

THEMATIC PROJECTS



SÃO PAULO RESEARCH
FOUNDATION

ADVANCES IN AGRARIAN SCIENCES AND VETERINARY

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ZOOTECNY



EXPLORATORY RESEARCH RECOGNIZED WORLDWIDE



Agronomy, zootechny, veterinary, fisheries resources. In these and other sub-areas of Agrarian and Veterinary Science, Brazilian scientists contributed with results recognized worldwide.

FAPESP, the São Paulo Research Foundation, is one of the main Brazilian agencies for the promotion of research. The foundation supports the training of human resources and the consolidation and expansion of research in the state of São Paulo.

Thematic Projects are research projects that aim at world class results, usually gathering multidisciplinary teams around a major theme. Because of their exploratory nature, the projects can have a duration of up to five years.

SCIENTIFIC OPPORTUNITIES IN SÃO PAULO, BRAZIL

Brazil is one of the four main emerging nations. More than ten thousand doctorate level scientists are formed yearly and the country ranks 13th in the number of scientific papers published.

The State of São Paulo, with 40 million people and 34% of Brazil's GNP responds for 52% of the science created in Brazil. The state hosts important universities like the University of São Paulo (USP) and the State University of Campinas (Unicamp), the growing São Paulo State University (UNESP), Federal University of São Paulo (UNIFESP), Federal University of ABC (ABC is a metropolitan region in São Paulo), Federal University of São Carlos (UFSCar), the Aeronautics Technology Institute (ITA) and the National Space Research Institute (INPE).

Universities in the state of São Paulo have strong graduate programs: the University of São Paulo forms two thousand doctorates every year, the State University of Campinas forms eight hundred and the University of the State of São Paulo six hundred.

In addition to the three state universities the state has 19 research institutes, three federal universities of international research level and most of Brazilian industrial R&D. The state houses more than 10 thousand fulltime faculty and 130 thousand students. São Paulo alone, produces more scientific papers than any country in Latin America, except for Brazil.



FAPESP: SUPPORT FOR RESEARCH IN SÃO PAULO

São Paulo Research Foundation (FAPESP) promotes scientific research in the State of São Paulo, Brazil. Through a robust program of fellowships and research grants it supports fundamental and applied research.

Created in 1962, the foundation is entitled by the State Constitution to 1 per cent of the tax revenues of the state of São Paulo. FAPESP has a sizable endowment and has already supported, over these 48 years, 100,000 fellowships and 90,000 research awards.

In 2010 FAPESP will invest US\$ 415 million in fellowships and research grants. The success rate for proposals in the fellowship programs ranges from 40 per cent to 63 per cent. In the grants programs the proposal success rate ranges from 40 per cent to 60 per cent, depending on the particular type of grant.

OPPORTUNITIES AND CHALLENGES

One of FAPESP's goals is the broadening and diversification of the research system in the state of São Paulo, strengthening the existing centers of excellence, by supporting their research, and stimulating the creation of new centers or research groups tackling new lines of activity. This is achieved mainly by funding Young Researchers Awards, the Biota-FAPESP Program, the FAPESP Research Program on Bioenergy (BIOEN), The FAPESP Research Program on Global Climate Change (RPGCC) the RIDC (Research, Innovation and Dissemination Centers) Program and the Thematic Projects.

All of these have in their teams, in addition to experienced scientists, young researchers as post-doctoral fellows, from Brazil and from abroad. FAPESP supports more than one thousand post-doctoral fellowships.





RESULTS OF GREAT IMPACT

When the research program for Thematic Projects was created, in 1990, FAPESP's objective was to provide a qualitative leap in Brazilian scientific research and meet the state of São Paulo's own particular demands for development. Since then, 1,200 projects in all fields of knowledge have been selected and supported. Selection is through a stringent peer reviewing process, using multiple reviewers for each proposal.

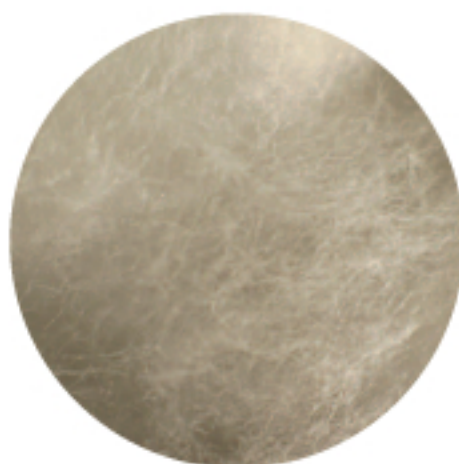
Thematic Projects are characterized by the breadth of their research and the boldness of their objectives. They are supported for five years (as opposed to two years for a regular research grant) and are lead by teams of experienced researchers.

Thematic Projects are funded, on the average, with 450 thousand dollars, plus fellowships. The salaries for the investigators and staff are not included in this amount since in Brazil they are paid by their universities. Each project is lead by 3 PI's and involves several undergraduate and graduate students.

Thematic Projects create opportunities for scientists in São Paulo to advance knowledge by creating internationally competitive science, while, simultaneously, educating a new generation of researchers.

CONTACT FAPESP

Contact FAPESP (www.oportunidades.fapesp.br/en) or a coordinator from the Thematic Project which interests you and see how to obtain a post-doctoral internship.





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GENETIC ANALYSIS OF *THEOBROMA CACAO* DEFENSE RESPONSES TO *Moniliophthora perniciosa*, CAUSAL AGENT OF THE WITCHES' BROOM DISEASE

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Figure 1. Witches' broom symptoms in 'Micro-Tom' tomato inoculated with biotype-S isolate. A. Comparison between control (left) and inoculated plant (right). B-D. Various strong symptoms of infection, including swollen and branched shoots, and rotten fruits with thick pedicels. Unpublished results.

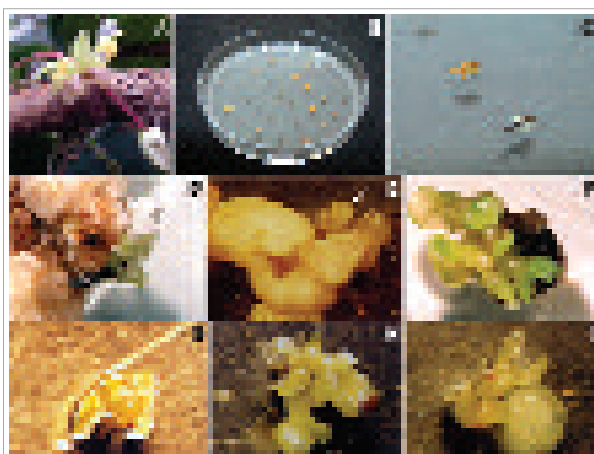


Figure 2. Various morphological steps to obtain somatic embryogenesis of *Theobroma cacao* from floral parts (petals and staminoids). Unpublished results.

Witches' broom, caused by the basidiomycete *Moniliophthora perniciosa* is an important disease of cacao (*Theobroma cacao* L.). The characterized sources of resistance contain distinct associated genomic regions and display different kinetics of defense gene expression. To dissect the defense response using resistant or susceptible cacao genotypes inoculated with isolates from various pathogen biotypes will be evaluated by DNA sequencing of interaction libraries or by amplification using conserved sequence regions. The pattern of gene expression and single nucleotide polymorphism (SNPs) in individuals from a segregating population for resistance will be used to locate these genes associated with defense in relation to known quantitative trait loci. The promoter region of genes induced by infection will be characterized and functionally analyzed, after optimization of cacao genetic transformation. The tomato 'Micro-Tom' cultivar, a genetic model susceptible to the *M. perniciosa* biotype-S will be employed to investigate the role of hormones in pathogenesis using available mutants, and also for functional analysis of candidate genes associated with defense by transgenesis. *M. perniciosa* isolates from the Brazilian Amazon will be classified using molecular markers, including microsatellites. These isolates will also be characterized accordingly to aggressiveness to differential cacao genotypes, and their putative virulence and/or pathogenicity genes will be analyzed for polymorphism and *in vitro* expression under stress condition. Fungal cell wall proteins already identified will be expressed heterologously and their effects on defense response activation will be evaluated. The project intends to demonstrate that the defense response of *T. cacao* to *M. perniciosa* is regulated by the jasmonate/ ethylene pathway, triggered by LRR receptor-like kinases with some of the investigated genes co-localized with identified resistance QTLs.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The analysis of differential expression of cacao genes, associated with pathogen perception, signaling and defense between resistant and susceptible plants from a segregating population identified few candidates with greater expression in resistant individuals. Genes encoding for flavonoid biosynthesis enzymes involved with phytoalexin production were analyzed and one potential candidate gene appeared as differentially expressed between resistant and susceptible individuals of segregating population. The same genes are targets to contain single nucleotide polymorphisms (SNPs), which will allow their genetic mapping, compared to previously established quantitative resistance loci (QTLs). The promoter regions of the genes associated with pathogen perception, signaling and defense are being cloned to investigate regulatory motifs, and to develop witches broom inducible promoters. The promoter of the E3 ubiquitin ligase SCF complex subunit/SKP1 gene contains motifs associated with response to hormones and biotic stress, and it is being prepared to be tested, driving a reporter gene in tomato cv Micro-Tom. Two putative pathogenicity genes from *M. perniciosa* have been identified, and their complete sequence have been obtained. No viable *E. coli* recombinant cells expressing these genes heterologously were recovered.

Cacao, a woody tropical perennial species cannot be considered an easy model system to investigate host-pathogen interactions. Thus, the first challenge of this project was to develop a system to investigate the pathogenesis of *Moniliophthora perniciosa* using the Micro-Tom model. We were able to identify and collect an isolate from *Solanum lycocarpum*, which produces spores under controlled conditions. An inoculation method was successfully adapted, and the isolate shows to be highly virulent to Micro-Tom. Currently, we are screening a series of tomato mutants for synthesis and perception of hormones introgressed into a Micro-Tom background.

An efficient Micro-Tom transformation system was developed being an enabling tool to test our hypothesis concerning the role of hormonal imbalance in *M. perniciosa* pathogenesis. For instance, the involvement of auxin and/or cytokinin ratio in witches' broom pathogenesis and symptom development is currently being addressed by inoculating auxin and cytokinin mutants (*dgt* and *brt*), as well as a transgenic line over-expressing a cytokinin oxidase gene. Similarly, somatic embryogenesis has been obtained from various cacao genotypes, which are able to produce plantlets. This protocol is now being tested to generate transgenic cacao plants.

MAIN PUBLICATIONS

Pino-Nunes LE, Figueira A, Tulmann Neto A, Zsögön A, Piotto FA, Silva JA, Bernardi WF, Peres LEP. 2009. Induced Mutagenesis and Natural Genetic Variation in Tomato 'Micro-Tom'. *Acta Horticulturae* **821**: 63-71.

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MOLECULAR EPIDEMIOLOGY AND INTEGRATED MANAGEMENT OF HUANGLONGBING (*ASIATICUS* AND *AMERICANUS*) IN THE STATE OF SÃO PAULO

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Liberibacter-like particles in sieve tube on Citrus spp. in São Paulo, Brazil. (photo by Francisco A.O. Tanaka)

This research project has ten subprojects. Its joint analysis will allow a better understanding of the structure and behavior of the Huanglongbing pathosystem in São Paulo, a *sine qua non* condition for the definition of a more rational disease management. The proposed subprojects are: (1) development, evaluation and application of real time PCR for diagnosis and detection of *Ca. Liberibacter asiaticus* and *Ca. L. americanus* in plants and insect vectors and in the study of the vector (*Diaphorina citri*) infectivity; (2) spatial and temporal molecular epidemiology of HLB (*asiaticus* and *americanus*) in greenhouse conditions considering two different inoculum sources; (3) spatial and temporal analysis of HLB epidemics in commercial orchards; (4) determination of acquisition and inoculation periods of *Ca. Liberibacter americanus* and *Ca. L. asiaticus* for *Diaphorina citri*; (5) determination of incubation and latent periods of HLB (*asiaticus* and *americanus*) after transmission by *Diaphorina citri*; (6) effect of temperature and geographic region on the progress of HLB (*asiaticus* and *americanus*); (7) geographic distribution of *Ca. Liberibacter americanus* and *Ca. L. asiaticus* in the São Paulo State; (8) progress of HLB colonization (*asiaticus* and *americanus*) in different citrus varieties with different ages; (9) determination of natural infectivity of the vector (*Diaphorina citri*) in orchards with different HLB (*asiaticus* and *americanus*) incidences; (10) strategies of HLB control in commercial orchards based on more favorable times for elimination of symptomatic plants and vector control. The project core is the HLB epidemiology, always considering both, *Ca. L. americanus* and *Ca. L. asiaticus*. All subprojects will be assessed through visual inspection and molecular methods. These methods will be applied for the first time in the epidemiological study of HLB. The final objective is to control the disease in a more rational way.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Assessments of diseased trees by visual symptoms were made in 36 groves from 8 farms in the Central citrus region of São Paulo State. 124 HLB spatial maps (varying from 0.1 to 34.8% of disease incidence) were analysed, considering squares sizing 2x2, 4x4, 6x6 and 8x8 trees, by the binomial index of dispersion and the binary form of Taylor's power law. The dispersion binomial index for various squares sizes suggested aggregation of HLB-symptomatic trees for about 40% of the plots. The relationship between log of observed variance and log of binomial variance was highly significant for the four squares sizes (R^2 of 0.99, 0.97, 0.93, and 0.88 for squares sizing 2x2, 4x4, 6x6 and 8x8 trees, respectively). Estimated parameters of the binary form of Taylor's power law provided an overall measure of aggregation of HLB-symptomatic trees for all square sizes tested. All estimates of a and b values were statistically different from 1, which indicated a general and significant symptomatic plants pattern aggregation for all squares sizes tested. B values were 1.03, 1.05, 1.10, and 1.09 for squares sizes of 2x2, 4x4, 6x6 and 8x8 trees, respectively. Log a values were 0.10, 0.24, 0.45 and 0.45 for squares sizes of 2x2, 4x4, 6x6 and 8x8 trees, respectively. The degree of aggregation was positively related to disease incidence.

