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COMPARATIVE EFFICACY OF BOTANICALS AND INSECTICIDES ON TERMITES IN SUGARCANE

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ABSTRACT

Termites are a major pest that cause damage to many crops, including sugarcane. The use of chemical insecticides to control termites has resulted in environmental and health hazards. Therefore, there is a need to investigate alternative methods of termite control. This study aimed to compare the efficacy of botanicals and insecticides on termites in sugarcane. The botanicals included neem, garlic, and turmeric, while the insecticides included chlorpyrifos and fipronil were used. The efficacy of botanicals as first application on setts in furrow against termites in sugarcane was compared with insecticides and control. The botanicals were used in 1:20 in water. The study was conducted in a randomized block design, and the data were analyzed using Statistix 8.1. The results showed that neem, garlic, and turmeric were effective in controlling termites in sugarcane. Neem had the highest efficacy, followed by garlic and turmeric. Chlorpyrifos and fipronil were also effective in controlling termites, but they had a negative impact on the environment. Therefore, it is recommended to use neem, garlic, and turmeric as an alternative method of termite control in integrated pest management of sugarcane.

Keywords: Sugarcane, termites, botanicals

INTRODUCTION

Sugarcane is an important crop that contributes significantly to the economy of many countries. However, it is susceptible to damage from pests, including termites. Termites are social insects that live in large colonies and feed on cellulose-based materials, including sugar cane. The use of chemical insecticides to control termites has resulted in environmental and health hazards. Therefore, there is a need to investigate alternative methods of termite control.

Sugarcane is the very important sugar-producing crop of the world, which is under attack of many insect pests (Verma, *et al.,* 2018). Borers and termites are the most important insect pests of sugarcane worldwide. Subterranean termites are the major problem which affect the sugarcane crop from its germination through shoot emergence and finally on the quality of canes. At germination stage, termite losses up to 90-100 percent have been recorded in sugarcane (Adeyemi and Adedire, 2022). For the control of termites, many methods have been adopted, among which chemicals were dominated means of the control since long. However, chemicals are expensive and have many harmful effects. The insecticides in liquid or dry formulation viz., Chloropyrifos, imidacloprid and fipronil are being applied as sett treatment in furrows at the time of sowing of sugarcane (Paul *et al.,* 2018). The success of such treatment with insecticides is highly variables. In order to find alternates to persistent insecticides it is thought that termites should be repelled by the time of successful shoot emergence. Many plants have been found to contain chemicals, but their potential has not been explored for field use (Akbar *et al.,*2021). The reduction in infestation of termites in sugarcane with neem and Calotropis extracts has been reported (Chakraborti, 2017).

Plant parts and plant extracts can be used effectively because these are less expensive and biodegradable and hence environmentally suitable (Roychoudhury, 2016). Botanicals are plant extracts that have been used traditionally for pest control. Neem (*Azadirachta indica*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*) are botanicals that have been reported to have insecticidal properties (Majeed *et al.,* 2020). Neem is a tree that is native to the Indian subcontinent and has been used for centuries for its medicinal properties. Garlic is a bulbous plant that is widely used in cooking and has also been used for medicinal purposes. Turmeric is a spice that is used in cuisines and has been reported to have anti-inflammatory and antioxidant properties.

Insecticides are chemical compounds that are used to control insects. Chlorpyrifos and fipronil are insecticides that have been used for termite control (Kambrekar, 2021). Chlorpyrifos is an organophosphate insecticide that has been used for over 50 years. Fipronil is a relatively new insecticide that has been used for termite control since the 1990s.This study is aimed to compare the efficacy of botanicals and insecticides on termites in sugarcane.

MATERIALS AND METHODS

The study was conducted at Entomological Research Institute (Ayub Agricultural Research Institute, Faisalabad). in a randomized block design with five treatments and four replications. The total experimental area was 6,000 m^2, which was divided into 20 equal plots. The size of each plot was 300 m^2 with dimension 20m x 15m = 300 m^2. Variety CPF-253 was sown and Row-to-Row distance was 90 cm. The treatments included neem, garlic, turmeric, chlorpyrifos, and fipronil. The botanicals were prepared by grinding the leaves of neem, garlic, and turmeric and mixing them with water to form a suspension (Nisar *et al.,* 2020). Chlorpyrifos and fipronil were applied as per the recommended dose. At the time of application, treatments were sprayed on setts at the time of sowing in furrows, in the same way, chemical pesticides were sprayed in furrows. The setts were wetted to the soaking condition. After application, the data on termite count were carried out by random digging in each plot on a weekly basis. After 15 days of sowing, germination and bud damage were also recorded. Bud damage was recorded from places in a plot where there was gap of > 1 m between two seedlings. The efficacy of the treatments was evaluated based on the percentage of termite mortality. Ten sugarcane stalks were randomly selected from each replication, and the number of live and dead termites was counted. The mortality percentage was calculated using the formula: (number of dead termites/total number of termites) x 100. The data were analyzed using ANOVA, and means were separated using Duncan's multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

The results showed that all treatments were effective in controlling termites in sugarcane (Table 1). Neem had the highest efficacy, with a mortality rate of 93.25%, followed by garlic (85.75%) and turmeric the efficacy of chlorpyrifos and fipronil was also significant, with mortality rates of 82.5% and 78.5%, respectively. However, the use of chemical insecticides can have a negative impact on the environment and human health. The data also showed significant differences between the treatments ($p<0.05$). Neem had a significantly higher efficacy than garlic, turmeric, chlorpyrifos, and fipronil. Garlic had a significantly higher efficacy than turmeric, chlorpyrifos, and fipronil. Turmeric had a significantly higher efficacy than chlorpyrifos and fipronil. There was no significant difference in efficacy between chlorpyrifos and fipronil.

The results of this study suggest that botanicals, particularly neem, garlic, and turmeric, are effective in controlling termites in sugarcane. This is consistent with previous studies that have reported the insecticidal properties of these botanicals against a range of pests.

Neem contains compounds such as azadirachtin, which have been reported to have insecticidal and antifeedant properties. Garlic contains compounds such as allicin, which have been reported to have insecticidal and repellent properties. Turmeric contains compounds such as curcumin, which have been reported to have insecticidal and antifeedant properties.

The efficacy of chlorpyrifos and fipronil in controlling termites is also consistent with previous studies. However, use of chemical insecticides can have a negative impact on the environment and human health. Chlorpyrifos has been classified as a possible human carcinogen, and fipronil has been reported to have a negative impact on non-target organisms, including honeybees.
Therefore, use of botanicals as an alternative method of termite control in sugarcane is recommended. Botanicals are environmentally friendly, safe and non-target organisms and readily available. Use of botanicals can reduce the risk of resistance development in termites.

