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Determinants of Adoption of Sawah Rice Technology among Farmers in Ashanti Region of Ghana

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Abstract: The purpose of this study is to identify the determinants of adoption of sawah technology in Ashanti region of Ghana. It identifies those independent variables that explain adoption and can also facilitate further dissemination and adoption of sawah technology. Sawah refers to leveled rice field surrounded by banks with inlet and outlet for irrigation and drainage. This study uses data from 108 farmers randomly selected from 198 sawah farmers in the study area. Regression analysis showed that age, educational level and year of experience in rice production were the personal factors that determined the adoption of sawah technology among the respondents. The result further showed that contact with extension agents, attendance in previous sawah trainings, land tenure arrangement, yield from sawah farm and attributes of sawah technology determined adoption. This study suggested that adoption of sawah technology should be targeted at young, educated and experienced farmers. Also, security of land must be ascertained by farmers in order to enhance adoption, continuous and sustained use of sawah technology.

Key words: Adoption, Ashanti, determinants, farmers, sawah.

1. Introduction

The agriculture sector in Ghana continues to serve the traditional role of providing food security, supplying raw materials to industry, creation of employment opportunities, and the earning of foreign exchange. This sector is still the largest foreign exchange earner and the largest contributor to Ghana's Gross Domestic Product (GDP). Its dominant role in the economy makes this sector a target for national development programmes and strategies [1, 2]. Ghana is 51% self-sufficient in cereal production while rice self-sufficiency is estimated at 30% in 2009 [2]. Rice is an important cereal to Ghana's economy and the second most important cereal next to maize in terms of consumption. Rice constitutes 58% of all cereal imports. The rice import bill is estimated at US$500 million annually and has become a source of concern to government. Ghana's increasing dependency on rice imports and the consequent negative impact on foreign exchange balance will continue to increase if there is no significant strategy and policy shift in support of the local rice industry [2].

Ghana was found to have a comparative advantage in the production of paddy rice over the other countries in the sub-region [3]. Rain-fed rice contributes 84% of total current production, generating average paddy yields of 1.0-2.4 metric tons per hectare. Irrigated production totals only 16%, but produces average paddy yields of 4.5 metric tons per hectare. Due to poor yields from rain-fed rice and the lack of irrigation facilities, domestic rice production

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has not grown as fast as domestic demand. As a result, rice imports from Thailand, Vietnam, the U.S., India and Pakistan have grown considerably to fulfill Ghana's increasing demand and preferences. From a steady level of 7-8 kg per year before 1990, per capita rice consumption increased to 11.5 kg per year on average during the 1990s and climbed considerably to 27 kg per year for the period from 2001 to 2005 [4]. Future increases are projected by the Ministry of Food and Agriculture (MOFA) based on a combination of overall population growth, rising income, and increasing urbanization. Based on demographic trends and income growth, Ghana's Ministry of Food and Agriculture estimates that demand for rice in Ghana will increase at a compound annual growth rate of 11.8% from 939,920 metric tons to 1,644,221 metric tons between 2010 and 2015.

In view of food security and foreign currency savings, increased production of domestic rice with higher competitiveness against imported rice is paramount to Ghana's agricultural sector development [5]. Improvement in rice productivity potential will no doubt play a critical role in feeding the population that is expected to double during the next two decades. Therefore, there is a need to support farmers to increase rice productivity rather than acreage cultivated, if Ghana is to meet the short-fall in rice production.

In addressing, the challenges faced by farmers in increasing rice production, sawah rice technology was introduced to farmers in Ghana. Sawah refers to leveled rice field surrounded by banks with inlet and outlet for irrigation and drainage. The introduction of sawah technology with soil improvement, water management and high yielding characteristics has been viewed as a strategy to increase and maintain rice production levels. Sawah as a low-cost innovation that is highly sustainable, not requiring large capital investments and relatively easy to implement can help poor farm households become more productive by improving the fertility of the soil and increasing yields [6]. Lowland sawah systems can sustainably produce more than 2 t/ha paddy without any chemical fertilizer application [7, 8]. In addition, lowland sawah systems can support rice cultivation continuously for decades, centuries or more without any fallow period [6].

According to Tsujimoto et al. [9], sawah approach offers low-cost irrigation and water control for rice intensification with sustainable paddy yield of more than 4 t/ha but with improved agronomic practices, such as the System of Rice Intensification (SRI) with the sawah systems, paddy yield can reach more than 10 t/ha. The sawah approach involves site selection and site-specific sawah system design; skills for cost-effective sawah system development using a small hydro-power tiller; co-ordination of farmers' group formation and land tenure arrangements to sustain sawah development; sawah-based rice agronomy, including best variety selection and management to realize at least the sustainable paddy yield of more than 4 t/ha, and establishment of institutional training and dissemination systems for sawah eco-technology transfer [10].

Various research programmes and extension projects have been carried out to improve the adoption and diffusion of sawah rice technology ranging from on-farm adoption trials, on the job training and workshops. This study intends to examine the determinants of adoption of sawah technology among the farmers in Ashanti region of Ghana. A wide range of variables influence adoption of such technology. It is important to understand the role of these factors to ensure the sustainable development and use of sawah technology. To achieve self-sufficiency in rice production, sawah technology should be disseminated to farmers across all regions in Ghana and the implication is that, in disseminating sawah technology, policy makers must bear the major findings of this study in mind to enhance effective adoption.