An evaluation of control methods was also carried out. A total of 716,476 citrus plants (*Citrus sinensis*), from five to ten years old, distributed in 357 blocks (mean of 2006.9 trees per block) were submitted to different number of insecticide sprays (3 to 12) during three growing seasons. Insecticide sprays were aimed to control citrus huanglongbing (HLB) throughout the control of its vector, *Diaphorina citri*. Eradication of symptomatic trees was carried out in the whole area 4 to 8 times per growing season. Incidences of HLB in all blocks ranged from 0.0 to 8.35 % of symptomatic trees. The relationships between the number of eradicated plants and the number of insecticide sprays were investigated considering (i) the three growing season, (ii) the grouping blocks according to classes of initial disease incidence, and (iii) eradicated plants in the last season (to minimize the influence of a long latent period) and the total number of insecticide sprays in all seasons. We have not found any significant negative relationship between the number of sprays and HLB incidence. We suggest that, in the farm conditions, the low incidence of HLB was mainly due to eradication of symptomatic trees than to insecticide sprays.

MAIN PUBLICATIONS

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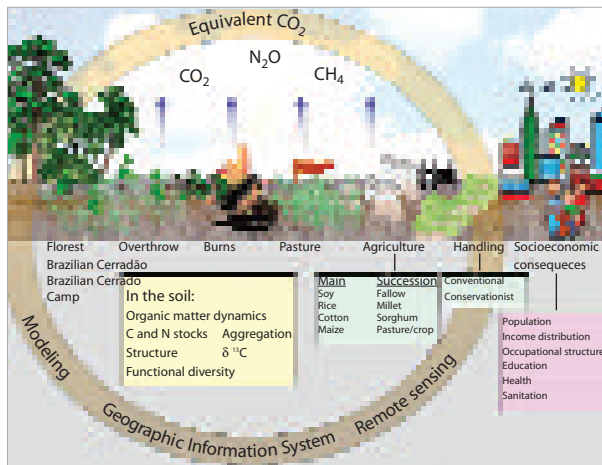
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ENVIRONMENTAL IMPACT OF AGRICULTURAL EXPANSION IN SOUTHWEST AMAZONIA

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The increasing call for grains, fibers and meat for exportation is significantly shifting the Brazilian agricultural scenario. Pristine areas are being occupied with pasture and agriculture. Older pastures are being converted to crop lands, while these are submitted to more intensive and technologically advanced management practices. The first two uses and land-use changes are occurring in high scales and very quickly in the Amazonian legal area, and particularly in the southwest, embracing Rondônia and Mato Grosso. This agricultural frontier is, probably, the world most extensive, with more than 1 million km² in these states. Land-use changes during the last 30 years are certainly causing important global environmental impacts, mainly in what concerns atmospheric warming. This research assumes that the agricultural expansion in the southwest Amazonia is causing important environmental impact by reinforcing greenhouse gas (GHG) emission to the atmosphere during deforestation and soil degradation (mainly of the soil organic matter), although important social and economic regional benefits are, at least temporarily, achieved. The main objective is to evaluate the environmental impact due to land use and land-use change in the southwest Amazonia during the last 30 years, and preview by modeling the consequences of increased GHG (CO₂, CH₄ and N₂O) emissions, in terms of CO₂eq. Concerning soil degradation, we will evaluate the effect on soil organic matter (SOM), which is the natural source of GHG. The estimated GHG emission during the last 30 years (more intensive anthropic activity) will be done for the total area of Rondônia and Mato Grosso states. The obtained results will be associated to others from the literature and used to model SOM dynamic and GHG emissions according to actual, retrospective and prospective scenarios. The product will constitute an important environmental, social and economic data base, which will be available to the community. Results will be important tools for decision makers interested in public policies to mitigate global warming and to maintain the regional sustainable development.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Deforestation and consequent land use for agriculture and husbandry are the main activities that cause greenhouse gas emissions in the Brazilian territory. In the last 30 years these practices have been more intense at the Amazon deforestation arch, particularly in the States of Rondônia and Mato Grosso. Presently, the region represents the largest area of agricultural expansion in the world; therefore, it is necessary to better assess its greenhouse gas emissions. In order to evaluate those emissions, it is initially necessary to estimate carbon stocks in soils and vegetations, previously to human intervention. The main objective of the present research was to estimate soil carbon stocks for Rondônia and Mato Grosso states and then assist in the future calculations of greenhouse gas emissions due to land use changes. The present estimates were performed by developing the following tasks: constitution of a georeferenced data base with information compiled from the literature, estimate soil bulk density for profiles in which this information does not exist, standardization of soil layers through vertical adjustment of soil profile depth and finally, calculation of soil carbon stocks. The surface of Rondônia and Mato Grosso states was divided in 11 ecoregions generated by overlying thematic maps of soils, climate, native vegetation, topography and others through a Geographic Information System. This division in ecoregions is a recommendation from the guideline produced by the Intergovernmental Panel on Climate Changes that brings the official procedures to estimate the greenhouse gas emission for national scales. The soil carbon stock estimates were done for each one of the 15 soil groups in each of the 11 ecoregions. The mean soil carbon stocks (expressed in kgCm^{-2}) for each ecoregion presented the following values: Alto Xingu (8.7); Parana Sedimentary Basin (9.6); Chapada dos Parecis (12.3); Depressão Araguaia (6.7); Depressão Cuiabá-Paranatinga and Serrana region (10.4); Depressão Guaporé (15.2); Northeast of Mato Grosso (11.2); North of Mato Grosso (9.8); North of Rondônia (8.7); Pantanal (7.5) and Central Rondônia (10.4). The integrated soil carbon stock calculations of each one of the 11 ecoregions that cover $1.128.000 \text{ km}^2$ showing values of $5.7 \pm 0.7 \text{ Pg C}$ and $10.4 \pm 1.3 \text{ Pg C}$, for the 0-30 cm and 0-100 cm soil layers, respectively. Considering only the 0-100 cm soil layer it is possible to infer that the total of 10.4 Pg C represents 0.7% of the total carbon stored in the world soils (1576 Pg C). This is a meaningful value since it is concentrated in an area of $1.12 \text{ million km}^2$, i.e., 0.008% of the global soil surface (about $135.21 \text{ million km}^2$). The information generated in the present research is essential for the thematic project that has been carried on by the Environmental Biogeochemical Laboratory/Cena/USP, related to the CO_2 emissions to the atmosphere, generated from soil organic matter decomposition due to deforestation and agricultural use of soils from Rondônia and Mato Grosso states.

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MANAGEMENT OF CITRUS LEPROSIS

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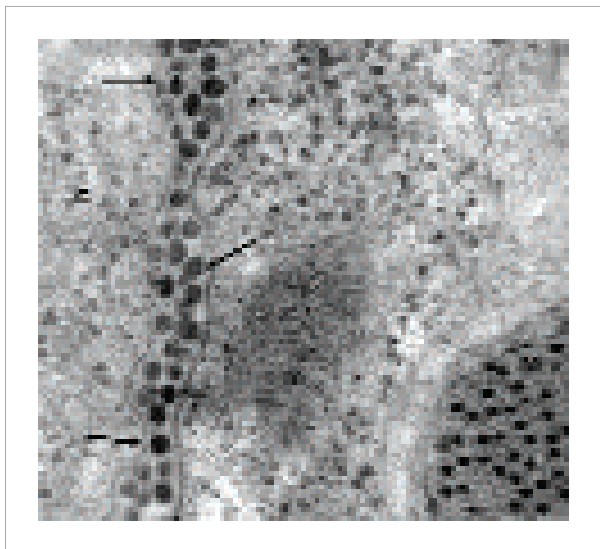


Figure 1. Transmission electron micrograph of particles of CiLV-C (arrows) between membranes of caeca epithelial (C) and interstitial (I) cells near the prosomal glands of a viruliferous *Brevipalpus phoenicis* adult female. M- muscle cell; m- mitochondrion

Leprosis is considered one of the main citrus diseases in Brazil. It is caused by a virus transmitted by the polyphagous and cosmopolitan tenuipalpid mite *Brevipalpus sp.*

This mite has been the main target for the control of leprosis, essentially by large scale use of chemical acaricides, resulting in high financial and environmental cost, which could be much smaller with a rational disease management in the field. In the last ten years, impressive progresses have been made on the understanding of the citrus leprosis pathosystem including molecular tools for virus detection, viral genome, biology of the mite vector, geographic distribution of the disease, genetic resistance, host range and natural predation, etc. This project intends to use the available information to implant new integrated and cost effective leprosis management concentrating the control in the prevention of the virus entry and localized control of the focus, playing with all parts of the pathosystem chain. These comprehensive, multidisciplinary and multinstitutional works include evaluation of the lesion type as source of inoculum for the mite, the efficiency of different pruning type associated with the use of acaricides and its cost/benefit, the mite population in organic orchards, the spatial distribution of the mites in the field, the role of alternative hosts on the epidemiology, the resistance of different citrus types, of the virus/vector relationship, and the role of predator mites on the population of *Brevipalpus* mites.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Some of the subprojects have already produced relevant informations. The effects of pruning combined with chemical control, started in 2003, clearly have indicated that a light pruning combined with acaricides (spirodiclofen and cyhexatin) is more cost effective. Turk's hat (*Malvaviscus arboreus*), among the plants used as wind break in citrus orchard, is susceptible to *Citrus leprosis virus C* (CiLV-C) and excellent host for *B. phoenicis* mites under field conditions. This plant may play a role on the epidemiology of this virus. Transmission electron microscopy led to the detection of CiLV-C particles in the mite tissues, confirmed by *in situ* immunolabeling. The pattern viral distribution within the mite body is suggestive of a circulative rather than circulative/propagative type of virus/vector relationship. The use of bean (*Phaseolus vulgaris*) as indicator plant results in necrotic local lesions five days after infestation by mites viruliferous for CiLV-C. This permitted the establishment of some parameters as the virus acquisition, inoculation feeding period and efficiency of viral acquisition by the vector. Experimental infection was

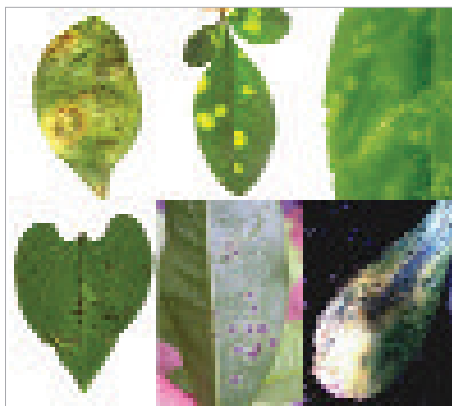


Figure 2. Localized leaf lesions caused by CiLV-C in natural (A-Valencia sweet orange; B-Swinglea glutinosa) and experimental (C-Hibiscus rosa sinensis; D-Phaseolus vulgaris; E-Glycosimis pentaphyla; F-Arabidopsis thaliana) infection

study on the variability of the CiLV-C from varied origins (Argentina, Bolivia, Colombia, Panama, Costa Rica, Mexico and several parts of Brazil) have indicated that genetic variability of this virus is rather low.

achieved also on *Arabidopsis thaliana*, which will open opportunities to follow the gene activation and metabolic changes during the infection process. A retrospective study, based on published images and samples kept in herbarium, strongly suggests that the citrus leprosis that occurred in Florida might have been of the nuclear (CiLV-N) rather than cytoplasmic type. A