CONCLUSION

In conclusion, this study compared the efficacy of botanicals and insecticides on termites in sugarcane. The results showed that neem, garlic, and turmeric were effective in controlling termites in sugarcane, with neem having the highest efficacy and the repellency of neem plant against termites is well documented. Chlorpyrifos and fipronil were also effective, but their use can have a negative impact on the environment and human health. Therefore, it is recommended to use neem, garlic, and turmeric as an alternative method of termite control in sugarcane. Further studies are needed to investigate the long-term efficacy of these botanicals and their impact on sugarcane yield.

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COMPUTATIONAL IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF NOVEL GENES INVOLVED IN SUGARCANE DROUGHT TOLERANCE

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ABSTRACT

Drought stress is a major constraint on sugarcane production and understanding the molecular mechanisms underlying drought tolerance is crucial for developing drought-resistant varieties. In this study, we applied an integrative computational approach to identify novel genes and pathways involved in sugarcane drought tolerance. We integrated multi-omics data, including transcriptomics, proteomics, and metabolomics from drought-tolerant and drought-sensitive sugarcane genotypes and performed differential expression analysis to identify candidate genes involved in drought tolerance. Using gene co-expression network analysis, we further identified potential transcription factors and regulatory pathways involved in sugarcane drought tolerance. We functionally characterized these candidate genes using CRISPR/Cas9-mediated gene editing and demonstrated their role in drought tolerance. Our results reveal several novel candidate genes involved in sugarcane drought tolerance, which could be used for developing drought-resistant sugarcane cultivars using genetic engineering. Our study highlights the power of integrative computational approaches in identifying key genes and pathways involved in complex biological processes such as drought tolerance.

INTRODUCTION

Sugarcane (Saccharum spp.) is an economically important crop that is cultivated worldwide for sugar and bioenergy production. It is a highly polyploid and complex genome plant species, making it difficult to study using traditional experimental techniques (Zhao *et al.,* 2020). In recent years, the advent of high-throughput sequencing technologies has revolutionized the field of genomics, enabling researchers to study the molecular mechanisms underlying various biological processes in sugarcane at unprecedented resolution (Misra *et al.,* 2022).

One of the major challenges faced by sugarcane growers and breeders is drought stress, which is a significant constraint on sugarcane productivity worldwide. Drought stress reduces sugarcane growth and yield, and the impact of drought is expected to worsen with climate change (Mall *et al.,* 2022). To develop drought-tolerant sugarcane cultivars especially in Pakistan, it is essential to understand the molecular mechanisms underlying sugarcane drought tolerance (Chen *et al.*, 2023).

In recent years, computational biology has emerged as a powerful toolset for analyzing large-scale genomic and transcriptomic data and gaining insights into complex biological processes (Namwongsa *et al.,* 2019). Computational biology combines statistical and computational approaches to model and analyze biological data, thereby enabling researchers to generate novel hypotheses and test them using experimental techniques (Khonghintaisong *et al.,* 2018). In this study, we applied an integrative computational approach to identify novel genes and pathways involved in sugarcane drought tolerance. We integrated multi-omics data, including transcriptomics, proteomics and metabolomics, from drought-tolerant and drought-sensitive sugarcane genotypes and performed differential expression analysis to identify candidate genes involved in drought tolerance (Xu *et al.*, 2023). Using gene co-expression network analysis, we further identified potential transcription factors and regulatory pathways involved in sugarcane drought tolerance. We functionally characterized these candidate genes using CRISPR/Cas9-mediated gene editing and demonstrated their role in drought tolerance. This study provides new insights into the molecular mechanisms underlying sugarcane drought tolerance and identifies several novel candidate genes and pathways that could be targeted for crop improvement as previously identified by (Liu *et al.,* 2021). By leveraging the power of integrative computational approaches, we demonstrate the utility of these methods in identifying key genes and pathways involved in complex biological processes such as drought tolerance (Ribeiro *et al.,* 2013). Our results have significant implications for developing drought-resistant sugarcane cultivars, which are essential for maintaining sugarcane productivity under changing climatic conditions.

MATERIALS AND METHODS

Plant material and growth conditions

Three drought-tolerant (DT) and one drought-sensitive (DS) sugarcane (Saccharum spp.) varieties were used on farmers field in Faisalabad District. The DT cultivars included CPF-253, CPF-246 and NSG-59 which were previously identified as highly drought-tolerant in a field study. The DS variety was SPF-234, which is commercially grown in the region but is known to be sensitive to drought. Plants were initially grown in a greenhouse under controlled conditions (temperature: 28 ± 2 °C, relative humidity: 60 ± 5%, photoperiod: 14 h light/10 h dark) in 3-gallon pots filled with commercial soil mix. Plants were watered daily with tap water and fertilized with a commercial fertilizer (20-10-20 N-P-K).

Drought stress treatment

After planting in the open field, at the six-leaf stage, half of the plants of each cultivar were subjected to drought stress treatment by withholding water for 14 days, while the other half of the plants were maintained under well-watered conditions. Leaf samples were collected from both groups of plants at three time points: 0 days, 7 days and 14 days after the start of the drought stress treatment (Sanji *et al.,* 2002). Samples were immediately frozen in liquid nitrogen and stored at -80 °C until further processing.

RNA extraction & sequencing

Total RNA was extracted from leaf samples using a Trizol-based method according to the manufacturer's instructions (Invitrogen, USA). RNA quantity and quality were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) respectively. RNA sequencing (RNA-seq) was performed using the Illumina HiSeq platform with 150-bp paired-end reads at a sequencing depth of 30 million reads per sample.

Protein extraction and mass spectrometry

Proteins were extracted from leaf samples using a phenol-based method as previously described (Khonghintaisong *et al.,* 2018). Protein concentration was determined using the Bradford assay (Bio-Rad, USA), and protein quality was assessed using SDS-PAGE. Proteomic analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, USA).

Metabolite extraction and analysis

Metabolites were extracted from leaf samples using a methanol-chloroform-water method as previously described (Mall *et al.,* 2022). Metabolite extracts were analyzed using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific, USA).

Data analysis

Quality control of RNA-seq data was performed using FastQC (Chen *et al.*, 2023) and reads were trimmed and filtered using Trimmomatic (Xu *et al.*, 2023). Transcript abundance was quantified using Kallisto and differential expression analysis was performed using edger (Liu *et al.,* 2021). Proteomic data were analyzed using MaxQuant and metabolomic data were analyzed using MzMine2 (Chapae *et al.,* 2020). Gene co-expression networks were constructed using the Weighted Gene Co-expression Network Analysis (WGCNA) package in R (Liu *et al.,* 2021).