2. Literature Review

Increasing agricultural productivity is critical to economic growth and development of any nation and this can be achieved through the introduction of improved agricultural technologies and management systems [11]. Adoption of improved agricultural technologies has become a critical avenue for increasing productivity in developing countries [12], but subject to various factors, the farmers consider before the adoption. Adoption is the mental process an individual passes through from first hearing about an innovation to final adoption [13]. Adoption studies have consistently emphasized the importance of various farmer and farm characteristics in determining whether such technologies will be adopted. The decision of a farmer to adopt a technology is complex and involved two mutually exclusive processes; the first involves making the decision to adopt the specific technology in the first place, while the second involves deciding on the level or intensity of use of the same technology [14]. According to Mcdonald and Brown [15], farmers may reject or abandon many technologies, which have been proved useful, and adopt others in their place since they consider a variety of factors in deciding whether or not to adopt particular innovation. On the other hand, after adopting a technology, farmers also decide on the level at which they use the technology.

Quite a number of studies have been carried out to identify the factors that determine the adoption and the level of adoption of agricultural technologies. All these studies have reported both the farmers-specific factors, farm-specific factors, institutional factors, innovation-specific factors, economic factors and non-economic factors. Anderson and Thampapillai [16] found that a wide variety of factors including land tenure arrangements, access to credit and farmers risk attitudes influence the rationality of adopting soil conservation practices among farmers. Hwang et al. [17] also reported that poor access to credit and lack of secure tenure, as well as low output prices, were limiting the adoption of soil conservation practices among farmers. Sall et al. [14] reported that both farmers' perceptions, as well as farm and farmer characteristics, were found to be important in determining the decision to adopt and the intensity of adoption of the improved rice varieties. According to De Souza Filho et al. [18], membership of farmers' organizations, contacts with nongovernmental organizations (NGOs), availability of family labor, soil conditions and farm size determine the level of adoption of agricultural technology. Faltermeier [19] also reported that access to credit, project involvement, family land/labour ratio, age, reported good results, as well as soil type and retention capacity influence the adoption decision of bunds in the lowland rice production systems.

Thapa and Rasul [20] found that institutional support, including land tenure, extension services and credit facilities, productive resource base and the distance to the market and service centres were found to be the major factors influencing agricultural systems. Motivation by Governmental Organizations (GOs) and Non Governmental Organizations (NGOs), motivation by community members and farmers' groups, attendance in training, also determined the adoption of technology [21]. According to Reimer et al. [22], perceived high levels of relative advantage (e.g., reduced inputs, time-savings and on-farm and environmental benefits), compatibility (with farm system and needs of producer) and observability (observing practice's advantages) are the most important in increasing adoption of conservation practices. To He et al. [23], farmers' educational background, active labor force size, contacts with extension, credit obtained, assistance obtained, technical training received and positive attitudes towards technology are some of the variables that have positive effects on adoption of technology. Lapar and Ehui [24] reported that farmers who are more educated, have higher income, and have access to credit are more likely to adopt the dual-purpose forages. Adrian et al. [25] found out that attitude towards agricultural technologies, perceptions of net benefit, farm size and farmer educational levels positively influenced the intention to adopt precision agriculture technologies.

3. Methods

3.1 Study Area

The study was carried out in Ashanti region of Ghana. Ghana is located on the west coast of Africa, about 750 km North of the equator 800 N, 200 W. The population in 2010 is estimated at 24,339,838, with a population growth rate estimated at about 1.8%. Ghana has a total area of 239,460 km^2 with land area of 230,020 km^2. Land use pattern is made up of arable land (6.26%), permanent crops (9.67%) and others (74.07%). The study was carried out in Ashanti region of Ghana. The Ashanti region is centrally located in the Middle belt of Ghana. It lies between longitudes 0.15 W and 2.25 W, and latitudes 5.50 N and 7.46 N. The region shares boundaries with four of the ten political regions, Brong-Ahafo in the North, Eastern region in the East, central region in the South and Western region in the SouthWest. The region occupies a total land area of 24,389 km^2 representing 10.2% of the total land area of Ghana. It is the third largest region after Northern (70,384 km^2) and Brong Ahafo (39,557 km^2) regions. The region has a population density of 148.1 persons/m^2, the third after Greater Accra and Central Regions. More than half of the region lies within the wet, semi-equatorial forest zone. Agriculture provides employment to more than half of the economically active population in the region. The major occupation in all the districts is agriculture/animal husbandry/forestry. The region has an average annual rainfall of 1,270 mm and two rainy seasons. The major rainy season starts in March, with a major pick in May. There is a slight drop in July and a pick in August, tapering off in November. December to February is dry, hot and dusty. The average daily temperature is about 27 °C Celsius. Much of the region is situated between 150 m and 300 m above sea level.

3.2 Sampling and Data Collection

Sites for dissemination of sawah technology were carefully selected based on the availability of inland valleys suitable for production. The availability of inland valley is a prerequisite for the adoption of sawah technology. Data used in this study were collected at the sawah sites within Ashanti region of Ghana namely: Adugyama, Amaekrom, Asuade, Baanekrom, Biemso 1, Biemso 2, Nsutem, Potrikrom and Sokwae. A list of rice farmers in the villages where sawah system was disseminated was compiled. A total of 108 sawah farmers were randomly selected from the population of 198. A well structured interview guide was used to elicit information from the farmers. The data for this study were obtained from a survey using face-to-face interview with the randomly selected sawah farmers in 2011.

