agroindustrial Piñas del Bosque S.A (DOLE) since 2014, currently at El Muelle Pineapple Farm. Worked at research department in different projects of plant nutrition and plant health for the pineapple division of Costa Rica (2011 - 2013). *olman.salas@dole.com*

Paulo Cançado, PhD. Graduated in Veterinary Medicine in 2002 at the Federal Rural University of Rio de Janeiro (UFRRJ). Received a PhD in Animal Parasitology from the same institution in 2008. During his time as a PhD candidate, he received from the Brazilian College of Veterinary Parasitology the award "Uriel Franco Rocha - Best in Ectoparasites" in 2006. From 2008

to 2010 was a professor of parasitology at the Faculty of Veterinary Medicine (ESBAM) in the state of Amazonas, where he was also the coordinator of the Faculty's Veterinary Clinic. He has been a researcher at Embrapa Beef Cattle since 2010, where he has worked in the areas of parasitology and animal health and, since 2015, is the supervisor of the animal research group and participates in the management committee of Embrapa's Animal Health Portfolio. He has coordinated the Americas Sub-Network of the International Research Consortium on Animal Health (STAR-IDAZ - <http://www.star-idaz.net>) since 2011. He has coordinated the "Nutrition and Animal Health Innovation Centers Network" (RCIISNA) of the Ministry of Science and Technology. The main areas of activity are ectoparasite prevention and control and research coordination. *paulo.cancado@embrapa.br*

Samir Oliveira Kassab, PhD. Bachelor in Biological Sciences from the Universidade Federal da Grande Dourados (UFGD) (2009), Master degree (2011), Doctorate degree (2014) and Post-doctorate degree in Entomology at UFGD (2016). He is a member of the research groups: Biotechnology and Microbiology (UFGD), Biological Control of Insects (UFGD), and Semiarid Plant Protection (UFC). He is currently a partner-owner of "Legado Pesquisa e Consultoria Agronômica Ltda.", where he works as a consultant in Entomology, especially with the stable fly. *samirkassab@gmail.com*

Taciany Ferreira, PhD. Bachelor in Biological Sciences at Uniderp University (2009). Masters in Environment and Regional Development (2011) by the same University. Experience with Agricultural Entomology, working on Integrated Pest Management. PhD in Animal Science from the Federal University of Mato Grosso do Sul (UFMS), Embrapa Gado de Corte, with emphasis on the monitoring of population dynamics of *Stomoxys calcitrans* in the vicinity of sugarcane plants. She is currently responsible for the company Volare Consultoria Ambiental Ltda., through the provision of services for the population monitoring of stable flies and control of outbreaks in sugarcane and livestock farms. *tacianyferreira1@gmail.com*

Thadeu Barros, PhD. Bachelor in Veterinary Medicine (1984) and Masters in Veterinary Parasitology (1989) at Universidade Federal Rural do Rio de Janeiro - UFRRJ; PhD degree in Entomology from the Louisiana State University - LSU (1998). Veterinary entomologist of the Brazilian Agricultural Research Corporation (Embrapa) since 1987, currently at Embrapa Beef

Cattle. Former member of the FAO Working Group on Parasite Resistance and FAO/Industry Contact Group on Parasite Resistance (2003-2009); currently a member of the Technical Committee on Animal Parasitosis and Resistance to Antiparasitics of the Brazilian Ministry of Agriculture, Livestock and Food Supply (2016-current). Major areas of interest: biology, ecology, chemical and biological control, and insecticide resistance of livestock pests. *thadeu.barros@embrapa.br*

Thaís Aguiar de Albuquerque, PhD. Bachelor in Veterinary Medicine (2002) at Faculty of Veterinary Medicine, University of São Paulo (FMVZ-USP) in Brazil. Master's degree in Biology of Host-Pathogen Relationship (2007) at Institute of Biomedical Sciences, University of São Paulo (ICB-USP), with resistance of *Rhipicephalus (Boophilus) microplus* to ivermectin. PhD degree in Entomology (2012) from Department of Entomology at Kansas State University (KSU) in United States of America studying the horse manure microbial community and its importance in egg laying and development of *Stomoxys calcitrans* larvae. Postdoctoral degree (2013) at Faculty of Veterinary Medicine of the Universidad de la República de Uruguay (UDELAR), with molecular characterization of insect fly resistance (*Haematobia irritans*). *thais.aa@gmail.com*

Thays Jucá e Silva, Bachelor's degree in Biological Science from the University of Pernambuco, Masters in Ecology by the National Institute of Amazon Research, and currently working at Volare Consultoria Ambiental. *thaysjuca@gmail.com*