Functional characterization of candidate genes

Candidate genes involved in drought tolerance were identified based on their differential expression patterns and their co-expression with known drought tolerance genes. CRISPR/Cas9 genome editing was used to knock out selected candidate genes in the DT cultivars to determine their role in drought tolerance. The sgRNA design and cloning were performed using the CRISPResso2 software (Zhao *et al.,* 2020). The CRISPR constructs were transformed into sugarcane cultivars using Agrobacterium mediated transformation as previously described (Babu *et al.,* 2019). Transgenic plants were selected using hygromycin resistance and verified by PCR and sequencing. The transgenic plants were subjected to drought stress as described above and their physiological and molecular responses were compared to those of the wild-type plants.

Physiological and biochemical assays

Physiological and biochemical assays were performed to determine the drought tolerance mechanisms in the DT cultivars and the effect of the candidate genes on these mechanisms. Leaf water potential, stomatal conductance, and chlorophyll fluorescence were measured using a pressure chamber (PMS Instrument Company, USA) a LI-COR 6400XT portable photosynthesis system (LI-COR Biosciences, USA) and a Handy PEA chlorophyll fluorometer (Hansatech Instruments, UK) respectively. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were assayed using spectrophotometric methods (Zhao *et al.,* 2022). Proline content was determined using the acid-ninhydrin method (Misra *et al.,* 2022). Soluble sugar content was measured using the anthrone method (Prasitsom *et al.,* 2019).

Statistical Analysis

Statistical analysis was performed using R software (Team, R. C. 2021). Significant differences between treatment groups were determined using atwo-way ANOVA with Tukey's post hoc test ($p < 0.05$). Heatmaps and clustering analysis were performed using the heatmap.2 function in R. Principal component analysis (PCA) was performed using the prcomp function in R.

RESULTS AND DISCUSSION

RNA-seq analysis was performed on the leaf samples of three DT (CPF-253, CPF-246 and NSG-59) and one DS (SPF-234) sugarcane cultivars subjected to drought stress for 0, 7, and 14 days to identify the genes involved in drought tolerance. A total of 1.2 billion reads were obtained, and 98.6% of the reads passed the quality control criteria. The reads were mapped to the sugarcane reference genome, and 41,672 genes were quantified. Differential gene expression analysis revealed a significant variation in the number of differentially expressed genes (DEGs) between cultivars and time points. The DS cultivar SPF-234 showed the highest number of DEGs at all time points, while the DT cultivars showed a lower number of DEGs. The number of upregulated genes was higher than the number of downregulated genes in all cultivars and time points. Gene ontology (GO) analysis showed that the upregulated genes were enriched in stress response, oxidation-reduction process, and carbohydrate metabolism, while the downregulated genes were enriched in photosynthesis, chloroplast organization, and carbon fixation. Proteomic and metabolomic analysis of drought-tolerant and drought-sensitive sugarcane cultivars:

To complement the transcriptomic analysis, we performed proteomic and metabolomic analysis on the same leaf samples used for RNA-seq. A total of 4,869 proteins and 691 metabolites were identified and quantified. The abundance of the identified proteins and metabolites showed a significant variation between cultivars and time points.

Comparative analysis of DT and DS cultivars showed that the DT cultivars had higher levels of stress-related proteins, such as heat shock proteins, late embryogenesis abundant (LEA) proteins, and pathogenesis-related (PR) proteins, compared to the DS cultivar. The DT cultivars also showed higher levels of osmoprotectants, such as proline, soluble sugars, and betaine, compared to the DS cultivar. The DT cultivars had lower levels of reactive oxygen species (ROS) and higher levels of antioxidant enzymes, such as SOD, CAT, and POD, compared to the DS cultivar. The DT cultivars showed better photosynthetic performance, as indicated by higher levels of chlorophyll fluorescence, stomatal conductance, and leaf water potential, compared to the DS cultivar.

Functional characterization of candidate genes

CRISPR/Cas9 genome editing was used to knock out selected candidate genes in the DT cultivars to determine their role in drought tolerance. The sgRNA design and cloning were performed using the CRISPResso2 software (Chapa e*et al.,* 2020). The CRISPR constructs were transformed into sugarcane cultivars using Agrobacterium mediated transformation. Transgenic plants were selected using hygromycin resistance and verified by PCR and sequencing. Transgenic plants with knocked out candidate genes showed a significant reduction in drought tolerance compared to the wild-type plants. The physiological and molecular responses of the transgenic plants to drought stress were compared to those of the wild-type plants. The knockout of candidate genes involved in stress response, such as LEA and PR proteins, resulted in a significant reduction in osmoprotectant accumulation and antioxidant enzyme activity, leading to increased ROS accumulation and decreased photosynthetic performance (Misra *et al.,* 2022). The knockout of candidate genes involved in ROS detoxification, such as SOD and CAT, also resulted in increased ROS accumulation and decreased drought tolerance. Our study aimed to identify the molecular and physiological mechanisms underlying drought tolerance in sugarcane cultivars. We performed a comprehensive transcriptomic, proteomic, and metabolomic analysis on three DT and one DS sugarcane cultivars subjected to drought stress. Our results showed that the DT cultivars had a better response to drought stress compared to the DS cultivar, as indicated by higher levels of stress-related proteins, osmoprotectants, and antioxidant enzymes, and better photosynthetic performance. Our transcriptomic analysis showed that the up-regulated genes in the DT cultivars were enriched in stress response, oxidation-reduction process, and carbohydrate metabolism, while the down-regulated genes were enriched in photosynthesis, chloroplast organization, and carbon fixation. These findings suggest that the DT cultivars reprogram their gene expression in response to drought stress to enhance stress tolerance and conserve energy. Our proteomic and metabolomic analysis complemented the transcriptomic analysis by identifying the proteins and metabolites that were differentially accumulated between DT and DS cultivars under drought stress. The DT cultivars had higher levels of stress-related proteins, osmoprotectants, and antioxidant enzymes, which are known to play a critical role in stress tolerance. These findings provide further evidence for the importance of these proteins and metabolites in sugarcane drought tolerance. The functional characterization of candidate genes using CRISPR/Cas9 genome editing confirmed the role of selected candidate genes in drought tolerance. The knockout of genes involved in stress response, osmoprotectant accumulation, and antioxidant enzyme activity resulted in a significant reduction in drought tolerance, indicating their importance in sugarcane drought tolerance. In conclusion, our study provides valuable insights into the molecular and physiological mechanisms underlying drought tolerance in sugarcane cultivars. Our findings can be used to develop new strategies for enhancing drought tolerance in sugarcane and other crops. Further studies are needed to validate the role of the identified candidate genes and to develop molecular markers for marker-assisted breeding of drought-tolerant sugarcane cultivars.