3.3 Variable Selection and Measures

3.3.1 Dependent Variable

The dependent variable for this study is adoption of sawah technology. This is determined by listing all the aspect of sawah technology similar to Marenya and Barrett [26] and asked farmers to indicate their level of adoption. The aspects of sawah technology are bund construction, power tiller use and puddling, levelling, smoothening, nursery, canal construction, irrigation and flooding, dyke construction and use of sand bags. This was defined on a 3-point Likert Scale of full adoption (3), partial adoption (2) and discontinued/not adopted (1) following Alarima et al. [27]. Scale scores were computed by summing across responses to items in the scale.

3.3.2 Explanatory Variables and Hypotheses

As suggested by literature [28], adoption of agricultural technology is affected by social, economic and non-economic factors. The following are the explanatory variables that are hypothesized to influence adoption of sawah technology among the farmers in the study area. These variables ranged from personal factors, institutional factors, farming factors to the attributes of innovation (sawah technology) as shown in Table 1.

Personal Factors

Age: this was measured in years as a continuous variable. It is hypothesized that young farmers have a greater chance of absorbing and applying new technology and older people will be less likely to adopt, therefore age is expected to have significant influence on adoption.

Educational Level: this measures the level of education of the farmer. This was measured ordinally as: no formal education, primary education, secondary education and tertiary education. It is expected that farmers with higher levels of educational attainment are more likely to adopt new technologies than less educated farmers. Hence, it is expected that educational level has a significant impact on adoption of sawah technology.

Household Size: this measures the size of the family. Household size was determined by the actual number of persons in a household. A relatively large household size of the farmers could serve as a viable source of farm labor, hence it is hypothesized that household size has a significant influence on the adoption of sawah technology.

Farm Size: farm size measures the area of land put into sawah production. This was measured using a geographic positioning systems (GPS) instrument in hectares. It is expected that size of farm dedicated to sawah production will significantly affect level of adoption.

Table 1 Description of the variables of the study.

Acronym	Description	Measurement	Min	Max	Mean	SD
ADOP	Adoption level	3-point likert scale of full adoption (3), partial adoption (2) and discontinued/not adopted (1)	10.00	24.00	17.80	3.75
Personal factors						
AGG	Age	Continuous variable in years	26	62	39.96	7.69
EDD	Educational level	No formal education (1), Primary education (2), secondary education (3), and Tertiary education (4).	Mostly Secondary Education			
HHSIZ	Household size	Number of persons in the household	3	16	6.11	3.44
FSZ	Farm Size	Continuous variable in hectares	0.01	1.00	0.37	0.28
YREXP	Years of experience	Continuous variable in years	1	34	11.93	8.79
LABR	Labour source	Family labour (1), hired labour (2) and both family and hired labour (3)	Mostly family labour			
INCM	Income	Continuous variable in Cedi	630.00	7,303.00	3,043.80 (2,029.2 USD)	1,728.38
Institutional factors						
EXTN	Contact with Extension Agents	Dummy (1 if yes, 0 if no)	0	1	0.96	0.19
TRN	Attendance in training on Sawah	Dummy (1 if yes, 0 if no)	0	1	0.65	0.48
ASSM	Membership of farmers associations	Dummy (1 if yes, 0 if no)	0	1	0.89	0.31
Farming factors						
LTNR	Land tenure	Inheritance (1) and rentals (2)	Mostly by rent			
YELD	Yield	Continuous variable in kg	720	8,040	3,406.15	1,940
ATTR Attributes of innovation						
High yield		Dummy (1 if yes, 0 if no)	0	1	0.96	0.20
Disease and pest control		Dummy (1 if yes, 0 if no)	0	1	0.96	0.20
Fertilizer management		Dummy (1 if yes, 0 if no)	0	1	0.94	0.24
Water management		Dummy (1 if yes, 0 if no)	0	1	0.92	0.27
Weed control		Dummy (1 if yes, 0 if no)	0	1	0.92	0.27
Good tillering		Dummy (1 if yes, 0 if no)	0	1	0.81	0.039

Years of experience: this measures the number of years farmers have been involved in rice production. It is expected that farmers with long years of experience in rice production will be in better position to adopt sawah technology and hence it is hypothesised that years of experience has a significant influence on the adoption of sawah technology.

Labour source: labor source measures the source of labour available to the farmer. This was measured ordinally as: family labour, hired labour and combination of family and hired labour. It is expected that labour source will eventually affect the profit margin of the farmer. Thus, labour is expected to have a significant influence on the adoption of sawah [26].

Income: income was assessed as the total amount realized from both on-farm and off-farm activities in a given year in the local currency (Cedi). The local currency was converted to the dollar using the prevailing exchange rate at the time of this survey. Higher level of the income implies the ability to invest in sawah technology especially the purchase of tools like power tiller which is the only power-driven tool that is effectively being used for sawah activities and other inputs. A significant relationship is expected between adoption of sawah technology and income.

Institutional Factors

Contact with Extension Agents: this is measured as a dummy variable which measures whether or not the farmer has contact with extension agents (1 if yes, 0 if no). Farmers who have frequent contacts with extension agents and easy access to information about sawah technology can regularly upgrade their knowledge of technology. They will be able to relay their problems and challenges, they face to the extension agents thereby improving on their farming activities. It is expected that contact with extension agents will significantly influence adoption.

Attendance in previous Sawah training: this is measured as a dummy variable which measures whether or not the farmer has attended a previous training on sawah development (1 if yes, 0 if no). As in the case of education, farmers who have attended previous trainings on sawah technology will have a better understanding of sawah and hence it will have significant influence on their level of adoption.