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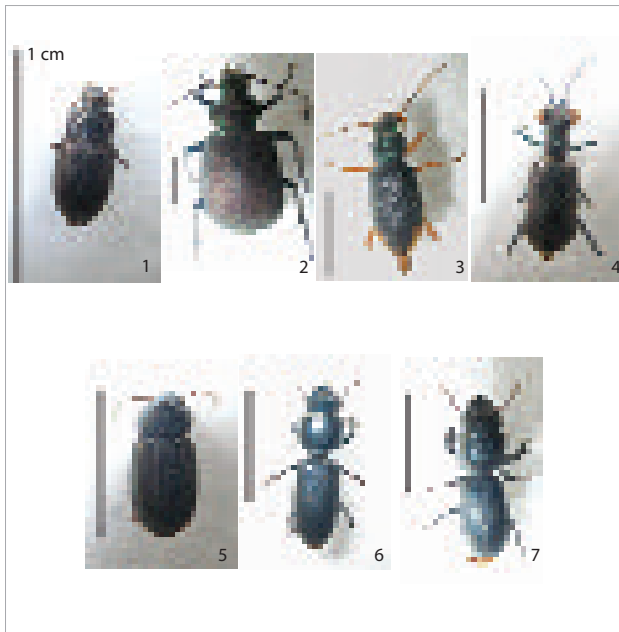
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DISTRIBUTION OF CARABIDAE AND STAPHYLINIDAE (COLEOPTERA) IN FOREST FRAGMENTS, AND CROPS, AND POTENTIAL OF PREDATION IN THE NORTHEASTERN REGION OF SÃO PAULO STATE

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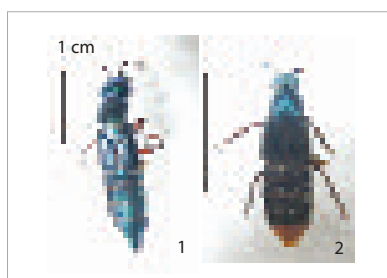


Carabidae dominant species

The families Carabidae and Staphylinidae (Coleoptera) include important predator species associated to the soil of agro ecosystems. Due their predatory nature, those insects have potential to reduce populations of agricultural pests. The diversity and the abundance of insect predators are related with the kind of vegetation at the crop adjacencies. The presence of alternative habitats has been considered important component in agro ecosystems because they can favor the occurrence of high density of carabids and staphylinids. The project is evaluating Carabidae and Staphylinidae communities associated with soil in five areas constituted each one of a forest fragment adjacent to: (i) soybean/corn field under no-tillage system (Jaboticabal and Guaíra municipalities); (ii) soybean/corn field under conventional tillage system (Guaíra municipality) and (iii) orange orchard (Gavião Peixoto and Descalvado municipalities). This study objectives are: (a) to determine whether the forest fragments are supporting a species assemblage different from that of the surrounding crops; (b) the extent the species, present in the forest fragment, crop and habitats boundary; (c) to analyze the populational fluctuation of dominant species; (d) to evaluate the potential of carabids and staphylinids as predators of fourth instar larvae of *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Noctuidae), and (e) to evaluate the effects of arboreous and herbaceous plants phenology on the occurrence of carabids and staphylinids.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A total from 32 to 53 Carabidae species and 18-41 Staphylinidae species were observed in the areas. Among dominant carabid species, the following species were dominant in at least two areas: *Abaris basistriatus*, *Selenophorus seriatoporus*, *Megacephala brasiliensis*, *Odontochila nodicornis*, *O. cupricollis*, *Scarites sp.3*, *Selenophorus alternans*, *Calosoma granulatum* and the staphylinids species *Eulissus chalybaeus* and *Xenopygus sp.2*. The highest species diversity was observed in areas with soybean under no-tillage system or orange orchard, the opposite occurred in the soybean area under conventional tillage. Those results have indicated that communities of carabids and staphylinids were best structured and contained lower number of dominant



Staphylinidae dominant species

species in cultivated areas whose soil was not disturbed by cultivation. In most of areas, species richness was high at the edge of the forest fragment with the cultures, while the distribution of species inside the cultures was always superior than the ones in the fragments. Most of species showed preference for the culture and the edge of the forest fragment with the cultures. The carabids species that showed high capacity to consume *A. gemmatalis* larvae were: *A. basistriatus*, *Notiobia cupripennis*, *Selenophorus discopunctatus*, *S. seriatoporus*, *C. granulatum*, *M. brasiliensis*, *Megacephala sp.*, species of the genera *Galerita* and *Scarites* and the estaphylinids *E. chalybaeus* and *Xenopygus sp.2*. Those species have an enormous potential to be included in studies aiming the development of conservation biological control programs, which promote sustainable agro ecosystems. In general, carabid number tended to increase starting from the sprout phase to the green fruit phase of the arboreal plants, occurring the opposite with Staphylinidae species. Usually, the populations of carabids *Notiobia amethytinus*, *Helluomorphoides squiresi*, *Sphalera plaumani*, *Tetragonoderus laevigatus* and most of *Selenophorus* species increased in number during the reproductive phases of the herbaceous plants. Those ground beetles have shown potential as herbaceous plants seeds predators being important to weeds biological control programs.

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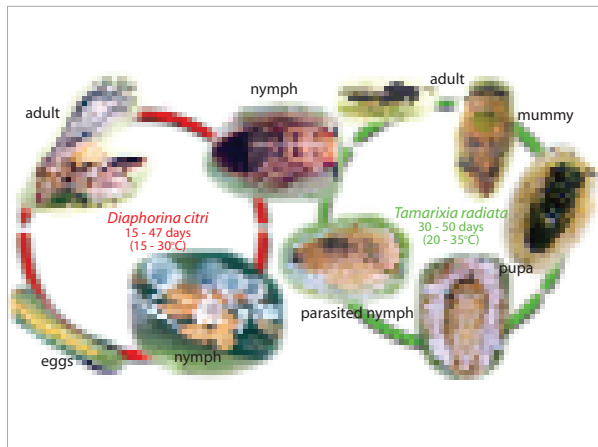
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BIOECOLOGY AND ESTABLISHMENT OF CONTROL STRATEGIES FOR *Diaphorina citri* KUWAYAMA, 1908 (HEMIPTERA: PSYLLIDAE), CITRUS GREENING (HUANGLONGBING) CAUSING AGENT

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Brazilian citriculture is responsible for one third of the world's orange production with São Paulo State accounting for 80% of the nation's yield. The entire production can be compromised by several phytosanitary problems, including native and exotic pests. The bacteria *Candidatus Liberibacter asiaticus* and *Ca. L. africanus* are the main citrus disease-causing agents worldwide. In Brazil, a new species, *Ca. L. americanus* was detected in association with an unidentified phytoplasma in citrus greening symptomatic plants in São Paulo State. In Brazil, greening transmission has been associated with the psyllid *Diaphorina citri*. Although *Diaphorina citri* has been reported since 1942, few studies have been conducted until 2004 since this vector has been considered a secondary pest. There is a great concern on São Paulo citriculture being decimated by greening disease, similarly to what happened in some Asian countries, if vector, disease control and management measures are not quickly developed. The objectives of this project are: to study insect vector, including its taxonomy; to determine regions where the pest could be of major concern; to study vector x bacterium interactions and their population dynamics, as well as the population dynamics of native natural enemies, and to establish control strategies, including alternative (biological, microbial, plant volatile compounds, plant resistance) as well as chemical methods, especially the use of selective products. These studies will allow the development of a series of measurements (a technological package) that can be used to manage the greening insect pest in citrus groves in São Paulo State.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Murraya paniculata and *Citrus limonia* are the most suitable hosts for rearing *D. citri*. The pest's temperature requirements and biological studies have shown that *D. citri* can withstand higher temperatures in Brazil than its counterpart in the USA. However, there has been an indication that insect development is impaired above 30°C. It has been also observed that low RH values affects the nymphal stage of *D. citri*. Based on genotypic variation analysis of the mitochondrial cytochrome oxidase I gene (Col) from *D. citri* populations, we concluded that distinct biotypes do occur. All *D. citri* symbionts, identified at the molecular level, are vertically transmitted. Comparative analyses between aposymbiont and associated lines pointed out that some of those symbionts play a role in the process of development and reproduction of *D. citri*, indicating their potential for the development of alternative control methods against the pest, by breaking down *D. citri* – symbiont interactions. Feeding behavior studies using the "Electrical Penetration Graph" technique and additional evaluations, demonstrating transmission of the bacterium *Candidatus Liberibacter spp.* for several weeks after acquisition, indicated a persistent and propagative relationship between the bacteria and *D. citri*.

The population dynamics of *D. citri* is similar in the various areas studied, although population levels have changed between regions. In 2005 - 2006 more *D. citri* individuals were captured in the summer (followed by fall and spring, with smaller populations in winter). Population peaks have occurred during the spring, but in some years peaks have occurred in the winter, possibly due to more frequent chemical control applications in the spring and summer periods, when vegetative growth is more intense. At high population densities, a clumped distribution pattern can be observed, whereas a random distribution occurs at low populations.

By using a Geographic Information System, we were able to obtain monthly distribution maps for the development of *D. citri* and its parasitoid *T. radiata*. Around 13 to 20 *D. citri* generations, and 27 to 35 *T. radiata* generations may occur throughout the year in the Northeast citrus-growing regions of São Paulo State. In the Southeast and Southwest regions, however, the number of generations ranges from 6 to 13 and from 19 to 27 for the pest and parasitoid, respectively. *T. radiata* was released in citrus-growing areas of Araras, Cordeirópolis, and Limeira cities, where the parasitism ranges from 40% to 80%. This results have shown that this parasitoid is quite promising. A promising isolate of the pathogen *Beauveria bassiana* (Esalq PL63), affecting *D. citri* nymph's physiology during its metamorphosis period, as well as the insect's immune system, has also been selected for *D. citri* control.

The repellent effect of guava-derived volatiles (*Psidium guajava*) has been demonstrated for *D. citri* adults. The identification and synthesis of such compounds are currently underway. Resistance management monitoring revealed significant differences in the susceptibility of *D. citri* populations to neonicotinoids and high toxicity of those products to *T. radiata*. We expected that these results and continued research will allow the generation of a technological package in order to control this important citrus disease.

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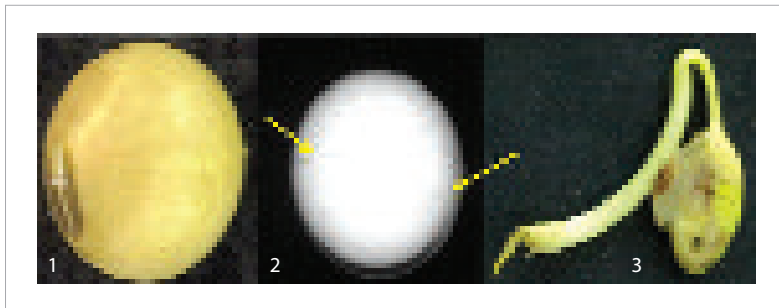
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SEED IMAGING ANALYSIS IN SEED TECHNOLOGY RESEARCH

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Internal splits caused by mechanical injuries that cannot be detected by naked eye are detected by x-ray analysis. Lesions usually promote decreases in seed performance (Flor, 2003).

1. External image; 2. Radiography; 3. Abnormal Seedling

Seed testing is an important component of seed production systems. It is a dynamic activity as shown by the improvement of the available procedures and the development of new approaches and modern methodology. These tools comprise relatively new techniques such as seed and seedling imaging analysis that have indicated consistent potential to be used in basic and applied research.

This proposal was designed with the collaboration of recognized seed technology researchers and promising younger professionals that have demonstrated

sufficient qualification to guarantee the success of future actions in seed research. The members of this group represent important national and international institutions, comprising universities and research institutes located in São Paulo and Minas Gerais states, Brasília and USA.

The central theme is Seed Imaging Analysis and the proposal includes different approaches such as:

- a) evaluation of seed morphology by X-ray analysis and its possible association to performance as influenced by environment and seed physiological potential;
- b) use of an automated computer imaging system (SVIS®) to assess seed vigor; studies on seed maturation and seed priming in association of imaging analysis.
- c) association between physical and physiological characteristics of seeds identified by imaging analysis;
- d) identification of developmental stages during seed maturation and its relation to seed performance;
- e) evaluation of mechanical injuries and effects on seed physiological potential;
- f) identification of the relation between physical and physiological characteristics of seed embryo and the effects on seed performance;
- g) evaluation of the damage caused by insects, pathogens and adverse environmental conditions;
- h) the use of seed imaging analysis as a support on Molecular Biology research.

Different grain crops, fruits, and vegetable are included in the proposal such as maize, soybean, field bean, papaya, cucurbits, okra, bell pepper, castor bean and also some and Brazilian native species.

Results will produce useful information to seed companies mainly to identify factors that affect seed performance, improvement of some available procedures to determine seed quality and establish consistent diagnosis necessary to solve problems.

Depending on the information provided by initial results, the inclusion of new approaches and species will be considered, but all possibilities of distortion to the main focus will be avoided.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

X-ray imaging tests is an effective tool to identify the seed structure and the action of factors that negatively affect seed performance such as immediate and latent mechanical injury and other disturbances that may occur during and after seed maturity. It is a non-destructive test applicable to evaluate seed structure of several species.

The X-ray results can be associated with those from germination and vigor tests, providing consistent information to diagnose seed quality and to allow efficient procedures during different phases of seed production, processing and testing.