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A REVIEW OF ENVIRONMENTAL EFFECTS ON SUGARCROPS

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ABSTRACT

Sugarcane is the second most important cash crop of Pakistan after cotton, belonging to *Saccharum* species. It is noted that patterns of trade influence elements of sugar production that have an impact on the environment. It is not the main intention to compare cane and beet systems, although comparisons are sometimes made for contextual purposes. The environmental impacts of the processing (but not cultivation) of sugar crops have been summarized previously and other texts on aspects of sugar production often include some coverage of environmental issues. However, this appears to be the first attempt to collate and review information on the environmental impacts of sugar production as a whole. The emphasis is on an environmental perspective although agronomic priorities are generally acknowledged where appropriate. The one area where a consistent difference in viewpoints has become apparent is in relation to soil quality. From an environmental perspective, soil nutrient balance is seen as degradation; this only tends to be the case from an agronomic perspective when the effect is sufficient to reduce yields. In particular, there appears to be a lack of data on air pollution (and human health impacts) arising from poorly managed aerial application of agrochemicals. Similarly, short-term water pollution events arising directly from an application of fertilizer or pesticide appear not to be reported. In broad terms, the literature on environmental aspects of cane sugar production is dominated by contributions from Australia, South Africa and to a lesser extent India, Mauritius and Pakistan. This should not be taken to suggest that environmental impacts, or measures to reduce them, are necessarily of greatest significance. The purpose of this review is to study environmental effects (ongoing cultivation, water, soil and air pollution) on sugarcane crops.

INTRODUCTION

Sugarcane, a tropical grass resembling bamboo and belonging to the Saccharum species, stores sucrose in its stem. There are two confirmed wild species, *S. spontaneum* found in tropical Oceania, Asia, and Africa, and *S. robustum* limited to Papua New Guinea and neighboring islands. The domesticated species include *S. officinarum* (noble cane, one of the first cultivated for chewing, now grown in limited locations), *S. edule* (found mainly in Melanesia and Indonesia), *S. barberi* (used for the first sugar production) and *S. sinense*. Most commercial sugarcane varieties are hybrids resulting from selective breeding of these species, which has significantly increased cane sugar yields from 1-1.5 t/ha to 8-17 t/ha during the 20th century, benefiting the sugar industry. The sugar industry is often praised for its positive environmental features, including the remarkable efficiency of sugarcane in converting solar energy to biomass. Sugarcane has the highest harvest index among all crops, meaning it utilizes a large proportion of the material grown in the field. This exceptional efficiency is attributed to several factors, as described by Alexander (1985) and Payne (1991).
Firstly, Saccharum species, including sugarcane, readily interbreed, providing a wide range of options for plant breeders and facilitating the spread of adaptive traits. Furthermore, sugarcane and related species employ the C4 photosynthetic pathway, which allows them to exploit lower concentrations of carbon dioxide and a wider range of light intensities, while eliminating photorespiration. Sugarcane has also been shown to utilize a broader range of wavelengths of solar radiation

within the visible spectrum compared to most plants. Physiological adaptations, including the use of sucrose as a principal photosynthate for easy carbohydrate translocation, contribute to the efficiency of sugarcane in fixing solar energy.

As a result of these adaptations, sugarcane fixes approximately four times as much solar energy as most temperate crops, leading to a yield potential of around 50 t dry matter/ha/year. This is supported by Paturau (1989) and UNEP (1982), which estimate that 1 million kcal of energy in the form of sugar requires only 0.07 ha of sugarcane for production, whereas the same amount of energy in the form of beef requires 7.70 ha. These findings highlight the remarkable yield efficiency of sugarcane as a crop, further emphasizing its positive environmental characteristics.

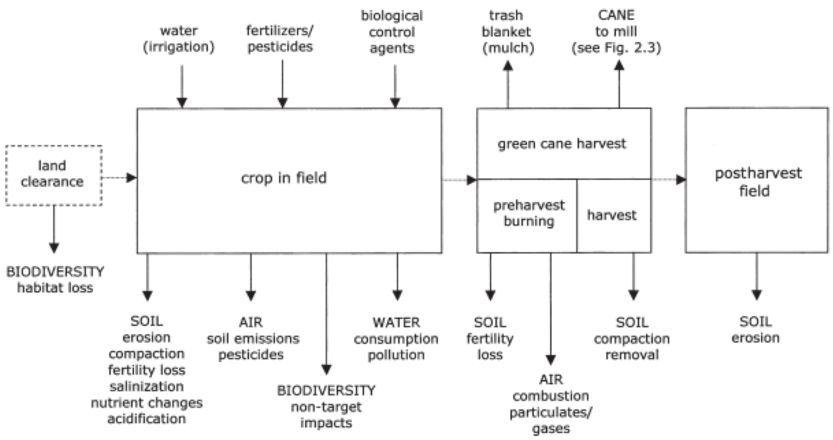

Figure-1 Sources of environmental impacts relative to key processes and inputs in the cultivation of sugarcane

Impacts on ongoing Cultivation

Sugar crop cultivation can have environmental impacts that extend beyond the farmer's field, potentially affecting biodiversity. These impacts can include waterway sedimentation from soil erosion and eutrophication resulting from nutrient leaching and runoff. Areas under cultivation generally have lower levels of indigenous species compared to adjacent natural habitats, although crop and soil invertebrates can exhibit considerable diversity, and micro-organismal biodiversity is often overlooked. The use of pesticides can directly harm non-target organisms and indirectly affect other species that rely on them for food or shelter. Inappropriate biological control methods, such as the introduction of non-native species like the mongoose in the Caribbean or the cane toad in Australia, can also have negative biodiversity impacts. However, responsible biological control programs can have positive effects. Concerns have been raised about the potential biodiversity impacts of cultivating transgenic crops, including sugar beet.

Impacts on Water

Cane cultivation is heavily reliant on irrigation in many areas, which has raised concerns about the increasing quantities of water used. In Pakistan, for instance, cane cultivation is seen as putting pressure on available groundwater resources. Similarly, in Australia, water extraction for cane irrigation has led to the overuse and degradation of river systems. Cane is known for its highwater consumption, with an estimated 7.5 Ml/ha of water needed for a cane crop of 100 t/ha. This demand can only be met by rainfall in some areas, leading to substantial irrigation requirements in others. Unfortunately, irrigation systems are often found to be inefficient, resulting in water wastage. Irrigation may also exacerbate other cultivation impacts, including soil salinization. While sugar beet is relatively insensitive to soil moisture, around one-fifth of the world's beet cultivation is still irrigated, which may be essential in some dry areas but provides little benefit in others.