Membership of farmers associations: this is measured as a dummy variable which measures whether or not the farmer belongs to a farmers' association (1 if yes, 0 if no). Farmers in rural areas form farmers associations and groups. This helps them in accessing loans and credit facilities and other farm inputs such as fertilizer, seed and simple farm tools from the government and other Non Governmental Organizations (NGOs), hence, it is expected to have significant effect on adoption.

Farming Factors

Land tenure: land tenure measures status of land ownership. This was measured ordinally as inheritance for those that use the land belonging to their family and rentals for those that hired the land they use for sawah development. In the literature, there are divergent views on the importance of tenural arrangement as it affects adoption decisions. Some reported that land tenure has effect on adoption [29] while other reported no effect on adoption [30], hence, it is difficult to predict whether land tenure will have a significant effect on adoption. However, for the purpose of this study, it is hypothesized that land tenure will significantly influence adoption.

Yield: the yield was determined by measuring in kilogram, the paddy harvested from the cultivated sawah area. High yield from sawah farm is expected to increase the level of adoption of sawah technology. A significant relationship is expected between adoption of sawah technology and yield.

Attributes of Innovation

This was determined by asking the farmers to indicate the attribute of sawah technology that motivated them to adopt the technology. This is measured as a dummy variable as 1 if yes, 0 if no for the identified attributes of sawah which include high yield, disease and pest control, fertilizer management, water management, weed control and good tillering [6, 27]. It is hypothesized that positive perception of these attributes will significantly influence adoption.

3.4 Data Analysis

The obtained data were subjected to descriptive and inferential statistics. Descriptive statistics were used to analyze the personal and farming characteristics of the farmers. Regression analysis was used to determine the relationships between adoption and predictor variables as specified in the equation below:

$$ADOP = a + \beta \times AGG + \beta \times EDD + \beta \times HHSIZ + \beta \times FSZ + \beta \times YREXP + \beta \times LABR + \beta \times INCM + \beta \times EXTN + \beta \times TRN + \beta \times ASSM + \beta \times LTNR + \beta \times YELD + \beta \times ATTR$$

Table 1 also shows the detail description of the variables in the regression equation.

4. Results and Discussion

4.1 Descriptive Statistics

The study as shown in Table 1 reveals that the adoption score range between 10 and 24 with a mean of 17.80 (SD = 3.75). The level of adoption among the farmer is considerably high which may be due to benefits the farmers derived from using sawah technology. Age of farmers ranged between 26 and 62 years with a mean of 39.96 (SD = 7.69). Majority of the farmers have secondary education which means they can read and write while the mean household size is 6 (SD = 3.44). The average farm size of the respondents is 0.37 ha (SD = 0.28) while mean years of experience in rice production is 11.93 years (SD = 8.79). Average annual income of farmers is 3,043.80 Ghana Cedi (2,029.2 USD) (SD = 1,728.38). Farmers mostly use family labor for their farm activities while average rice yield of the farmers is 3,406 kg/ha of sawah field. Most farmers in the study area rent the land they use for sawah rice production. This study revealed that there is always a tenancy arrangement between the tenant farmers and the land owners before the land is used. This arrangement is considered as mutually beneficial for both landlord and tenant and the nature of agreement is believed to be fair on the part of both parties. The duration of the agreement ranges from 5 to 15 years which is renewable.

4.2 Adoption Regression Results

Table 2 reports the regression result for the variable that determined the adoption of sawah technology among the respondents. The result of this study reveals that age has a negative impact on adoption of sawah technology, which suggests that the probability of adoption of sawah technology is higher among the young farmers than older farmers. This could be because of resistance to change by aged farmers [31, 32]. According to Marenya and Barrett [26], older farmers are less likely to adopt technology because their planning horizon shrinks and their incentives for them to invest in future productivity of their farms diminish. Older farmers find it difficult to change from their former way of doing thing for a new method. The younger farmers may be inquisitive, wanting to learn more, hence, increase their level of adoption. This may also be connected with the strenuous nature of sawah technology which may need relatively healthier and stronger younger farmers to adopt than older counterparts [29]. Education has a positive significant relationship with adoption of sawah technology suggesting that more educated farmers are more likely to adopt sawah technology than less educated farmers. According to Chianu and Tsujii [33] sited from He et al. [23], targeting young farmers and a systematic increase of farmers educational attainment can increase probability of agricultural technology adoption. Year of experience in rice production has a positive significant relationship with adoption of sawah technology. The knowledge gathered by farmers in long years of rice production can also be useful in the adoption of sawah technology. However, contrary to expectations, household size, farm size, labor source and income do not significantly influence farmers' adoption of sawah technology.

Table 2 Regression between adoption and predictor variable

Variables	Unstandardized coefficients (B)	Standardised coefficients (β)	S.E.	*t*-ratio (B/S.E.)
AGG**	-17.766	-0.104	4.275	-4.156
EDD**	3.305	0.454	0.854	3.869
HHSIZ	0.139	0.183	0.115	1.202
FSZ	1.578	0.131	1.541	1.024
YREXP**	2.714	0.445	0.873	3.109
LABR	-0.237	-0.334	1.673	-0.141
INCM	0.026	0.017	0.053	0.500
EXTN**	0.160	0.436	0.049	3.240
TRN*	5.070	0.497	1.768	2.869
ASSM	0.436	0.081	0.597	0.730
LTNR**	3.404	1.296	0.838	4.062
YELD*	0.152	1.261	0.079	1.927
ATTR*	2.855	0.242	1.031	2.770
Constant*	-6.062		3.211	-1.888

$R = 0.86$; $R^2 = 0.74$; Adjusted $R^2 = 0.62$; df = 13/30; F value = 6.42; Sig. = 0.00;
*significant at $P < 0.05$; **significant at $P < 0.01$.