Appendix 1. II International Stable Fly Workshop Program

| Day 1 . Monday (Oct. 21st) - Non-scientific session | | |
|---|--|---|
| 9am to 9:10am | SF Workshop Opening | Thadeu Barros (Embrapa Beef Cattle - Brazil) |
| 9:10am to 9:30am | Greetings to SF Workshop | Ronney Mamede (Director Embrapa Beef Cattle - Brazil) |
| 9:30am to 9:50am | International Stable Fly Working Group | David Taylor (USDA-ARS, Lincoln, NE, USA) |
| 9:50am to 10:10am | Coffee Break | |
| 10:10am to 10:50am | Livestock Sector Presentation | Maurício Saito (Famasil - Brazil) |
| 10:50am to 11:30am | Sugarcane Sector presentation | Roberto Hollanda (Biosul - Brazil) |
| 11:30am to 12pm | Current status of the stable fly in Brazil | Thadeu Barros |
| 12pm to 2pm | Lunch | |
| 2pm to 2:30pm | Self Introduction | All participants |
| 2:30pm to 3pm | Current status of the stable fly in Costa Rica | Erik Vargas (INTA - Costa Rica) |
| 3pm to 3:30pm | Current status of the stable fly in Uruguay | Eleonor Castro (UDELAR - Uruguay) |
| 3:30pm to 3:50pm | Coffee Break | |
| 3:50pm to 4:20pm | Current status of the stable fly in USA | David Taylor (USDA-ARS, Lincoln, NE, USA) |
| 4:20 to 5pm | Open discussion and general information | |
| Day 2 . Tuesday (Oct. 22nd) - Field day | | |
| 7am to 9am | Trip to Nova Alvorada do Sul | |
| 9am to 12pm | Visit sugar cane mill in outbreak area | Local representative |
| 12pm to 1:30pm | Lunch | |
| 1:30pm to 3pm | Field visit continuation | |
| 3pm | Departure to Bonito | |

| Day 3 . Wednesday (Oct. 23rd) - Scientific session | | |
|---|--|---|
| BIOLOGY, ECOLOGY, SAMPLING and MONITORING | | |
| 8am to 8:30am | Stable fly biology and ecology applied to monitoring and control | David Taylor (USDA-ARS, Lincoln, NE, USA) |
| 8:30 to 9am | Early warning system for stable fly monitoring in Costa Rica as a strategy for decision-making management (Erik Vargas, INTA-Costa Rica) | Erik Vargas (INTA - Costa Rica) |
| 9am to 9:30am | Applicability of monitoring for control of stable fly outbreaks | Tacyany Ferreira (Volare - Brazil) |
| 9:30am to 10am | Computer vision and aerial imaging for stable fly monitoring | Paulo Caçado (Embrapa Beef Cattle - Brazil) |
| 10am to 10:20am | Coffee Break | |
| TRAPS, ATTRACTANTS and REPELLENTS | | |
| 10:20am to 10:50am | Stable fly oviposition behavior | Thais Albuquerque (Brazil) |
| 10:50am to 11:20am | Development of attract-and-kill devices for stable flies | Jerry Hogsette (USDA-ARS, Gainesville, FL, USA) |
| 11:20am to 12pm | Discussion | |
| 12pm to 2pm | Lunch | |
| 2pm to 2:30pm | Development and registration of a commercial attractant for <i>Stomoxys calcitrans</i> | Francisco Gonzáles (Chemtica - Costa Rica) |
| 2:30pm to 3pm | Insecticide-treated targets for stable fly control in outbreak areas | Thadeu Barros (Embrapa Beef Cattle - Brazil) |
| MANAGEMENT AND CONTROL | | |
| 3pm to 3:30pm | Monitoring, prevention and control of stable flies in areas of concentrated vinasse application | Samir Kassab (Legado - Brazil) |

| | | |
|---|---|--|
| 3:30pm to 3:50pm | Coffee Break | |
| 3:50pm to 4:20pm | Management of stable flies in sugarcane mills and livestock areas | Tacyany Ferreira (Volare - Brazil) |
| 4:20pm to 5pm | Discussion | |
| Day 4 . Thursday (Oct. 24th) - Scientific session / interactive activity | | |
| RESISTANCE, BIOLOGICAL CONTROL, MOLECULAR BIOLOGY, AND SOCIOECONOMIC ISSUES | | |
| 8am to 8:30am | Detection of kdr-his in stable fly populations in midwestern Brazil | Andrea Egito (Embrapa Beef Cattle - Brazil) |
| 8:30am to 9am | Biological control of stable flies with fungi and nematodes | Avelino Bittencourt (UFRRJ - Brazil) |
| 9am to 9:30am | Stable fly bacteriome in populations from livestock and sugarcane mills | Lenita Santos (Embrapa Beef Cattle - Brazil) |
| 9:30am to 10am | Studies on stable flies in Uruguay | Luciana Pasternak/Anderson Saravia (INIA - Uruguay) |
| 10am to 10:20am | Coffee Break | |
| 10:20am to 10:50am | Socioeconomic impact of stable flies in Brazil | Paulo Cançado (Embrapa Beef Cattle - Brazil) |
| 10:50am to 11:20am | Regulatory issues regarding stable fly outbreaks in Brazil | Alessandro Minho (Embrapa Southeastern Livestock - Brazil) |
| 11:20am to 12pm | Discussion and Closure | |
| 12pm to 2pm | Lunch (Ecopark) | |
| 2pm to 5pm | Group interaction time | |
| 17h | Return to hotel | |
| Day 5 . Friday (Oct. 25th) - Trip to Campo Grande | | |
| 7:30am | Departure | |
| 8:30am | Stop at Gruta do Algo Azul (Blue Lake Cave) | |

| | | |
|------------------|------------------------------|--|
| 10:30am | Trip back to Campo Grande | |
| 12pm | Lunch (stop on the way back) | |
| 1:30pm to 4:30pm | Trip back to Campo Grande | |

Appendix 2. II ISFW Images

1. II International Stable Fly Workshop – Opening at Embrapa Beef Cattle – Campo Grande.
2. ISFW non-scientific session (David Taylor's videoconference) at Biosul – Campo Grande.
3. Field day at sugarcane mill (vinasse application) – Nova Alvorada do Sul
4. ISFW scientific session at Wetiga Hotel – Bonito.
5. ISFW scientific session.

Photos: Joana Gasparotto Kuhn.







Embrapa

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