The cultivation of sugar crops can result in pollution of watercourses and aquatic habitats due to agrochemicals and sediment runoff. Additionally, fertilizers applied to the crops may lead to nutrient leaching and contamination of ground water. These impacts can have far-reaching effects on downstream ecosystems, such as coastal zones. While examples of water quality impacts from cane cultivation are found in areas where the crop is grown, such as Australia, South America, and the USA, it can be challenging to attribute these impacts solely to cane growing due to other land uses. Similarly, concerns have been raised regarding beet cultivation, but it is difficult to pinpoint water pollution specifically to the cultivation of this crop since it is only one component of a broader crop rotation.

Impacts on Soil

Cultivating cane on slopes and leaving beet fields bare over winter are practices that can increase erosion risks, particularly in certain areas. The extent of erosion problems varies depending on local conditions. Soil erosion losses under sugarcane have been estimated to range from approximately 15 to over 500 tons per hectare per year, depending on the study (Prove et al., 1995). Beet fields left bare over winter can be vulnerable to both wind and water erosion, with estimates of soil losses ranging from 13 to 49 tons per acre per year due to wind erosion in the USA and 0.3 to 100 tons per hectare per year due to water erosion in European beet-growing regions (De Ploey, 1986; Morgan, 1986).

Harvesting crops can result in soil being removed from the field, in addition to erosion. In sugarcane cultivation, about 1-15% of the material delivered to the mill may consist of extraneous material, including soil. However, the nature of beet harvesting leads to larger quantities of soil being removed from the crop. Studies have estimated a soil 'tare' of 10-30% for harvested beet, with some suggesting soil losses of 9 tons per hectare per harvest (Poesen *et al*., 2001). Over large areas, these losses can accumulate to substantial amounts, with published estimates indicating 3 million tons per year for the EU and 1.2 million tons per year for Turkey alone (Oztas et al., 2002). Sugar crop cultivation can result in soil compaction, which increases bulk density and reduces porosity, negatively affecting soil fauna. Reduced porosity also leads to increased runoff, which exacerbates erosion problems. The risks of compaction associated with cane and beet cultivation differ according to the cultivation systems used (monoculture and rotation, respectively). Beet cultivation poses particular concerns due to the number of field operations used in field preparation and the fact that soils are often wet during harvesting. Sugar crop cultivation can also lead to other soil quality impacts, such as loss of soil organic matter, changes in nutrient levels, salinization, and acidification. Loss of organic matter and changes in nutrient levels occur in both cane and beet cultivation. Salinization, which is associated with poor water management and drainage, and acidification, mainly resulting from the application of inorganic fertilizers, are more prevalent in certain cane-growing areas than in beet cultivation. Combined impacts on soil quality can lead to a loss of fertility which is generally grown as a continuous monoculture. Several countries have experienced a decline in cane yields due to the loss of soil fertility.

Impacts on Air Quality
Air Pollution

Burning cane before harvest results in air pollution and contributes to environmental impacts. Additionally, the application of fertilizers can worsen nitrogenous emissions from sugar crop fields. Burning bagasse as a fuel source for cane processing operations can lead to unwanted emissions, although this is a form of utilizing a by-product and may be a more environmentally friendly option than other

alternatives. The wastes produced during the processing of cane and beet can also cause significant odor issues due to the emission of noxious gases.
The environmental impacts of the processing of sugar crops are summarized below, and their sources relative to key processes and inputs are illustrated in Figure-2 (for cane) and Figure-3 (for beet).

Positive Environmental Aspects of Sugar Production

Crop characteristics

Sugarcane is known for its high productivity in terms of yield per unit area and per unit of water consumed. Due to its ability to fix large amounts of atmospheric carbon, it has gained interest as a renewable fuel source, whether in the form of biomass or alcohol. The use of bagasse, the waste fiber from cane processing, as a fuel source in many regions has already made cane sugar production relatively carbon-neutral. Although sugar beet does not have the same level of productivity as cane, it has been explored as a potential biofuel source due to its efficient root system, which allows it to scavenge nutrients effectively.

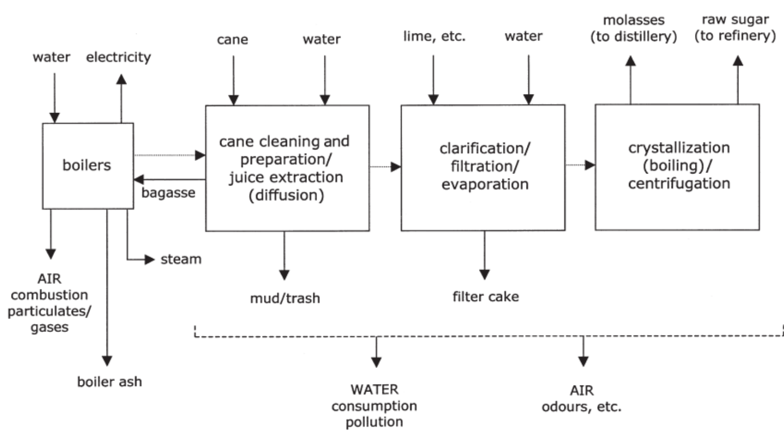

Figure-2 Sources of environmental impacts relative to key processes and inputs in the processing of sugarcane

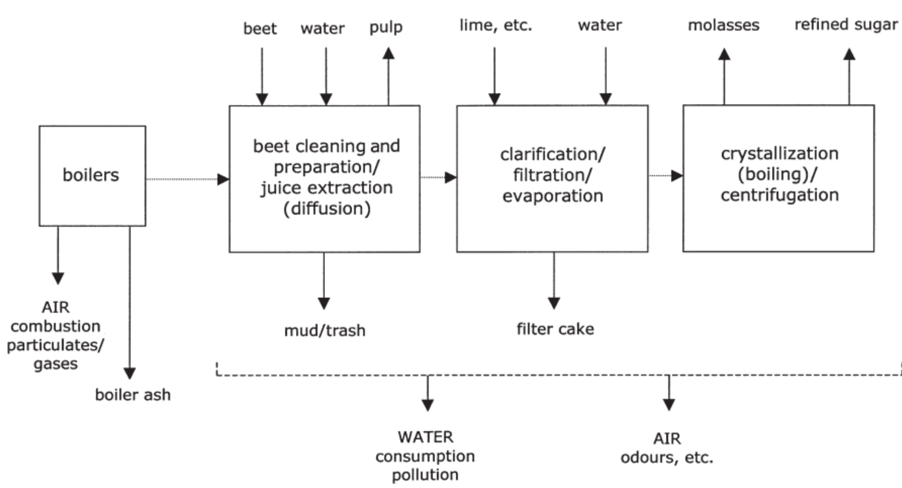

Figure-3 Sources of environmental impacts relative to key processes and inputs in the processing of sugar beet

By utilizing waste materials generated from sugar cultivation and processing, particularly from cane, the amount of waste output can be reduced, potentially mitigating the negative impacts of other sugar production activities. Utilizing by-products instead of environmentally damaging alternatives, such as mulches or soil amendments, can also have positive environmental effects. The burning of bagasse as a renewable fuel to generate power, for instance, can replace the consumption of fossil fuels. It should be acknowledged, however, that the utilization and further processing of by-products can have negative environmental consequences, making an overall cost-benefit analysis complex. Such concerns extend to waste and by-products from secondary processing activities. For instance, the use of molasses as a feedstock for alcohol production creates a secondary waste material, vinasse, which may be either a pollutant or a valuable by-product, depending on how it is handled.