As expected, contact with extension was found to have a significant positive effect on adoption of sawah technology, meaning that farmers who have contact with extension agents and institutions are likely to adopt sawah technology than those that do not have contact with extension agents. Contact with extension agents allows farmers greater access to the latest information as regard agricultural practices and also avail them opportunity to participate in field demonstration hence increase the probability of adoption of any agricultural technology [23, 29]. As hypothesized, attendance in previous sawah training was also found to have a significant positive effect on adoption of sawah technology. Training farmers on how to construct bunds, canals, sawah basins and the operation of power tiller for instance is expected to have a positive effect on the adoption of sawah technology. Contact with extension and attendance in previous trainings can provide farmers with knowledge and information on sawah technology, which can lead to higher competence among the farmers [29]. However, contrary to expectations membership of farmers' association does not significantly influence farmers' adoption of sawah technology.

As hypothesized, land tenure has a significant relationship with adoption of sawah technology. Land as an important factor of production needs to be secured due to the critical role plays in adoption. Emanating from this study is the fact that land tenure arrangements significantly affect the adoption of sawah technology by farmers. Land tenure security determines whether people will invest in and adopt sawah technology and can therefore be regarded as an important ingredient in adoption of sawah technology. According to Oladele and Wakatsuki [29], the probability of adoption of sawah technology increases if the plot of land used for sawah is acquired through inheritance, by purchase, having long tenancy period and if the rent paid for the land is low. Yield realized by farmers from their farm is also found to have a significant positive effect on adoption of sawah technology. This result is corroborated by Ali-Olubandwa [34] who reported a statistically significant relationship between adoption and yield among small scale farmers in Western province of Kenya. According to Wakatsuki et al. [6], sawah systems can sustain paddy yields higher than 4 t/ha through various macro-scale natural geological fertilization processes and micro-scale mechanisms to enhance the supply of various nutrients. With the application of advanced agronomic practices, sustainable paddy yields above 10 t/ha can be achieved in lowlands with quality sawah and soil and water management.

The result further shows that attributes of sawah technology as hypothesized were found to have a significant positive effect on adoption of sawah technology. These attributes include high yield, disease and pest control, fertilizer management, water management, weed control and good tillering. As mentioned above, farmers are sure of paddy yields higher than 4 t/ha and when advanced agronomic practices are adopted, the yield may rice to 10 t/ha. Sawah system encouraged the growth of various aquatic algae and other aerobic and anaerobic microbes in addition to rice growth, which increase nitrogen fixation in the sawah system through increase of photosynthesis as multi-functional wetlands. The result of this study is also in agreement with Fu et al. [35] who reported that higher yield, better water and weed control, have been recognized by participating farmers as the factors affecting the adoption of sawah technology among the Nupe farmers in Nigeria.

5. Conclusions

The result of this study reveals that age, education year of experience in rice production, contact with extension agents and attendance in previous trainings, land tenure arrangements, yield were found to have a significant effect on adoption of sawah technology. The study further revealed that the benefits of sawah technology must be clearly perceived by the farmers in order to improve on the adoption of sawah technology. Further dissemination and adoption of sawah technology must therefore be targeted at young and educated farmers. Training should also be organised for the farmers to improve on their adoption. Also, ensuring high levels of tenure security is important for sustainable adoption of sawah technology. Success of sawah technology based on the findings of this study centres on land availability to the farmers with long-term security.

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References

[1] G. Kranjac-Berislavjevic, Some features of rice production in Ghana, multiagency partnerships for technical change, in: West African Agriculture, National Workshop on Rice Production in Ghana, Ho, Upper East Region, Ghana, 2000.

[2] Coalition for African Rice Development (CARD), Mapping of Poverty Reduction Strategy Papers (PRSPs), sector strategies and policies related to rice development in Ghana [Online], 2010, http://www.riceforafrica.org/downloads/CARD%20DOCS/Ghana_Mapping_report_Final_version.pdf.

[3] B. Asuming, Profitability, competitiveness and welfare effects of trade liberalization on the rice sub-sector in Ghana, structural adjustment and agriculture in West-Africa, COPESRIA Book Series, Senegal, 1998.

[4] West Africa Rice Development Association (WARDA), Africa Rice Trends 2007, Cotonou, Benin, 2008.

[5] JICA, The study on the promotion of domestic rice in the Republic of Ghana, National Development Planning Commission (NDPC), 2005 Growth and Poverty Reduction Strategy (GPRS II), 2007.

[6] T. Wakatsuki, M.M. Buri, S.E. Obalum, R. Bam, O.I. Oladele, S.Y. Ademiluyi, et al., Farmers' personal irrigated sawah systems to realize the green revolution and Africa's rice potential, paper presented at 1st International Conference on Rice for Food, Market, and Development (Rice-Africa), Abuja, Nigeria, Mar. 3-5, 2011.

[7] H. Hirose, T. Wakatsuki, Restoration of Inland Valley Ecosystems in West Africa, Nourin Tokei Kyoukai, Tokyo, 2002, p. 600.

[8] T. Wakatsuki, M.M. Buri, O.I. Oladele, West African green revolution by sawah eco-technology and the creation of African SATOYAMA systems, Kyoto Working Papers on Area Studies No. 63 (G-COE Series 61) [Online], Center for Southeast Asian Studies, Kyoto, Japan, p. 30, ISBN: 978 4 901668 63 7, http://www.humanosphere.cseas.kyoto-u.ac.jp/article.php/workingpaper61 2009.