The software "Seed Vigor Imaging System" (SVIS®) provides important advantages such as rapid measurement of seed physiological potential. This attribute clearly demonstrate that SVIS is an enhancement over traditional vigor tests for determining seed physiological quality.

The software "Tomato Analyzer" can be successfully used to evaluate the extent of embryo growth within non-endospermic seed species and is a promising alternative for the assessment of seed physiological potential by its indirect association with germination and vigor.

The association of X-rays and automated computer imaging system analysis comprises a modern and efficient resource to evaluate different aspects of seed quality. It is a significant improvement to be adopted in seed quality control programs.

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EPIDEMIOLOGY AND MANAGEMENT OF POSTBLOOM FRUIT DROP OF CITRUS IN NEW PLANTING AREAS IN SÃO PAULO STATE

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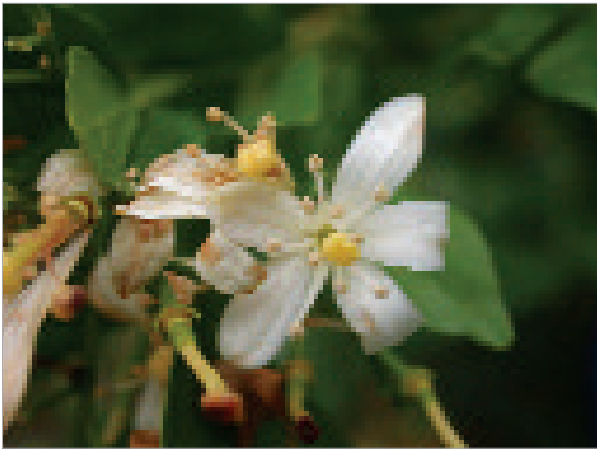


Figure 1. Flowers of *Murraya paniculata* with typical lesions, caused by *Colletotrichum acutatum* isolated from orange flowers (Spósito, M.B et al., unpublished)

Citrus crop in São Paulo State is migrating from North and Central regions to new areas in the Southeast. The main reasons for the migration are lower values of land in Southeast region, compared to traditional planting areas, and lower costs of production. Weather in this region is favorable to the occurrence of post bloom fruit drop, caused by *Colletotrichum acutatum*. This disease can cause the drop of up to 100% of young fruit. For this reason, its control is absolutely necessary in orchards where it occurs frequently. Control of post bloom fruit drop is made by preventive fungicide spraying during bloom. Costs of chemical treatment to disease control are estimated in US\$ 553/ha. Preventive control of the disease exclusively based on the phenological host stage commonly causes excessive fungicide spraying with adverse effects to the environment. The continuous application of the same fungicide in an orchard frequently selects resistant pathogen strains. In this case, the fungicide becomes inoperative. In order to improve disease management and to reduce the cost of fungicide spraying, epidemiological behavior of the pathosystem must be known. There is very few information available on the epidemiology of post bloom citrus fruit drop. It is still unknown the way and the period of inoculum survival between blooming, the environmental conditions favorable to infection and the inoculum variability inside the orchards. It has not been determined as well, the pattern and distances of inoculum dispersal, influence of weather variables in the space-temporal disease progress. Considering the lack of information, it is not surprising that forecast systems developed in Florida, to control this disease, have not been adopted by São Paulo citrus growers. The main objectives of this project are: (i) to characterize the spatial distribution of the inoculum and the disease progress in the orchards in the Southeast of São Paulo State; (ii) to understand different phases of disease cycle, particularly the mechanisms of inoculum survival, infection and colonization; (iii) to test different alternatives to the chemical control and to validate/develop disease forecast systems to São Paulo growers.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The most important results in the first year of this project are the description of a new host for the pathogen and a different *Colletotrichum* species causing the disease in citrus. According to our results, *Colletotrichum acutatum* can cause blossom blight (Figure 1) and fruit drop (Figure 2) in orange jasmine (*Murraya paniculata*), an evergreen, herbaceous perennial that belongs to Rutaceae family, as well as *Citrus* spp, which is commonly used as fencing in citrus groves in São Paulo State (Lima, W.G *et al.*, unpublished). In addition, 24 *Colletotrichum* isolates, from a population of 139 isolates from São Paulo State were identified as *C. gloeosporioides*. These isolates were mainly found in limes (*Citrus latifolia*) but when inoculated on orange flowers, they showed the same symptoms (blossom blight and post-bloom fruit drop) as *C. acutatum*. In addition, preliminary results indicate that the pathogen does not survive in soil orchards, but some weeds can play a role in its survival.



Figure 2. Calix of *Murraya paniculata* alter postbloom fruit drop caused by *Colletotrichum acutatum* (Spósito, M.B *et al.*, unpublished)

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FLOWERING AND FRUCTIFICATION IN “VALENCIA” ORANGE: ANATOMICAL, PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ASPECTS

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Orange orchards in São Paulo State show low yield efficiency and annual yield oscillation. Crop management, aiming to stabilize the annual orange yield, depends on the understanding of the interactions among different environmental factors and related physiological processes. As for all dicot plants, orange leaves are carbon providers (source) and, at least at one stage during its development, leaves, roots, branches flowers and fruits become carbon sinks, draining sucrose from the leaves. Leaves and stems are the main organs in plants accumulating photosynthesized carbohydrates, and at a given moment in time, they make it available for other organs acting as sinks. During spring, after flower induction, the orange tree blossoms in São Paulo State followed by a period of intense flowering and young fruit dropping. Photosynthesis and carbohydrate reserves seem to affect flower fertilization, and fruit development. In the summer, the environmental conditions favour the production of energy derived from photosynthesis, and leaves maintain the carbohydrate supply to sustain plant growth and the accumulation of storage products. Fruits do not accumulate starch in the juice sacs but accumulate organic acids, establishing a link between respiratory cycle and sucrose translocation. Almost nothing is known about nitrogen metabolism in the fruits, compared with the information available for nitrogen metabolism in leaves.

The aim of this project is to analyse the biochemical, physiological and molecular aspects related to carbon partitioning in Valencia orange during flowering, fruit setting and maturation. Experiments with photosynthesis, hormones, nitrogen and carbohydrate compounds will be performed from blooming season until fruit maturation. At specific stages, gene expression analyses will be performed with candidate genes using microarrays and *in situ* hybridization techniques.

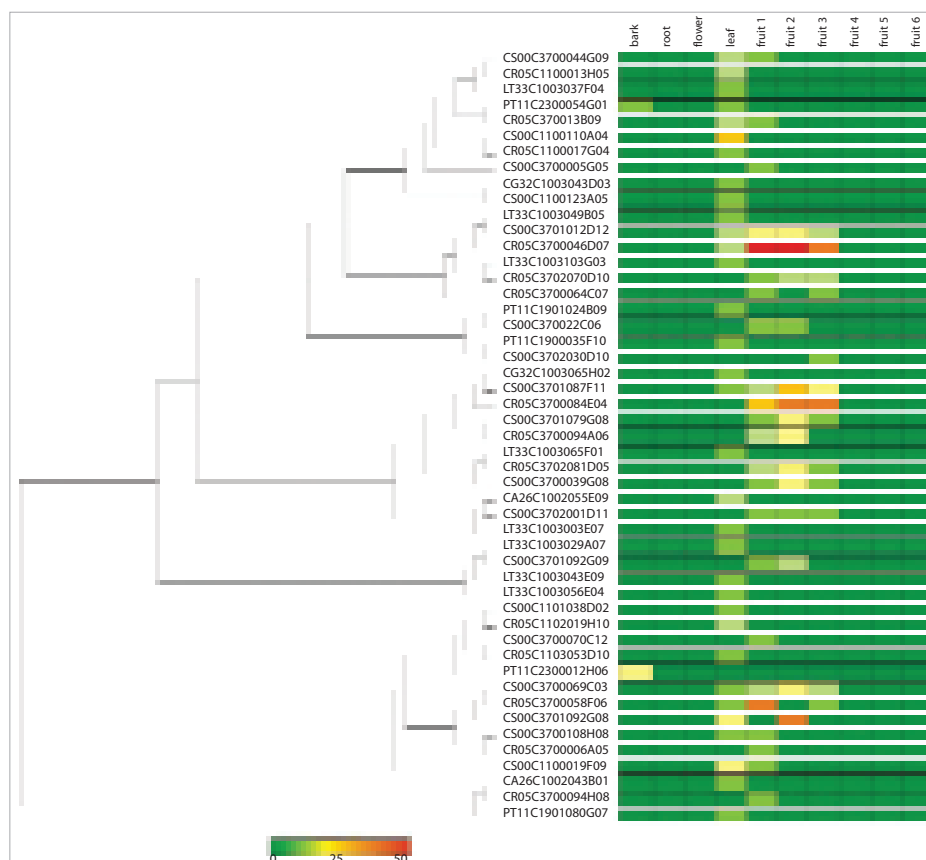


Figure 1. Expression profiles of the 49 *Citrus* putative terpene synthase EST clusters in selected cDNA libraries from the CitEST database. Data represent the relative number of reads from a specific library in each EST cluster after normalization. Each EST cluster is represented by a single row, and each library is represented by a simple column. The cladogram on the left represents the relatedness of all *Citrus* sequences and was build according to their relative genetic distances. CA refers to *Citrus aurantium* sequences; CG refers to *Citrus aurantifolia* sequences; CR refers to *Citrus reticulata* sequences; CS refers to *Citrus sinensis* sequences; LT refers to *Citrus latifolia* sequences; PT refers to *Poncirus trifoliata* sequences

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

To obtain a high production of citrus fruits in 2008, we induced heavy citrus flowering through the removal of fruits produced during the previous season (February/2007). These experimentally-prepared plants were compared with control plants bearing fruits. As expected, the experimental plants showed higher productivity than control plants in 2008. Several physiological and biochemical parameters were measured in the plants (sampling from August to December 2008).

Plant material was collected for microarray studies (based also on data previously obtained using the EST-Citrus databank, <http://biotecnologia.centrodecitricultura.br/>), and also for *in situ* hybridization to study genes related to flower bud differentiation. Other experiments were performed, in greenhouse conditions, to study the relation of water transport and flowering.

The field experiments have shown that the variation in carbon reserves in citrus depends on sink demand and photosynthesis, which may not be synchronized. Thus, under high carbon demand, carbon stored in the roots and stems may be remobilized. Also, this may be influenced by seasonal variation in climate and growth stages.

We observed significant changes in photosynthesis rates throughout the year in citrus plants. The photosynthesis rate, in warm and humid summer days, when intense vegetative and fruit growth occur, is 3 times the observed rate during winter. Our study showed that this might be related to the high and low carbon demand and that it is strongly related to temperature and water status. Sucrose exported from the leaves to the growing tissues clearly stimulates photosynthesis. On the other hand, this export is related to intense growth rates occurring during summer.

The *in situ* hybridization experiments, together with the morpho-anatomical description of samples showed the changes occurring in the lateral buds of branches, associating morphological modifications with gene expression patterns. Plants not bearing fruits have shown 15 days delay in the initiation of the reproductive development, followed of a delay in the expression of the *CsLFY* gene, responsible for the transition of the vegetative meristem to the flower meristem in *Arabidopsis*. Since *LFY* gene is the molecular activator of other floral development genes, it also has shown a delayed expression, as presented by the expression pattern of *CsAP1* and *CsPI*, homologous of *APETALA1* and *PISTILLATA*.