Reducing the environmental impacts of Sugar Production

The effectiveness of measures to mitigate environmental impacts can be maximized when they are incorporated into a comprehensive and practical system of sustainable management. The subsequent sections outline several measures that can be implemented to address the diverse environmental impacts of different activities. The responsibility for natural resource management should be shared transparently between the government, the community, and the sugar industry. Appropriate incentives should be established to promote the protection of natural resources and their use in an ecologically sustainable manner, with a mix of motivational, voluntary, property-right, price-based, and regulatory instruments tailored to the specific issue and local, regional, and social characteristics. The community, along with natural resource users and beneficiaries, should contribute to providing incentives to sugar producers who are primarily responsible for protecting the environment.

CONCLUSION

Sugar is a product that most of us consume on a daily basis. Sugar production contributes to development in many poor countries, by producing employment and reliable incomes for many, there are a range of negative issues associated with its production. The demand for sugar has continued to rise steadily, increasing by about 70% in total since 1980. The environmental impacts of sugar production have been largely ignored. Sugarcane plantations in many tropical and sub-tropical countries have led to perhaps the largest losses of biodiversity of any single agricultural product.

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SUGAR INDUSTRY ABSTRACTS

Application of remote sensing and GIS for age differentiation of sugarcane crop classes in Argentina

Carmina Fandos, Federico José Soria, Pablo Scandaliaris, Javier Ignacio Carreras Baldrés, Eduardo Raul Romero and Jorge Scandaliaris

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1491–1499, 2019.

As sugarcane yield generally decreases with increasing ratoon number, "crop class" is one of the most important factors influencing sugarcane productivity. Sugarcane is often replanted every 4-5 years to maintain economic production, but this period may be longer or shorter depending on crop performance. Information about the crop classes of sugarcane on a regional scale is scarce, and sugarcane monoculture makes it difficult to identify where cane is re-established using satellite images. However, when sugarcane crops are rotated with other crops or interspersed with fallow periods, it facilitates the identification of renewals or new cane fields. The rotation with soybean crops is a common practice in the sugarcane fields of Tucumán province in Argentina. This paper aimed to differentiate the classes of sugarcane crops by using this soybean-sugarcane rotation system and the cane available for harvesting in 2018 in Tucumán. A multi-temporal analysis of categorized images was conducted using GIS techniques. The thematic layers showing soybean and sugarcane crops for the period 20102018 were used as a baseline. These layers were the result of the multispectral classification of satellite images. Results indicated that 44% of the total sugarcane area available for the 2018 harvest in Tucumán had been rotated with soybean crops at least once between 2010 and 2017. Of this total, 21% area was in plant cane, 36% was in first to third ratoons and 43% was in fourth to seventh ratoon. The percentage distribution of different crop class groups reveals an aging of the sugarcane fields in Tucumán. Nevertheless, the analysis in those areas with high percentages of soybean/ sugarcane rotation show a predominance of young sugarcane fields in areas with the most serious water-deficit problems. This methodology contributes to the identification of areas under different crop age groups in sugarcane, information that allows for improved estimates of sugarcane yield given the association between the lower or higher age of sugarcane with the increase or decrease in production, respectively.

Reaction of sugarcane clones to cold using morphological, hysiological and biochemical characteristics related to cold resistance

M Fooladvand, A Ebrahimi, MR Jahromi and VS Joni

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 767–772, 2019.

Sugarcane (*Saccharum officinarum*) is a plant that grows to around 32°N and 32°S in different parts of the world. Sugarcane resistance to frost is not very high, and it limits its ability to be grown in sub-tropical regions where there is the probability of a cold occurrence below zero.
Since the development and improvement of plants for resistance to stress and thei r response to stress are strongly dependent on the genes involved in the resistance, these genes must first be identified. The most effective way to identify the genes involved in resistance to stress, including cold, is to examine the products (morphological, physiological and biochemical) of the gene or genes related to tolerance to cold. A morphological study in December 2014, and 1 week after a cold stress of -3°C was carried out where 454 sugarcane clones cultivated in an 18-m plot were rated for their tolerance to cold. The traits studied to identify tolerant clones included: green canopy percentage, dry canopy

percentage, leaf sheath status for vegetation, presence or absence of pith structure. Tolerant clones and clones sensitive to cold were identified. 54 clones resistant and tolerant to cold were selected and then planted in the second stage in October 2015 in a randomized complete-block design (RCBD) for further study. 15 months later and in January 2016, 1 week after occurrence of cold, clones were studied using the morphological indexes. The highest cold tolerance was in BR00-01 with an average of 13.9 for the "cold tolerance" and 88.76% for the "purity of syrup" (PTY) traits. Measurement of biochemical traits in tolerant and susceptible cultivars showed that morphological characteristics are related to cold resistance.

Diagnosis and sanitary status of sugarcane propagation material in different stages of the Vitro plants project during 2014/2018

CM Joya, RP Bertani, C Funes, S Chaves, DD Henriquez, MF Perera, V González, AP Castagnaro and PA Digonzelli

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 470–475, 2019

The Vitroplants Project of Estación Experimental Agroindustrial Obispo Colombres (EEAOC) has produced high-quality seed cane through *in vitro* meristem-culture techniques and AS Noguera micropropagation since 2001. In order to select healthy seed cane, it is necessary to verify the absence of pathogens both in the laboratory (meristem donor plants and micropropagated plantlets) and in seed-cane field propagation (Basic, Registered and Certified nurseries). In this study, results of molecular techniques (PCR and RT-PCR) in the laboratory stage to detect five sugarcane diseases of major importance in Tucumán, Argentina, ratoon stunting disease (RSD), leaf scald (LS), red stripe (RS), yellow leaf disease (YLD) and mosaic virus (MV), are presented. In addition, evaluations of a Tissue-Blot Immunoassay (TBIA) technique for diagnosing systemic bacterial diseases (RSD and LS) in the field propagation stage are given. During the five-year period analyzed, the disease that was the most common in micropropagated plantlets was YLD (17.57%); while in the meristem donor-plant collection the highest incidences were for RS (41.67%) and YLD (16.67%). RSD incidence level (number of infected stalks/total number of stalks) evaluated in the Registered nurseries during 2014/2018 resulted in a minimum value of 0.20% (2014) and a maximum of 0.43% (2016). In addition, LS incidence levels were between 0.09% (2016) and 0.33% (2017). In Certified nurseries, RSD incidence levels reached a maximum value (0.29%) in 2016 and a minimum (0.15%) in 2017. These results demonstrate the efficiency of the scheme of production and multiplication of high-quality seed cane implemented in the Vitroplants Project and drives a significant improvement in sugarcane crop sanitary conditions and yield. It is important to have access to sensitive diagnostic methods that allow detection of very low levels of pathogens.