[9] Y. Tsujimoto, T. Horie, H. Randriamihary, T. Shiraiwa, K. Hommaa, Soil management: The key factors for higher productivity in the fields utilizing the system of rice intensification (SRI) in the central highland of Madagascar, Agric. Sys. 100 (2009) 61-71.

[10] M.M. Buri, R.N. Issaka, T. Wakatsuki, The sawah system of rice production, CSIR-Soil Research Institute and Kinki University, Kumasi, Ghana, 2009, p. 147.

[11] C.R. Doss, Analyzing technology adoption using microstudies: Limitations, challenges, and opportunities for improvement, Agricultural Economics 34 (2006) 207-219.

[12] D.B. Mignouna, V.M. Manyong, J. Rusike, K.D.S. Mutabazi, E.M. Senkondo, Determinants of adopting imazapyr-resistant maize technologies and its impact on household income in Western Kenya, The Journal of Agrobiotechnology Management and Economics 14 (3) (2011) 158-163.

[13] E.M. Rogers, Diffusion of Innovations, 5th ed., New York: Free Press, 2003.

[14] S. Sall, A.D. Norman, B.A.M. Featherstone, Quantitative assessment of improved rice variety adoption: The farmer's perspectives, Agricultural Systems 66 (2000) 129-144.

[15] M. Mcdonald, K. Brown, Soil and water conservation projects and rural livelihoods: Options for design and research to enhance adoption and adaptation, Land Degrad. Dev. 11 (2000) 343-361.

[16] J. Anderson, J. Thampapillai, Soil conservation in developing countries: Project and policy intervention, World Bank Discussion Paper No. 78, World Bank, Washington, DC, 1990.

[17] S. Hwang, J. Alwang, G.W. Norton, Soil conservation practices and farm income in the Dominican Republic, Agricultural Systems 46 (1994) 59-77.

[18] H.M. De Souza Filho, T. Young, M.P. Burton, Factors influencing the adoption of sustainable agricultural technologies evidence from the state of Espı´rito Santo, Brazil, Technological Forecasting and Social Change 60 (1999) 97-112.

[19] L. Faltermeier, Adoption of water conservation and intensification technologies in the lowland rice production systems of Northern Ghana, in: Paper Prepared for Presentation at the MEIDE Conference, Maastricht, Netherlands, May 30, 2007.

[20] G.B. Thapa, G. Rasul, Patterns and determinants of agricultural systems in the Chittagong Hill Tracts of Bangladesh, Agricultural Systems 84 (2005) 255-277.

[21] G.B. Thapa, K. Rattanasuteeraku, Adoption and extent of organic vegetable farming in Mahasarakham province, Thailand, Applied Geography 31 (2011) 201-209.

[22] A. Reimer, A. Thompson, L. Prokopy, The multi-dimensional nature of environmental attitudes among farmers in Indiana: Implications for conservation adoption, Agriculture and Human Values 29 (2012) 29-40.

[23] X. He, H. Cao, F. Li, Econometric analysis of the determinants of adoption of rainwater harvesting and supplementary irrigation technology (RHSIT) in the semiarid Loess Plateau of China, Agricultural Water Management 89 (2007) 243-250.

[24] M.L.A. Lapar, S.K. Ehui, Factors affecting adoption of dual-purpose forages in the Philippine uplands, Agricultural Systems 81 (2004) 95-114.

[25] A.M. Adrian, S.H. Norwood, P.L. Maskc, Producers' perceptions and attitudes toward precision agriculture technologies, Computers and Electronics in Agriculture 48 (2005) 256-271.

[26] P.P. Marenya, C.B. Barrett, Household-level determinants of adoption of improved natural resources management practices among smallholder farmers in western Kenya, Food Policy 32 (2007) 515-536.

[27] C.I. Alarima, A. Kolawole, C.I. Sodiya, O.I. Oladele, T. Masunaga, T. Wakatsuki, Factors affecting the adoption of sawah technology system of rice production in Nigeria, Journal of Food, Agriculture and Environment 9 (3 & 4) (2011) 177-182.

[28] A. Amsalua, J. Graaff, Determinants of adoption and continued use of stone terraces for soil and water conservation in an Ethiopian highland watershed, Ecological Economics 61 (2007) 294-302.

[29] O.I. Oladele, T. Wakatsuki, Effect of land tenure on the adoption of sawah rice production technology in Nigeria and Ghana, Journal of Agricultural Science and Technology 3 (10) (2009) 47-53.

[30] S. Gavian, S. Ehui, Measuring the production efficiency of alternative land tenure contracts in a mixed crop-livestock system in Ethiopia, Agricultural Economics 20 (1999) 37-49.

[31] S.A. Adesoji, A.J. Farinde, A.O. Ajayi, Determinants of training needs of fadama farmers in Osun State, Nigeria and implication for extension work, Journal of Applied Sciences 6 (15) (2006) 3082-3088.

[32] A.O. Ajayi, Identification of training needs of women farmers in Oyo State, Unpublished M.Sc. Thesis, Agricultural Extension and Rural Sociology, O.A.U, Ile-Ife, 1995.

[33] J.N. Chianu, H. Tsujii, Determinants of farmers' decision to adopt or not adopt inorganic fertilizer in the savannas of northern Nigeria, Nutr. Cycl. Agroecosyst 70 (2004) 293-301.