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DIRECT AND RESIDUAL EFFECT OF N AND S FERTILIZATION ON SUGARCANE GROWN IN SUCCESSIVE CYCLES UNDER CONSERVATION SYSTEM

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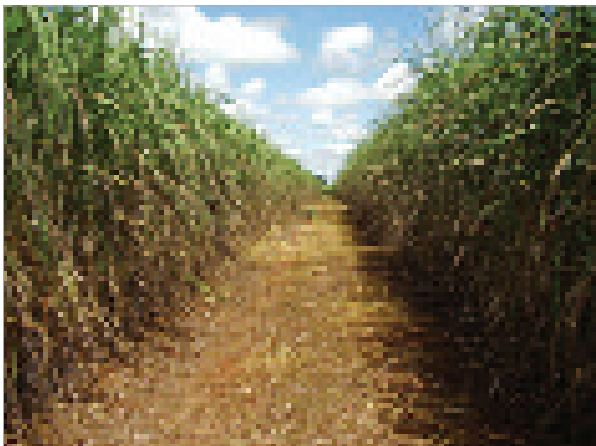


Figure 1. Green cane as a conservation system: sugarcane fields with soil covered with straw

Nitrogen (N) is the most expensive nutrient applied to sugarcane as mineral fertilizer. Its effect, on plant, in general, is related with the effects of sulfur. Besides, N use efficiency by plants is also regulated by crop management and by the extent of the N losses that may take place in the soil-plant system. Current knowledge on yield response of sugarcane to N and S fertilization is mostly based on one cycle experiment either of plant cane or ratoon crop. However, sugarcane is a semi-perennial grass and as such, crop new growth is dependent on energy and nutrient reserves of roots and rhizomes. Therefore, the main objective of this project was to evaluate, across several cycles of cuttings, the yield response of sugarcane grown under conservation system – with no burning of cane straw before harvesting and maintaining little soil disturbance – in relation to a combination of N fertilization applied to the plant cane and to the ratoon cycle. The transformations of fertilizer-N and -S in the soil-plant system were evaluated by ^{15}N and ^{34}S stable isotope techniques in order to establish the relationship between N and S nutrition and cane yield. Three field experiments are carried out in soils of different textural classes. Sugarcane was planted between January and April 2005 and evaluations will be done during four years. Rates of N (0, 40, 80, and 120 kg ha⁻¹) as urea applied to plant cane were combined with rates of N (0, 50, 100, and 150 kg ha⁻¹) as ammonium sulfate (AS) applied to the first ratoon cycle. Micro plots with ^{15}N -labelled urea for the plant cane cycle and with ^{15}N - and ^{34}S -labelled AS for the first ratoon cycle were set up in order to determine the N and S fertilizer use efficiency by sugarcane and the residual effects of the fertilizers on yield of subsequent cycles.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Usually high responses to N fertilization are observed in the first years after implementation of green cane system under the climatic conditions of the São Paulo State. Yield increases of both stalks and sucrose are likely to be obtained with N application. Considering the results of three experiments, the N rates that resulted in the higher economic return were between 40 and 80 kg ha⁻¹ of N, which are lower than those obtained in the ratoon cycles. In eutrophic soil, N fertilization of plant cycle enhanced sugarcane growth and nutrient uptake as well as increased biomass and industrialized stalk yields. However, most of the N accumulated in the cane plant came from other sources, especially the soil stock, because only 12% of the N content of the whole



Figure 2. Sampling of sugar cane plant ("green cane") at harvest to define the fate of ¹⁵N and ³⁴S labeled fertilizers

plant derived from fertilizer. Nitrate leaching below 90-cm depth of N derived from both soil N mineralization and N fertilizer was negligible in the plant cane cycle: 1.1 and 0.02 kg ha⁻¹ N, respectively, indicating that risks of ground water contamination were very low. Intense soil plowing before planting sugarcane promotes mineralization of soil organic N and of

sugarcane crop residues incorporated into the soil, which is a possible explanation for the lower response to N fertilization in the plant cane cycle. The N accumulated in the sugarcane plant was around 200 kg ha⁻¹, of which 45% (85 kg ha⁻¹ N) were in the stalks, 15% (30 kg ha⁻¹ N) were in the dry leaves, 20% (40 kg ha⁻¹ N) were in the plant tops and 6% and 13% respectively were found in roots and rhizomes. The N content of the above-ground part of the plant cane decreased with time: 11 to 13 g/kg dry mass in 5 to 6-month-old plants but only 3.2 to 3.3 g/kg after 14 to 16 months when plants were harvested. In the first ratoon cycle, under green cane management, which resulted in a thick mulch of crop residues of about 15 t ha⁻¹, yield response to N was high and optimum economic return was obtained with around 100 kg ha⁻¹ N. Nitrogen fertilizer applied to the plant cane cycle resulted in a residual effect that caused an increase in stalk yield of the first ratoon crop. However, N fertilizer application to the plant cane cycle did not affect the response to the N added to the first ratoon crop. In the plant cane cycle, sugarcane absorbed around 30% of the N fertilizer applied (¹⁵N urea), whereas in the ratoon cycle, the fertilizer N utilization (¹⁵N AS) was 40%. These figures indicate that most of the N, added as fertilizer, does not end up in the sugarcane plant at the harvest stage.

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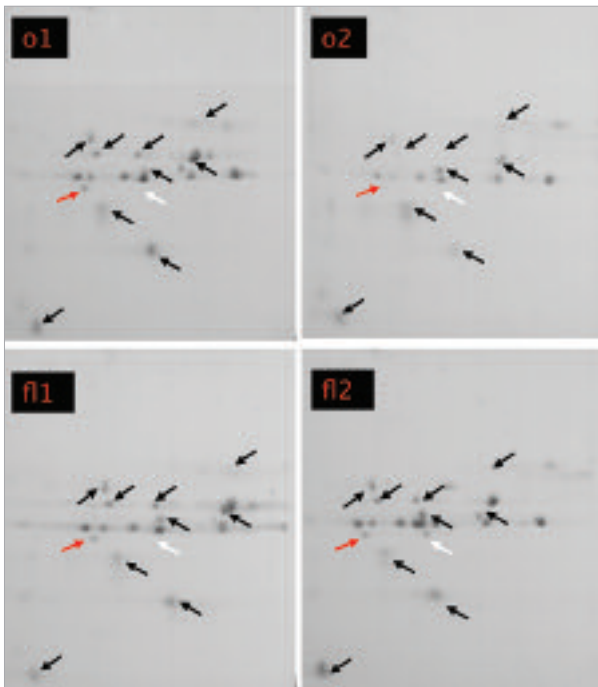
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UNDERSTANDING LYSINE METABOLISM IN CEREAL CROPS

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2D-PAGE of Zein Storage Proteins:
 Red and white arrows indicate examples of a drastically reduced expression of a protein (in the o2 maize mutant) and a newly expressed protein (in the fl2 maize mutant), respectively. Azevedo et al. 2003. European Journal of Biochemistry. **270**: 4898-4908

Hunger and malnutrition are among the most devastating problems affecting a large part of the world's population. Nearly 30% of people in the developing world are suffering from one or more forms of malnutrition. In fact, one billion people worldwide are affected by nutritional deficiencies, 800 million people are chronically undernourished, more than 40% of women in the developing world are underweight and/or anemic, and one third of the world's children are affected by delayed growth and development caused by malnutrition. The tragic consequence of malnutrition includes disability, stunted mental and physical growth and death, affecting all age groups. Protein quality is based on their origin (animal or vegetable), amino acid composition (particularly their relative content of essential amino acids) and their digestibility. Therefore, high quality proteins are those that are easily digested and contain the essential amino acids in quantities that correspond to human requirements. Amino acid and protein requirements are metabolic demands of the organism. The aspartate metabolic pathway is extremely important since aspartate is the precursor of four essential amino acids: lysine, threonine, methionine and isoleucine. The challenge of producing crops with a high-lysine concentration in the seeds appeared to be in sight a few years ago, however, apart from the quality protein maize lines currently commercially available, the release of high-lysine cereal crops is still in its first steps. We are left with the question: Is the production of high-lysine crops still a challenge? The main goal of this project is to better understand the regulation of lysine metabolism in cereal crops. Key enzymes involved in the regulation of the aspartate pathway, the biosynthesis and the accumulation of seed storage proteins of distinct cereal crops were the main focus of our attention. In parallel, we are also investigating nitrogen use efficiency and amino acids metabolism.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have been studying naturally occurring maize (opaque and floury) and sorghum high-lysine lines and quality protein maize (QPM) lines obtained by Brazilian Agricultural Research Corporation (EMBRAPA). The maize storage proteins, albumins, globulins, zeins and glutelins of the maize endosperms from *opaque1*, *opaque2*, *floury1* and *floury2* mutants and their wild-type counterpart, *Oh43+*, were analyzed by two dimensional gel electrophoresis (2D-PAGE, 8-18% gradient gel), which allowed us to detect several specific changes for each mutant. In addition, enzymes involved in lysine, threonine and methionine biosynthesis, and two enzymes involved in lysine degradation, were analyzed indicating that the high-lysine levels in the mutants cannot be solely explained by one particular event, but are due to a combination of modified enzyme feedback regulation and differential distribution of the storage proteins. We were also able to perform relative quantification of gene expression for some of the enzymes in some of the genotypes. For instance, in developing seeds, a *LL-DAP aminotransferase (LL-DAP-AT)* gene was induced in both QPM lines (designated L161o and L161q) at 14 days after pollination and an enhanced expression was observed at 20 days after pollination in L161o. At 24 days after pollination, L161q exhibited up-regulation for both, *LL-DAP-AT* and *threonine synthase (TS)* genes, but *LL-DAP-AT* was three times more expressed than *TS*. In collaboration with EMBRAPA, we have been able to undertake a new strategy in which we have introduced a high-lysine containing protein encoding gene (zeolin) into maize and expressed it specifically in the seed endosperm by using a γ -*kafirin* promoter. Preliminary PCR results of transgenic plants have indicated several positive transformed plants. SDS-PAGE biochemical characterization has indicated that protein is properly expressed in the endosperm affecting the overall protein concentration (70-160% increase in zeins). Further genetic and biochemical characterization will focus on the inheritance of gene, lysine levels in the seeds and agronomical performance of the putative high-lysine maize lines.

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ECOPHYSIOLOGICAL STUDIES OF THE FRESHWATER ANOSTRACA *Dendrocephalus brasiliensis* PESTA, 1921 AND ITS IMPLICATIONS ON ZOOPLANKTONIC AND NEKTONIC COMMUNITIES

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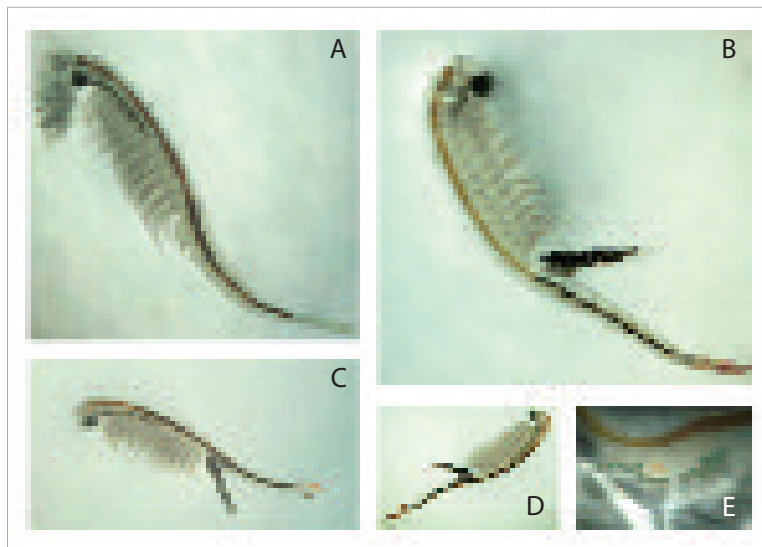


Figure 1. *Dendrocephalus brasiliensis*: A – male; B, C and D – females; E – sac of eggs (Photo by Renata M. dos Santos)

Larval rearing is considered the bottleneck for a sustainable development of aquaculture. Live food availability, particularly zooplankton, is essential for the development of young fishes and crustaceans. Nevertheless, there are serious difficulties to obtain zooplanktonic biomass enough to attend the growing world demand. The native tropical freshwater Anostraca *Dendrocephalus brasiliensis* (Figure 1) has a great potential for utilization in aquaculture due to its size (25mm) and great attractiveness for fishes. So, the present project aims to investigate ecological and physiological aspects of this species, at natural and experimental environments, in order to subsidize the development of technologies to produce resistance eggs and biomass. Its productive potential will be evaluated through studies

of secondary production (ecological and physiological approaches), growth rates, energetic balance, trophic efficiencies, life history characteristics, fecundity, egg viability, number of generations, longevity under different environmental and food conditions, body length and biomass. Studies on fish's food preference and nutritional values of *D. brasiliensis* compared to other zooplanktonic species will be performed, as well as the impact of each one in the production of cultivated tropical fishes. To optimize *D. brasiliensis* cultivation, it will be investigated the influence of microalgae nutritional quality on the animal biochemical composition (proteins, lipids and carbohydrates). Competition, predation and grazing interactions will be also investigated in order to found the real impact of this species on the other freshwater planktonic populations, mainly in São Paulo State environments.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

D. brasiliensis is rearing in laboratory to determine embryonic and postembryonic development time, to characterize the different instars morphology, to produce eggs large scale cultures and to analyze the stomach contents (Figure 2).

Microalgae are a source of natural products that support the growth of zooplankton in aquatic ecosystems. The biochemical composition of these photosynthetic organisms are manipulated through the variation environmental conditions, such as micro and macronutrients. Doing this, higher content of reserve molecules are produced and these cells, with higher energetic value, can be used as food source for zooplankton. Further, the zooplankton are used to feed fish larvae in aquaculture. In this project, we are currently investigating environmental factors to manipulate algae cells in culture as to obtain higher lipid content and, at the same time, higher biomass.

Physiological response of *Chlorella vulgaris* to environmental factors is being investigated. This microalgae is exposed to varying levels of nutrients (nitrates and phosphates), trace elements (copper and cadmium) and a combination of both nutrients and trace elements. Lipid class, total proteins, carbohydrates and biomass production are being studied at the set conditions for possible optimized large scale production.