Production of unicelular protein and reduction in contamination from the amount of vinasse

María F Lencina, Ada J Robinson, Samanta M Rearte and Patricia M Albarracín

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1646–1651, 2019.

Vinasse from ethanol distilleries is a highly contaminating industrial waste and is produced in large quantities. Through biotechnology, it can be used to produce protein while reducing contamination. A strain of the yeast *Candida utilis* was isolated and grown under different concentrations of aqueous formulations of vinasse, from 10 to 50% [mL/mL]. Experimental trials were carried out in "batch" reactor with vinasse. Growth of the microorganism was monitored using a carbon dioxide sensor (CO), and parameters measured were: total nitrogen, COD (chemical demand of oxygen), pH and conductivity, at the beginning and end of each trial. Every 2 hours, the OD (optical density in a liquid environment) was measured to determine cellular concentration. In total nitrogen there was an enrichment of 136% in the environment composed of 50% [mL/mL] of vinasse; the average removal of the chemical demand of oxygen was 43%; pH was 4% lower than at the beginning; and the conductivity was reduced to 9%. Monitoring of yeast growth by measuring the carbon dioxide concentration through time and the OD, showed a fermentation period of 21 hours. *Candida utilis* strain can develop in a batch reactor with vinasse, in aqueous solutions of 50% [mL/mL] and produce a protein enrichment as well as removing COD. The process reduces the contamination of the main industrial effluent of Tucumán.

Brazilian monetary carbon credits (CBio) optimized using sustainable mill technologies

José L Olivério, Fernando C Boscariol and Reinaldo E Ferreira

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 165–175, 2019.

In the 1990s, the Brazilian sucroenergy sector began to combine the positive externalities with sustainability. Until recently, such externalities were merely qualitative, without any financial benefit. In 2017, Brazilian legislation (RenovaBio) recognized the ethanol environmental qualities and defined criteria to transform reductions in Greenhouse Gases Emissions (GHGE) into a financial asset, a new income stream that can be earned by the sugarcane mill. Moreover, as the more efficient the mills can be in emissions reduction, the higher will be the monetary value of the ethanol traded. RenovaBio introduced a co-product of the sucroenergy mill: the *CBio-Crédito de Descarbonização por Biocombustíveis* (CBio–Decarbonization Credit Generated by Biofuels), where one CBio monetarily corresponds to one tonne of avoided CO emissions, an environmental financial reward whose value will be recognized and defined by the stockexchange market. This new co-product, CBio, will give an added impulse to the development of more efficient sugarcane mills regarding GHGE reduction, and these mills will utilize more sustainable equipment, processes and technologies. The Dedini Sustainable Mill (DSM), introduced to the Brazilian market in 2009 and internationally in 2010, has evolved with innovations since then to become a highly appropriate design to be used in new and/or retrofitted sugarcane mills to increase sustainability. The purpose of this work is to update the DSM and to determine if this design meets the current and future enlarged concepts of RenovaBio. The conclusion is that the DSM conforms to the Brazillian RenovaBio mill concept.

Energy characterization of sugarcane bagasse in Tucumán, Argentina

G del H Zamora Rueda, MG Mistretta, CE Gutierrez, MA Golato, D Paz and GJ Cárdenas

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1000–1004, 2019

The most important waste product of the sugar-alcohol industry of Tucuman is sugarcane bagasse, which is used as the main fuel for the generation of thermal and electrical energy in the processes of sugar and alcohol manufacturing. Its use as fuel in steam boilers requires its continuous energy characterization, mainly due to the variability of the climatic conditions that affect the soil and crops, as well as the different cane cultivars and harvesting systems. The aim of this study was to characterize the energy content of the bagasse samples collected from sugar mills in Tucuman during the 2017 and at the beginning of the 2018 harvest. The methodologies used follow the standards: ASTMD 5142-02 (thermogravimetric), ASTMD 2015-96 (higher calorific value), ASTME 776-87 and ASTMD 3177-02 (chlorine and sulfur, respectively). The average results obtained were: moisture of 52.54% with coefficient of variation (CV) of 5.30%; ash 5.77% on dry basis (d.b.) with CV of 2,57%; volatile solids 77.21% (d.b.) with CV of 2,25%; fixed carbon 16.65% (d.b.) with CV of 5.00%; higher calorific value 17,584 kJ/kg (d.b.) with CV of 2.77%; chlorine 0.14% (d.b.) with CV of 2.10%; and sulfur 0.16% (d.b.) with CV of 2.47%. These parameters are useful for the calculation and re-designing the steam generator systems.

Soil microbial community responses to different sugarcane management strategies as revealed by 16S metagenomics

PD Fontana, L Orru, CA Fontana, PS Cocconcelli, GM Vignolo and SM Salazar

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 502–510, 2019.

Sugarcane cultivation in Argentina is distributed in three geographic regions: Tucumán, Northern (Salta and Jujuy) and Littoral (Santa Fe and Misiones), covering about 376,223 ha. Tucumán has traditionally been the most important region with 68% of the total production. Since new agricultural techniques, such as green-cane harvesting and sugarcane crop rotation with soybean, were implemented in the last decade, changes in the agroecosystem of sugarcane, specific pathosystems and epidemiological parameters have been observed. A 16S metagenomics approach to investigate total bacterial communities associated with sugarcane rhizospheric soil when soybean was the predecessor crop in a cultivation area with a high incidence of red stripe disease was applied; soil from sugarcane monoculture was also included. Two commercial sugarcane cultivars (*Saccharum* spp. hybrids) with differential responses to red stripe infection (tolerant and susceptible) were evaluated. Sampling was carried out in 20132014 and in 2014-2015 (first and second ratoon, respectively) at 30, 90 and 180 days after harvest. Total soil DNA was obtained using FastPrep® technology. The 16S RNA gene (variable region V3-V4) was sequenced using a MiSeq platform Illumina. Taxonomic assignment revealed Bacillales, Rhyzobiales, Rhodospirilliales, Xanthomonadales and Acidobacteriales among the most abundant orders in all samples. Soil samples from sugarcane without soybean rotation showed a marked decrease in Bacillaes, Rhizobiales and Sphingomonadales. Cluster analysis grouped together samples from the tolerant genotype, while those from the susceptible genotype formed two subgroups that were distinguished according to sampling time after harvest. The analysis showed that samples from sugarcane under monoculture were grouped distant to the rest of the samples showing different microbiota composition. The sugarcane rhizosphere microbiome and its biotechnological potential open a new opportunity in the concept of sustainable crop management. The data contribute significant knowledge about the microbial diversity in agricultural ecosystems.