[34] A.M. Ali-Olubandwa, D. Odero-Wanga, N.J. Kathuri, W.A. Shivoga, Adoption of improved maize production practices among small scale farmers in the agricultural reform era: The case of western province of Kenya, Journal of International Agricultural and Extension Education 17 (1) (2010) 21-30.

[35] R.H.Y. Fu, M. Maruyama, O.I. Oladele, T. Wakatsuki, Farmers adoption and propensity to abandoned adoption of sawah-based rice farming in inland valley of central Nigeria, Journal of Food, Agriculture and Environment 7 (2) (2009) 379-382.

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Changing the Moisture Content of the *Spartina* and *Miscanthus* and Willow Shoots during Storage in Natural Conditions

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Abstract: The aim of this study was to determine the characteristics of the distribution of energy plant moisture content along the height of their shoots and the dynamics of moisture during storage in natural conditions. The shoots of *Spartina*, *Miscanthus* and willow were used in the study. Entire shoots were cut into sections of 10 cm and for each set in monthly cycles for six months moisture content was evaluated. After a month's storage of freshly cut shoots the biggest decrease of content moisture in the shoots of *Spartina* and *Miscanthus* was recorded, by 31% and 22%, respectively, and the lowest in willow shoots (12%). After sixth months of shoots storage the lowest moisture content (10%-12%) was reached in miscanthus. The most uneven moisture content along the height of shoots *Spartina* was characterized because on one third of the height from the bottom, the moisture content of shoots was 20%, and the top had moisture content in the range 5%-10%. Willow shoots were characterized by the smallest drop in moisture, and the final moisture content was about 23%, with the top part of moisture content of 10%-20%. The dynamics of moisture change during the six months of storage of grass shoots (*Miscanthus* and *Spartina*) in natural conditions under roofing was described by one power function regression, and willow by another one. Empirical models can be used to predict changes in moisture content of these plants in experiment conditions, since the coefficients of determination were 94.66% and 89.18%, respectively.

Key words: *Spartina*, *Miscanthus*, willow, moisture content, storage, natural drying.

1. Introduction

Perennial energy crops cultivated on agricultural land, depending on the species, can yield biomass in woody, semi-woody and strawy form with different energy parameters. These factors, along with the harvest periods and weather conditions have a major impact on the obtained biomass moisture, which farther determines its calorific value [1, 2]. Harvesting energy plants with woody biomass: Sprint and Wodtur one-year willow and rose multiflora; semi-woody: *Jerusalem artichokes*, Virginia Mallow, Japanese knotweed and Sakhalin knotweed, *Silphium perfoliatum*; strawy: *Spartina prairie*, sugar *Miscanthus*, Chinese *Miscanthus*, giant *Miscanthus* at two periods, it was found that at harvest in November, moisture of semi-woody and strawy species ranged from 39.4% for sugar miscanthus to 66% for the *Jerusalem artichoke*, and in March from 16% for sugar *Miscanthus* to 26.2% for prairie *Spartina*. In both harvest periods, willow and rose multiflora had a similar moisture content, 54.1% and 49.8%, respectively [3]. Frączek and Mudryk [4] observed the same regularity for energy willow harvested in November and March, because in both periods, the average moisture amounted to 52.5%. Much more effective was the storage of willow, because shoots harvested in November and stored without roofing

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reduced their moisture content to 50%, and under roofing to 43%. Thereafter, the dynamics of plants drying changed and at the turn of June and July moisture content of plants was similar (22%-23%), regardless of the storage method.

The shoots of plants harvested at high moisture and chopped immediately after harvest create a problem in storage due to biological processes, resulting in a decrease of the energy properties of biomass. For example, on the basis of previous research on wet wood chips in a pile with a side angle of 40° and a height of 1.5 m to 6 m, it was found that storing wood chips with a moisture content above 45% can be expected to increase the temperature inside the pile up to 60 °C [5]. In addition, freshly harvested wood chips stored in natural conditions in the pile do not dry efficiently and absorb precipitation water. The consequence of these factors is the rapid growth of mold and mildew, and loss of organic matter [5, 6]. If the plant material is used immediately after harvest and goes to heating plants, where it is burned in special fluidized bed boilers, the losses are the smallest and such technology is the most desirable.

From this it follows that action must be taken not only focusing on plantations, but also on organizing raw material storage, drying and distribution systems and ensuring the efficient use of biomass from energy crops [7].

Chips stored for a short period should be maintained on a compacted and aligned surface. If chips are to be stored for a longer period, as a raw material reserve, it is necessary to reduce their moisture by the use of forced, but expensive ventilation of piles or storage of biomass under roofing [8-10]. For example, wood chips stored under roofing for 4-6 months reduced their moisture from about 50% to 30%. Securing of raw fuel results from logistic needs and is dictated by harvest periodicity, but even at such a time one can expect unfavorable climatic conditions associated with torrential rains and heavy snowfall in the winter [11].

Willow shoots chopped into 5-7 cm sections can also be stored in openwork containers in which the lower part is made of mesh or grid, so that atmospheric air can be easily sucked into the center [12]. Samples stored under these conditions for 6 weeks in a container with a capacity of 1 m^3 reduced their moisture content from 46% to 19% and volume by about 7%-8%. In the two following two-week periods, moisture content decreased to 16% and 11%, respectively.