Several zooplankton species (*Daphnia laevis*, *Dendrocephalus brasiliensis*, *Artemia salina*, etc.) are fed with these manipulated algae. Nutritional values of algae and zooplankton, through the different manipulation conditions, is being determined in order to find the better biochemical composition of live food for tropical fishes' larvae.

Furthermore, we are evaluating food selectivity of larvae and young fishes of neotropical carnivores species with aquaculture potential fed with *Dendrocephalus brasiliensis* and other species of freshwater zooplankton, as well as determining feed rate, secondary production, growth and survival of these fishes.

We also are initializing ecological studies of *D. brasiliensis* in its natural ecosystems and competition experiments in controlled conditions.

We expect that the results obtained in this work, comparing *D. brasiliensis* with other native zooplanktonic species, will make possible the use of this Anostraca as alimentary source of young cultivated tropical fishes, improving Brazilian aquaculture. Moreover, we hope that our ecological studies will enable us to evaluate if the use of *D. brasiliensis*, in São Paulo State can be recommended or not for use in aquaculture. Detailed biochemical studies of several zooplanktonic and algae species will generate important information to produce alive food with greater nutritional values for a more efficient fish production.

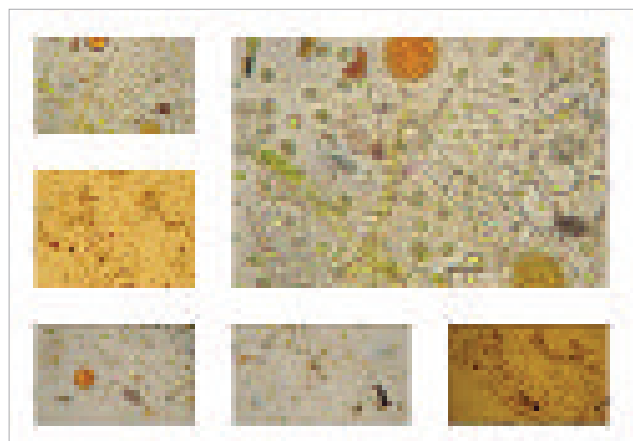


Figure 2. Food items in the digestive tract of *Dendrocephalus brasiliensis* (Photo by Renata M. dos Santos)

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CONTROL OF FOLLICULAR GROWTH AND OVULATION FOR ARTIFICIAL INSEMINATION, SUPEROVULATION AND EMBRYO TRANSFER

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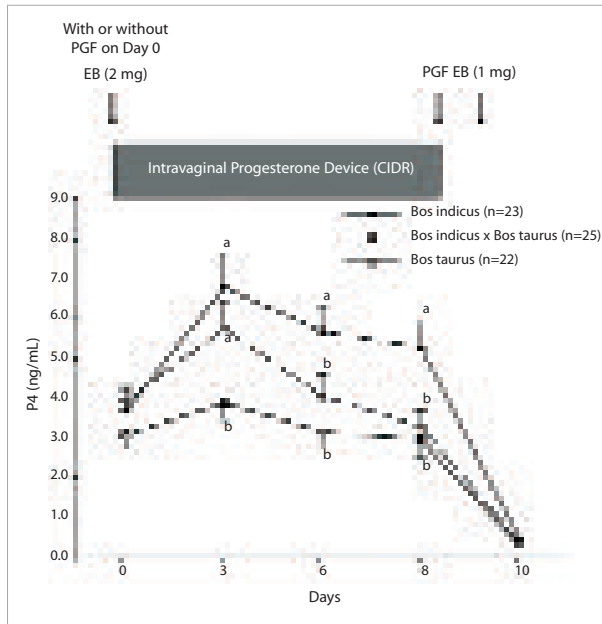


Figure 1. Serum progesterone (P4) concentrations in *B. indicus*, *B. indicus* x *B. taurus* and *B. taurus* heifers treated with an intravaginal progesterone-releasing device (CIDR) for 8 days, estradiol benzoate (EB) with or without PGF at device insertion (Day 0, data pooled), and PGF at device removal (Day 8). a and b within a day means that the distance between the lines (P) must be minor than 0.05 ($P < 0.05$). Carvalho et al., 2008.

Estrus detection is particularly challenging in Zebu breeds *Bos indicus* cattle *Bos taurus* and buffalo (*Bubalus Bubalis*). It limits the use of important technologies, such as artificial insemination and embryo transfer, for the genetic improvement of these species. Development of hormonal treatments that control precisely the timing of ovulation allows the use of fixed-time artificial insemination (FTAI). The main purpose of this project is to explore different aspects of the pharmacological control of estrous cycle. Studies have been conducted to provide the necessary understanding of physiological and endocrine events control. As a result, we have obtained the development of hormonal treatment strategies, which has improving the results of artificial insemination and embryo transfer in bovine and buffalo. Such strategies have been adopted successfully and are used extensively by veterinarians and practitioners in commercial beef and dairy operations in Brazil.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

As a result of this project, protocols consisting of a rational sequence of hormonal treatments were developed for the following purposes: 1) synchronization of ovulation for FTAI in beef cattle, dairy cows and buffalo females; 2) induction of multiple ovulations (super ovulation) in bovine and buffalo embryo donors and 3) synchronization of ovulation for fixed-time embryo transfer in bovine and buffalo embryo recipients. The protocols developed herein were made available to professionals and end users through numerous talks, seminars, workshops and lectures in local, national and international meetings related to animal reproduction. Most protocols utilized for FTAI, super ovulation of embryo donors and fixed-time embryo transfer, nowadays in Brazil, are based on the studies conducted in this project. From the practical and economical point of view, this represents a significant contribution for the use of artificial insemination and embryo transfer in the extensively raised herds of beef cattle (180 million) and buffalo (2 million) in Brazil. Indeed, it is estimated that FTAI and fixed-time embryo transfer are performed in 2.2 million and 100.000 recipients in Brazil, respectively.

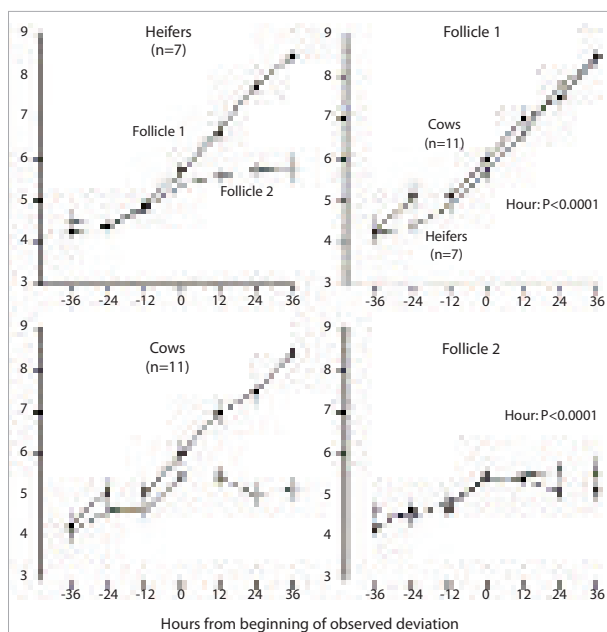


Figure 2. Mean \pm S.E.M. diameters of the largest (Follicle 1) and second largest (Follicle 2) follicles at 12-h interval in Nelore heifers and nonlactating cows after adjusting the follicle data for each heifer and cow to the beginning of observed deviation (0h). The left set of figures illustrates the diameter changes between follicles 1 and 2 within heifers and cows while the other set indicates the statistical comparisons between heifers and cows for follicles 1 and 2. Sartorelli et al., 2005

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THE VITELINE ENIGMA

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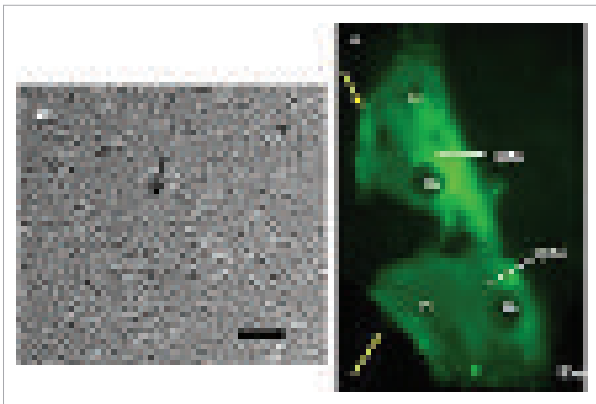


Figure 1. Immunofluorescence: positive for the antibody OCT-3 / 4 of the yolk sac cells of canine

The successive stages of pregnancy are characterized by two clear and distinct steps. The first one is related to early pregnancy in humans and corresponds to the first 3 months of pregnancy. During this period, important events occur, such as embryogenesis, organogenesis and placentation. Evidence found in animal models suggest that this phase of development is susceptible to some problems that may reflect the early pregnancy loss, and other effects compromising the pregnancy. The second phase corresponds to fetal development. Relations and regulation of the functional mechanisms involved in early pregnancy are not fully known, and many of them are coincident. The yolk sac is the only embryonic Annex present in all species. Complemented by other structures in viviparous, it is still unknown, among their functions, the mechanisms related to their actions and relations with the issue tropism nutrition, embryogenesis, placentation and organogenesis. Initially the group will focus on morpho-functional analysis related to sac activity in animal models showing vitelline placentation compared to those without vitelline placentation. Calf proteomics analysis, the mechanism of inversion sac absorption, insight, expertise and yolk transport will also be discussed. Yolk cells or yolk precursors are cultured in order to be able to establish yolk cell lines, and to study the *in vitro* vitellin cellular plasticity, including preclinical testing of cell therapy in genetic or acquired diseases in animals.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The yolk sac is the only embryonic structure present in all species. Our group will focus on analysis related to sac morphofunctional activity in animal models that have or have not vitelline placentation. A proteomic study of calf proteins, yolk sac proteins and primitive gut proteins are being held in embryos, to identify proteins and their functional aspects, as well as their changes after transduction. These information will contribute to the understanding maternal-fetal and to the improvement of biotechnologies such as artificial insemination and embryo transfer. The establishment and characterization of progenitor stem cells in horses supports joints diseases studies and the therapeutic perspectives for veterinary regenerative nerve and tendon injuries, arthritis and ruptured ligament braces. At the same specie, the potential of horse yolk sac pluripotent stem cells for differentiation into pancreatic cells in order to produce insulin for treatment of diseases. Dogs and horses embryos yolk sac stem cells have been differentiate in hematopoietic cell lines and endothelial cells, to elucidate problems related to blood diseases and to help heart transplants, vascular regeneration and repopulation of hematopoietic systems affected by disease. Finally, the characterization and differentiation of embryonic liver bud stem cells to establish liver cell lines for liver diseases treatment in animal models.

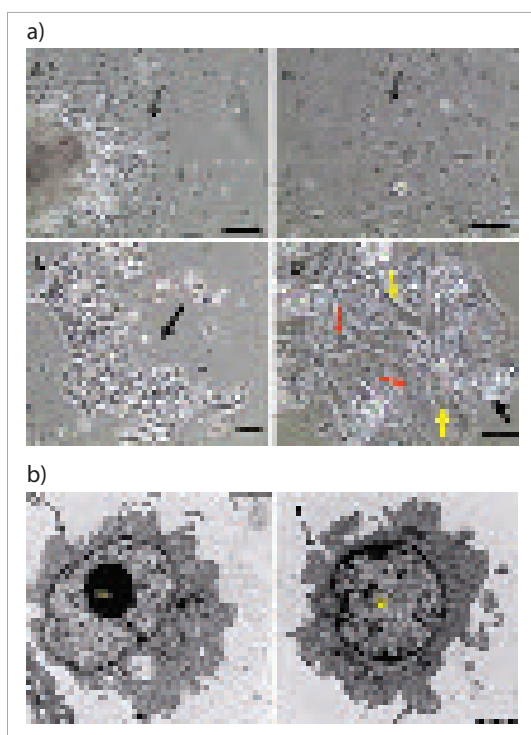


Figure 2. (a) Morphological diversity of canine yolk sac stem cells with 45 days. (b) Electron transmission of progenitor cells derived from the cultivation of canine yolk sac cells with 30 days of gestation

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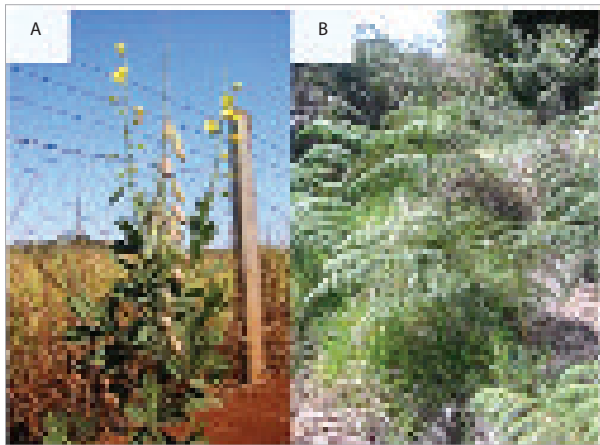
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TOXINS OF VETERINARY INTEREST: EVALUATION OF THE IMMUNOTOXICITY, NEUROTOXICITY AND TERATOGENICITY IN RODENTS AND GOATS

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A) *Crotalaria spectabilis*. B) *Pteridium aquilinum*.
In Górnica, S.L. *Poisonous plants of agronomical interest*.
Spinosa, H.S, Górnica, S.L., Palermo-Neto, J (eds) Manole,
Barueri, 2008.