Ramu stunt virus: genomic diversity across Papua New Guinea

Kathy Braithwaite, Chuong Ngo, Samuel Grinstead and Dimitre Mollov

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 948–952, 2019.

Ramu stunt disease, caused by the Ramu stunt virus (RmSV), is a devastating disease of sugarcane and currently confined to Papua New Guinea. Due to the proximity of other large sugarcane-growing areas, the disease is a quarantine concern outside of Papua New Guinea. In this study, the full-length genomes of five RmSV isolates from noble (*Saccharum officinarum*) and commercial (*Saccharum* spp. hybrids) sugarcanes were sequenced. Analyses revealed genomic differences among isolates and divergences were observed in the six genomic segments among all isolates. This information is useful to validate and improve existing diagnostic RT-PCR assays, as well as to better understand virus etiology.

Effects of tube dimensions and operating conditions on the heat-transfer coefficient of rising-film evaporators

OP Thaval, R Broadfoot, GA Kent and DW Rackemann

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 41–52, 2019.

Experimental investigations were undertaken in a single-tube rising-film pilot evaporator rig to determine the effects of tube dimensions and operating conditions on the heat-transfer coefficient. Nine tubes of different lengths and diameters were tested over a wide range of operating conditions that mimicked the conditions in the 1st, 3rd and 5th effect positions of a quintuple set of factory evaporators. For each set of processing conditions, heat-transfer rates at four different juice levels in the tube were measured. An optimum juice level existed to maximise the heat-transfer coefficient. 560 tests at different processing conditions were undertaken comprising an initial series of tests (based on the nine tubes) and a repeat series of tests (based on a four-tube subset). The study provided information on the boiling characteristics in different sections of the tube. The highest overall heat transfer rates were achieved when bubble/slug flow boiling conditions were achieved along the entire length of the tube. Conditions where there is insufficient wetting in the top section of the tube provided the lowest heat transfer rates. The information obtained provides a better understanding of how to maximise the operational performance and design of industrial Robert evaporators.

Effects of the host plants *Zizania latifolia* (Manchurian wild rice) and sweet corn on growth and development of the sugarcane shoot borer *Chilo infuscatellus* Snellen

DW Li, ZQ Qin, YW Luo, XP Song, CY Wei, HZ Ding and C Lin

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 330–334, 2019

The effects of Manchurian wild rice (*Zizania latifolia*) and sweet corn (*Zea mays*) on the growth and development of *Chilo infuscatellus* Snellen (Lepidoptera, Crambidae) were studied to provide data references for mass rearing of this borer and control techniques. Under laboratory conditions (26 ± 1°C), single newly hatched larvae were selected and put into the culture dish and fed with *Z. latifolia* or sweet corn, which were replaced every 3 days. The developmental and survival rates of *C. infuscatellus* larvae were observed every day. The sex and the weight of the pupa were recorded on the second day after the larva turned into pupa. The results showed that when feeding on *Z. latifolia* and sweet corn, the complete development duration of *C. infuscatellus* males was 22.54 and 26.29 days, respectively, while for females it was 24.69 and 29.77 days, respectively. The average duration for *C. infuscatellus* pupae were 8.68 days and 8.94 days, respectively, and the average life span of adults was 4.12 and 3.90 days, respectively. There was a significant difference between the average weight of pupae fed on wild rice and sweet corn with 0.0736 and 0.0651 g, respectively. The average survival rates from larvae to pupae were 49.3% and 38.0%, and those from larvae to adult were 46.0% and 36.0%, respectively. The sex ratios of *C. infuscatellus* fed on wild rice and sweet corn were 0.70 and 0.55, respectively. The results showed that feeding this borer on wild rice in laboratory conditions is more beneficial for its growth and development.

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INTERNATIONAL EVENTS CALENDAR

2023 CONFERENCES & MEETINGS

February 11-14, 2023	10th International Conference on Sugar and Integrated Industries (ICSII 2023), Luxor, Egypt
February 16-25, 2023	International Society of Sugarcane Technologists (ISSCT) XXXI Congress at Hyderabad India
Feb 27 - Mar 02, 2023	American Society of Sugar Beet Technologists (ASSBT) 2023 Biennial Meeting, Savannah, GA. USA
Mar 31 - Apr 02, 2023	8th International Symposium of International Society of Rare Sugars Takamatsu, Kagawa, Japan
April 17-20, 2023	Geneva Sugar and Biofuels Conference, Fairmont Grand Hotel Geneva, Switzerland
April 18-21, 2023	44th Australian Society of Sugarcane Technologists (ASSCT) Conference, Cairns Australia
May 07-11, 2023	Sugar Industry Technologists Annual Technical Meeting, New Orleans, USA
June 12-14, 2023	33rd ICUMSA Session, Friedrich-Wilhelm-Raiffeisen-Platz 1, 1020 Wien Vienna, Austria
June 13-15, 2023	American Society of Sugar Cane Technologists (ASSCT) Annual Joint Meeting Westin Savannah Harbor Savannah, GA. USA
June 23-26, 2023	25th Carbo Solutions International Sugar Conference Casablanca Morocco
July 5-7, 2023	2nd International Conference on Cane and Sugar 2023, Aswin Grand Convention Hotel Talad Bangkhen, Lak Si, Bangkok Thailand
August 4-8, 2023	38th International Sweetener Symposium Meritage Resort, Bordeaux Way, Napa, CA USA
August 15-17, 2023	95th South African Sugar Technologists' Association (SASTA) Congress Durban, South Africa
September 18-20, 2023	12th Congress ATALAC, Heredia Costa Rica
October 03-05, 2023	16th Annual Sugar & Ethanol Asia Conference Bangkok, Thailand
November 21-22, 2023	32nd International Sugar Organization (ISO) INTERNATIONAL SEMINAR, London United Kingdom

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