Poisonous plants and mycotoxins are two of the main causes of economic losses to Brazilian animal livestock industry. Besides animal death losses, other consequences from the prolonged exposure to toxins are the reduced animal body weight gains and production, reproductive impairments, abortions, birth defects, and immunosuppression with sub-clinical or short-term illness. Furthermore, plant-associated toxins may negatively impact food safety and contaminate human food.

The immune system is pivotal in host defense against infectious agents and neoplasia, which is a highly integrated network of cells. Xenobiotics that alter immune cells functions, can also potentially

injure these cells, disrupting the immune responses and altering host resistance. The aims of the present study includes the evaluation of the potential immunotoxicity and teratogenic effects of some plants and mycotoxins to both livestock and human health. In addition, we propose to improve current immune and teratogenic test protocols employed by regulatory agencies of risk assessment.

Pteridium aquilinum is a plant founded worldwide and epidemiological studies have revealed a higher risk of cancer in people who consume this plant directly or indirectly through milk from animals that are feeded with this plant species. In cattle, it has been showed that chronic exposure induces urinary bladder carcinomas and carcinomas of the upper alimentary tract. There are evidences of association between these carcinomas and chronic intoxication by *P. aquilinum* ingestion and bovine papilloma virus infection. Thus, it is plausible that the observed increasing in cancers diseases could be related to induction of an overall immunosuppression by this plant. Considering this, our study has evaluating the immunosuppressive effects of *P. aquilinum* in mice.

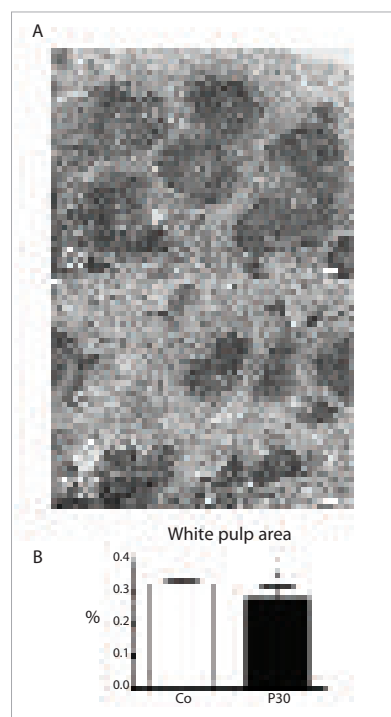
Monocrotaline (MCT) is a pyrrolizidine alkaloid found in a variety of plants, including *Crotalaria spp*, which are largely distributed in Brazil. The main symptoms of MCT toxicities in livestock are related to hepato- and nephrotoxicity. Although studies have shown that MCT can cause effects on cellular functions that would be critical to lymphocytes/macrophages during a normal immune response, no immunotoxicological study on MCT have been performed yet. Thus, the aim of the present study is to evaluate MCT effect on different branches of the immune system using mice as animal model.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Histological analyses of C57BL/6 mice administered with *P. aquilinum* extracts revealed a significant reduction in spleen white pulp area. A variety of immune response were analyzed in these animals including delayed-type hypersensitivity (DTH) and decreased IFN α production by NK cells during TH1 priming. The innate response in these hosts, assessed by analysis of NK cell cytotoxic functionality was also diminished in comparison to control animals in the assay. These results have confirmed the expected immunosuppressive effects of *P. aquilinum*. Thus, many

of the modulated immune responses can contribute to the increased risk of cancer in exposed hosts.

Rats treated with MCT have their lymphoid organs, acquired immune responses, and macrophage (MO) activity evaluated. No significative changes in the relative weight of lymphoid organs were observed. However, it was observed a decrease in the bone marrow cellularization in rats treated with MCT. Treatments with MCT caused no significant alterations in phagocytic function or in hydrogen peroxide production, however, the MCT causes compromised nitric oxide release by these cells. In conclusion, these results have shown that MCT causes myelotoxic effects and interferes in the formation of at least one product critical to the inflammatory process. Future experiments will be conducted to determine which bone marrow cell lines are



Effects in spleens of mice treated with 30 (P30) g/kg BW of *P. aquilinum* and supplemented with B1 vitamin in water (10 mg/ml) for 14 days. A) Representative spleen sections from the control group and from the P30 group. Observe smaller white pulp area in spleens from the P30 group. Bar = 100 μ m. B) Morphometric analyses showed a significant reduction in white pulp area in spleens from the P30 group ($p = 0.004$, Mann-Whitney test). Data are expressed as the mean \pm SD ($n = 9$). Latorre et al., in press

affected by MCT, and the roles of iNOS and NO in the inflammatory process. We will also analyze if the effects of this compound, on alveolar macrophages, could be a factor in the pulmonary hypertension known to be induced by this alkaloid.

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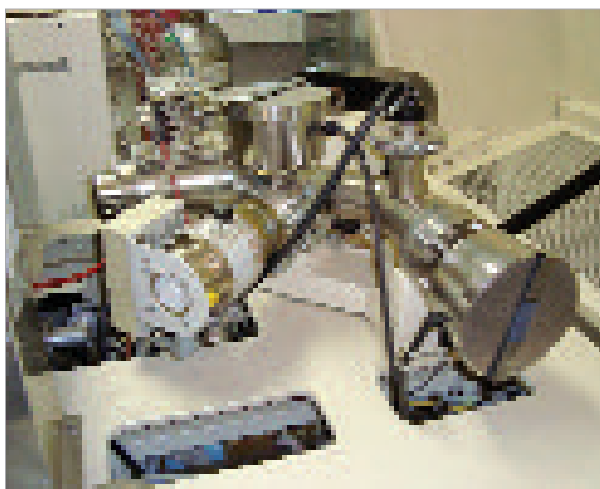
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ENVIRONMENTAL STABLE ISOTOPES IN ANIMAL SCIENCE

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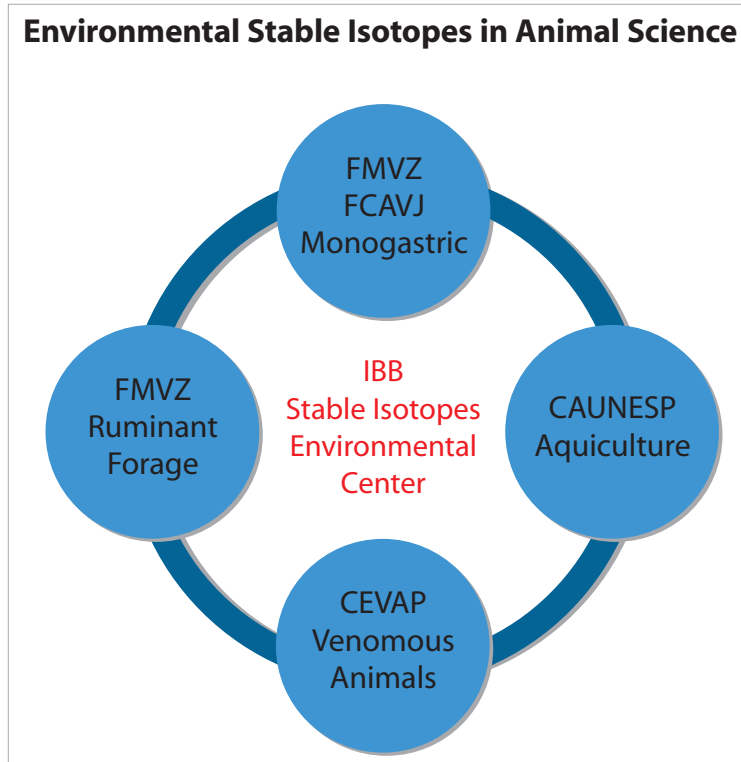


Mass Spectrometer. Application of traceability by stable isotopes of bioelements in areas of ruminant, non-ruminant and aquaculture

Traceability is the ability to find out food history, use or origin. It is fundamental for the concept of food safety, quality and animal welfare, to assure the food authenticity and food origin and final products (meat, milk, eggs), this allows the consumers to know and evaluate the food productive chain they consume, as the population expects more reliable information from products they purchase. Considering the meat production model, nearly 10% of the world meat production for human consumption is internationally commercialized, although there is a traceability program for production system through earings, registers and tattoos on the animals. This information is not given to the consumers. Nowadays, some markets do not accept the animal only with registers and programs. The traceability of the final product as a way to assure to the consumers the food safety and to protect the production of regions and countries is urgently due to safety questions. Analysis of stable isotopes of bioelements – Carbon, Hydrogen, Oxygen, Nitrogen and Sulfur – has been used to control food traceability for more than twenty years because it is non-radioactive and does not harm the environment. These bioelements are part of carbohydrates, proteins, lipids and nucleic acids structures being responsible for more than 90% of living tissues. The first products studies has included fruit, juice, honey, wine and alcohol, and for all of them, the official isotopic analysis method for detecting sugar or water addition, has been used since 1978 in the USA and 1995 in Europe. In Brazil, this technique started being used in Geochemistry, Hydrology and Agronomy areas due to the natural interaction between rock, soil, water and, consequently, the plants.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

There is a proposal to establish an official methodology through stable isotopes to detect the addition of prohibited food in animal diet, such as the animal meals, initially in products for exportation. The addition of these protein sources is forbidden by the European Union and Islamic Countries which expect to buy only products from animals exclusively fed with vegetal food due to the mad cow disease. Brazil has an important role in the world agribusiness scenery, and the traceability of final products through stable isotopes can make the certification program of Brazilian products more competitive in the international market, assuring to the consumers the concept of food safety. Another approach of the project is to evaluate the technique of environmental stable isotopes in physiology, nutrition and animal metabolism, providing a new tool to improve the search for information and concepts in the Animal Science area. Thus based on the facts described above, the implementation in Brazil of a new area of traceability system application through stable isotopes of bioelements in areas of ruminant, non-ruminant and aquaculture, is relevant.



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ENVIRONMENTAL IMPACT OF PHOSPHORUS EXCRETION IN LIVESTOCK: QUANTITATIVE ANALYSIS OF FLOW PHOSPHORUS USING BIO-MATHEMATICAL MODELS

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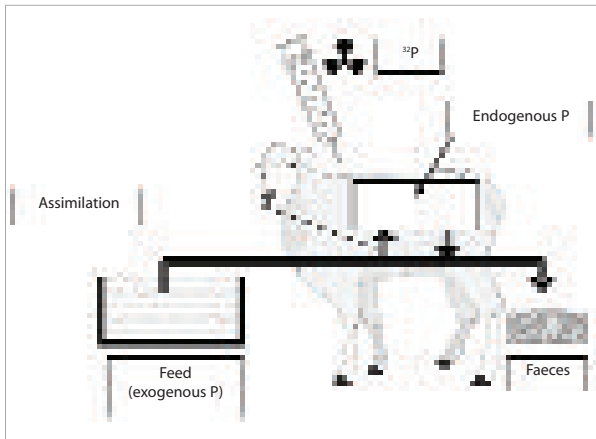


Figure 1. Schematic representation of the isotope dilution technique

Animal husbandry concentration in production areas greatly contributes to water and soil pollution through excessive nutrient dejection output. This study will be conducted to evaluate the environmental impact of phosphorus excretion in livestock and to obtain a better understanding and quantification of phosphorus excretion. Phosphorus metabolism will be evaluated by using radioisotope technique (Figure 1) and mathematical models. Various levels of phosphorus supplementation and/or different P sources will be given to the animals in their diet. After 21 days pre-experimental period, each animal will receive a single dose of 7.4 MBq ³²P in 1 ml sterile isotonic saline at the right jugular vein. Blood samples, fecal and urine samples will be taken for 7 days. Specific activities in plasma and feces will be determined. Endogenous losses and true availability will be determined. After the end of the experiment, the animals will be killed by intravenous injection of pentobarbital. Tissues (liver, heart, kidney and muscle) and bone samples (12th rib) will be collected. Phosphorus metabolism will be evaluated using mathematical models.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Quantitative aspects of phosphorous metabolism have been considered using balance studies and kinetic models based on experiments with radioactive tracers. Using data from balance and kinetic studies, a model of P metabolism in growing goats fed with increasing levels of P was proposed by Vitti *et al.* (2000).

The proposed of P kinetics model was revised considering the study of calcium (Ca) flows in growing sheep (Dias *et al.*, 2006) (Figure 2). Sheep weighing 32 kg were injected with ³²P and ⁴⁵Ca to trace the movement of P and Ca in the body. The original model had 4 pools representing the gut, plasma, soft tissues and bone. In the revised model, instantaneous values rather than averages for pool derivatives were incorporated, and the model was extended to represent absorption and excretion of phytate P. The amendments improved the model, resulting in higher flows between plasma and bone than between plasma and tissue. Therefore a more accurate representation of P metabolism was obtained. Phosphorus and Ca metabolism were then assessed conjointly using the revised model. Dias *et al.* (2006) found that phytate P digestibility in the forage used to feed the animals was only 47%, and P retention was negative, suggesting that a characteristic feed impairs P utilization, resulting in P deficiency. The models described contributed to a better understanding of P and Ca metabolism in ruminants and non-ruminants being a support tool for diet assessments and their efficiency of utilization or pollution impact.

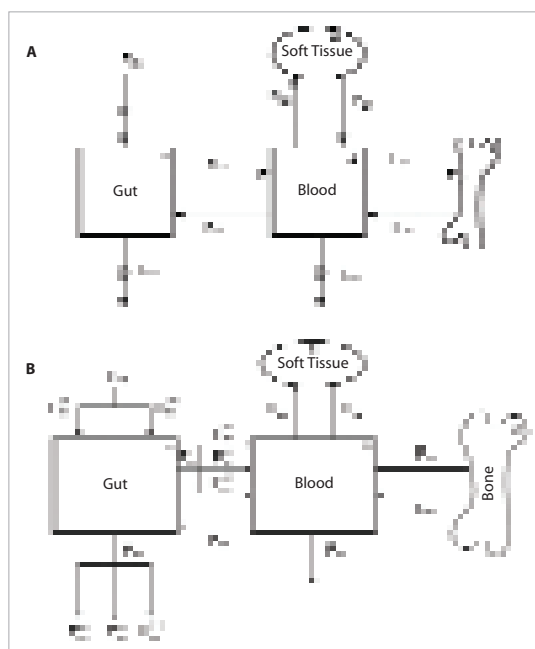


Figure 2. (A) Schematic representation of P metabolism model in goats. F_{ij} is the total flux of pool i from j , F_{i0} is an external flux into pool i and 0 a flux from pool j out of the system. Specific activities of pool i is represented by s_i and circles denote fluxes measured experimentally (Vitti *et al.*, 2000). (B) Revised model of P metabolism in growing sheep showing phytate P. F_{10} denotes ingestion of P, F_{01} excretion of P in faeces, F_{02} excretion of P in urine. The flows $F_{10}^{(p)}$, $F_{01}^{(e)}$, and $F_{21}^{(n)}$ are partitioned as shown, with superscripts (p), (e), and (n) indicating P of dietary phytate, re-cycled endogenous, and dietary non-phytate origin, respectively (Dias *et al.*, 2006).

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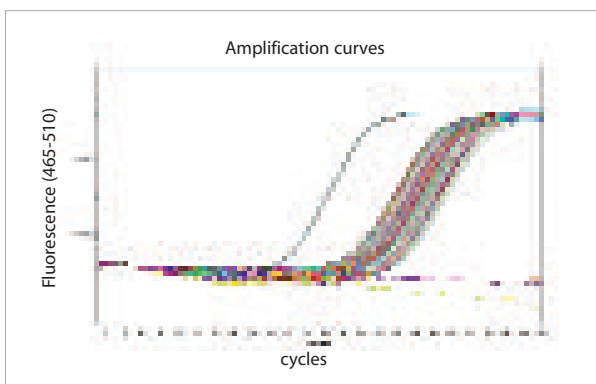
CHICKEN MEAT QUALITY: GENETICS, PRE-SLAUGHTER STRESS AND NUTRITION FACTORS

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Above, chickens in the experimental units; and on the left, monitoring chickens for heat stress



Quantitative PCR amplification curves

Chicken meat production is an important agrobusiness component, leading Brazil to a remarkable position in meeting the global market, as well as the domestic demand. Meat quality, considering fresh and processed broiler meat, is essential for the consumer acceptance of the product. As a background, elevated temperature during summer months can impact production efficiency and meat quality. In particular, pre-slaughter heat stress has been known to interfere with final product quality and was elected as a relevant aspect to be studied. Temperature, transportation and abattoir management are able to induce changes in the internal body regulation with consequences to the transformation of muscle in meat and on the meat functional properties. The main focus of this interdisciplinary project is to identify and characterize factors affecting chicken meat quality such as genetics, heat stress and nutrition, improving the comprehension of these aspects and opening the possibility of managing those factors. The specific objectives of this project include (a) the definition of an experimental model to relate pre-slaughter heat stress to chickens physiologic changes and meat quality; (b) to determine if different genetic lines of chickens have different physiological responses to heat stress conditions; (c) to investigate the gene expression pattern under pre-slaughter heat stress condition and its effect on breast meat quality; (d) to determine the chemical (pH, composition, susceptibility to oxidation) and physical (color, water holding capacity, tenderness) properties of chicken breast meat exposed to heat stress prior to slaughter and the effects on meat stored under refrigerated or frozen conditions; (e) to identify changes in proteolysis and migrations between the myofibrillar and sarcoplasmic fractions due to heat stress; (f) to determine the effectiveness of plant extracts and tocopherol, as natural antioxidants, on chicken performance and meat quality and stability during storage. This study represents a contribution to the productive chain of chicken meat in order to maintain its quality and competitiveness.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The experimental model to simulate the stressing conditions in chickens transport to the slaughterhouse was defined as 35°C and 85% relative humidity for up to two hours. Compared to chickens kept in therm neutral environment, the heat-stressed chickens have showed adaptive responses of increase in body temperature and respiratory frequency, followed by physiologic responses such as hemodilution to avoid hypovolemia that may occur due to weight loss represented mainly by transpiration. Differential water drainage among tissues was observed in the heat-stressed birds, with preservation of water in the breast muscle and loss in legs and wings.

In the breast muscle, differentially expressed genes were investigated by macroarray and qRT-PCR. Macroarray analysis revealed 259 differentially expressed genes, with 149 induced by heat stress. Nine genes were selected for qRT-PCR analysis: *Myostatin*, *Citrate Synthase*, *HSP 70* and *90* were induced by heat stress, while *Glycogen Pphosphorilase* was repressed.

The meat quality studies revealed that the heat stress provoked increased frequency of breast meat with higher pH and lower L* (lightness) values. Consequently, less cooking weight loss was found in the breast meat from heat-stressed chickens. A complementary study on the molecular aspects concluded that heat stress decreased the rate of myofibrillar fragmentation, but the proteolytic extension was not changed. Also, specific myofibrillar fragments were formed, with no alteration in shear force. Modifications in the sarcoplasmatic fraction were observed in pale meat.

The nutritional studies demonstrated that the addition of herbs extracts (rosemary, thyme, oregano, sage, bay and basil) and spices (cinnamon, clove and ginger) to broilers diets resulted in positive antioxidant effect in precooked chicken meat stored frozen, while not affecting the other meat quality parameters. Also, aqueous extracts of mate (*Ilex paraguariensis*) protected efficiently precooked meat balls from oxidative deterioration during storage. This strong antioxidant effect may be at least partially related to a synergistic interaction with vitamin E, which seems to be regenerated by the water soluble phenolic compounds from mate.

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MODELS TO ESTIMATE LYSINE, METHIONINE + CYSTINE AND THREONINE REQUIREMENTS IN POULTRY AND PULLETS

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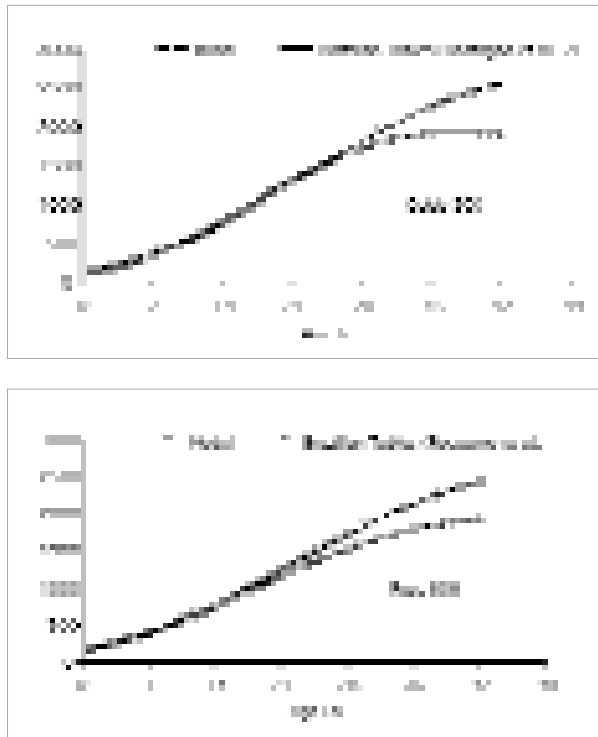


Figure 1. Lysine requirements estimated by the model elaborated in this study as compared to those of Brazilian Tables (Siqueira, J.C. et al., unpublished)

It is essential to supply diets with adequate nutritional requirements in order to allow poultry to express their genetic potential. Amino acids are particularly important as they are closely related to body protein synthesis and accretion. The main methods applied to study nutritional requirements are dose-response and factorial approaches. Dose-response is the most commonly used method and it is based on animal performance response to increasing dietary levels of a limiting amino acid. Nutritional requirement tables, such as the *Nutrient Requirements of Poultry* (1994) and the *Brazilian Tables for Poultry and Swine* (2000, 2005) are built on the results of such dose-response studies. However, genetics, nutrition, and environment may influence poultry nutritional requirements. The factorial approach is based on the principle that birds require amino acids for the maintenance of vital processes, growth, and/ egg production, fractioning total requirements into ratios for each of these purposes. In order to take into account weight and body composition differences, the factorial method allows building models to predict the nutritional requirements of poultry of different genetic lines and ages raised under various conditions. The models to estimate amino acid requirements provide economically feasible nutritional programs, as well as preventing excessive dietary amino acid supply and environmental pollution. Building these models requires knowledge on amino acid maintenance requirements and efficiencies of utilization. However, methodological variations have led to inconsistencies in their determination. The main objective of the present project is to elaborate prediction models of the amino acid requirements for broilers and layers based on the factorial method. Specific objectives include: (a) standardization of the method to determine amino acid maintenance requirements, (b) to compare poultry response to two diet formulation methods (graded amino acid supplementation and diet dilution), (c) to determine amino acid maintenance requirements, and (d) to determine amino acid utilization efficiencies in broilers and layers.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In order to standardize the experimental method and to estimate lysine maintenance requirements, metabolism trials were carried out with roosters from different genetic lines (Leghorn, ISA Label e Cobb 500) using nitrogen balance technique. Lysine maintenance requirements obtained were 44.9, 44.4, and 47.1g/kg^{0.75}/day for Leghorn, ISA Label, and Cobb, respectively. Considering the three trials, Lys maintenance requirement was estimated 45.1mg/kg^{0.75}/day or 151.2mg/BP_m^{-0.27} independently from genetic line. To compare diet formulation methods and to estimate the efficiency of lysine utilization, 4 trials were carried out with broilers in different phases (1 to 8, 8 to 22, 22 to 35, and 35 to 42 days). The results show that the efficiency of lysine utilization is not influenced by diet formulation method, and that it is estimated in 76.9%. Lysine requirement prediction model for broilers was elaborated based on these results and partitioning the requirements for maintenance and growth of body feather-free and feathers, described as:

$$\text{Lys} = [(151.2\text{BP}_m^{-0.27} \cdot \text{BP}_t) + (0.01 \cdot \text{FP}_t \cdot 18)] + [(75 \cdot \text{BPD} / 0.769) + (18 \cdot \text{FPD} / 0.769)]$$

Lys=digestible lysine requirement (mg/day), BP_m=body protein weight at maturity (kg), BP_t= body protein weight at time (kg), FP_t= protein feather weight at time (g), BPD=body protein deposition (g/day), FPD = protein deposition in feathers (g/day). The lysine content in feather-free body and feathers were considered to be 75 and 18 mg/g, respectively. The lysine requirements for growth of feather-free body and feathers were estimated considering the same k_{Lys} (76.9%).

The *Figure 1* shows lysine requirements estimated by the model as compared to those of Brazilian Tables for Poultry and Swine (2005), for two broiler strains. The proposed model estimated lower requirements than those of Brazilian Tables, after 27 days (*Figure 1*). Considering that during the starter phase, broilers require more amino acids for growth, and that body protein to fat ratio is reduced as bird ages, this model is able to predict requirements as a function of physiological development.

The theory applied to elaborate this model is based on protein growth potential of the body feather-free and feather. Genetic selection of broiler strains has enabled to increase protein deposition, resulting in precocious birds. Therefore, it is expected that the daily protein depositions decline early. In this sense, the proposed model was able to detect these changes accurately compared to Brazilian Tables model.

In addition, in broiler production, approximately 75% of the total feed is consumed after 21 days of age, increasing feeding costs. As the grower-finisher lysine levels estimated by the model were lower than those recommended by the Brazilian Tables, the developed model may contribute to adjust nutritional standards for broilers, thereby promoting poultry production profitability and sustainability.

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