The delayed harvest system is recommended, however, there is a significant loss of dry matter between the period that the crop matures in the fall and the field recovery of the harvested biomass in the spring. During winter, most of the leaves and the non-woody tops of the plant are broken off by wind [13]. In a review of *Miscanthus* yields in Europe, the peak yield in the fall was found to decline by approximately 0.30%-0.36% per day, which was connected with delayed harvesting in the spring [14, 15]. These losses vary considerably by location and year. In Germany, physical losses from the fall to spring harvest in March amounted to 15%-25% of the fall yield, while in the Netherlands losses of between 29% and 42% were recorded during the same period. The peak yield that can be obtained through the delayed harvest system in Europe was estimated to be 33% lower than fall yields at 12.6 t_{DM}/ha [10]. Additional losses occur from during the harvest process and in the crop stubble not removed during the mechanical harvesting process that could potentially be reduced. Estimates from Germany are that an additional 25% of the biomass is lost during the harvesting process [8]. In England, losses from using a mower conditioner and baler harvest system in the fall produced yields of only 1.5-6.2 t_{DM}/ha, a value 20%-27% lower than delayed spring yields [16]. Large losses of biomass from winter breakage and harvest losses during mowing and baling operations have also been reported with spring harvested switch grass in North America [17]. A further 17% of biomass is left in the stubble [8] and additional storage losses of 7%-10% dry matter (for spring harvested bales stored at 25%-30% moisture) can be expected if wet material is placed in storage [18]. Considering all of this, the total recovered biomass may amount as low as one third of the biomass available before winter [8].

More recently a new technique has been developed in Canada to fall mow switch grass and then directly bale the material from the windrow in the spring as a means to prevent field breakage and machine harvest losses [19]. This system has resulted in 21% higher yields recovered than when the material was spring mowed and baled. Machine harvested spring yields are approximately 30%-35% below the fall biological yield in the case of switch grass [14]. This technique if applied to *Miscanthus* could further increase its harvest yield because during winter the plant is loses a lot of leaves and panicle.

Hayes [20] reported that biomass yields of reed canary grass from an early-winter harvest will be 40% higher than those seen in the spring. Attributing this to the 20 t_{DM}/ha/yr (on an oven-dry basis) spring yields seen in high yielding mature plots in western Ireland, this paper predicts early harvest yields of 28 t_{DM}/ha/yr (on an oven-dry basis). Fall harvested reed canary grass has been shown to have a higher yield in older stands [21]. One study showed an average of 11.02 t_{DM}/ha for fall harvest compared to 8.82 t_{DM}/ha for spring harvest.

The non-destructive method showed a large increase in annual biomass production from the first to the second growing season [22]. Based on the harvested willow, average annual biomass production of the four clones ranged from 5.2 t_{DM}/ha/yr to 8.8 t_{DM}/ha/yr with a significant effect of both soil type and clone.

Landstrom et al. [23] reported reed canary grass yields of 2.72 t_{DM}/ha (dry matter) for spring harvest compared to 3.46 t_{DM}/ha during fall harvest. However, both chlorine and potassium concentrations were six times lower in the spring harvested crops making it a higher quality fuel for use in combustion machines [17]. Harvesting during the driest period is also recommended to avoid harmful soil compaction. Lodging of material in spring after heavy snow may restrict or limit harvests.

The quality of grasses used in biomass energy conversion systems is influenced by the composition of elements in the grass, which change with the seasons. Elements found in perennial grasses that can be harmful to biomass combustion boilers are potassium, chlorine, magnesium, and phosphorus. Perennial grasses harvested before senescence (spring/summer) possess the highest levels of combustion contaminants, which decrease as harvest is delayed and crops are allowed to senesce (fall/winter) [12, 24]. Further, aboveground contaminants are leached by rain and snow after senescence [25].

Moisture affects stable storage, stable transportation, and combustion efficiency of harvested materials. Moisture content decreases with time after the first frost with the lowest moisture content occurring in early spring [12].

Based on the available literature, it can be said that we have very little information about changing moisture content of energy plant shoots stored whole in natural conditions and how in these conditions the moisture content changes along the height of shoots, which affects the loads dynamics of working units of machines used to harvest and process biomass. The distribution of changes in moisture content along the height of shoots was pointed out by Igathinathane et al. [26] in relation to plant maize, variety 743 DeKalb during their growing season. Prior to harvest of silage maize, a sharp drop in the moisture occurs, especially in the lower region of the stem, followed by its stabilization and a fairly good alignment along the height of the stem. This coincides with a period of about 122 days after sowing, when the maize reaches the best maturity to harvest for silage. However, this depends on climatic conditions and maize varieties because our experience [27] shows that the harvest of Inagua variety within 127 days after sowing was slightly too early.

This information inspired the formulation of an investigation aim concerning the explanation of the dynamics of moisture change of selected energy crops along their shoots during six months storage in natural conditions.

The study assumed three hypotheses: (1) the characteristics of material moisture content distribution along the height of shoots and the dynamics of changes of this moisture are a feature of the plant species; 2) during natural drying material moisture content along the height of a signal shoot becomes evened out which indicates that water is internally redistributed; 3) the moisture change at the time of natural storage can be determined based on the adaptation of forced drying models.

2. Material and Methods

The investigation was conducted for plant shoots of prairie *Spartina*, giant *Miscanthus* and basket willow. The material was harvested from plots at the Experimental Station in Skierniewice, which belongs to Warsaw University of Life Sciences. The harvest of plants was held on October 13, 2011, using a hand secateur and a petrol brush cutter. Plants were cut at a height of about 0.05 m ± 0.03 m from the ground. After cutting, the plants were gathered in separate bundles of 30 shoots for each species. Shoots were arranged in a shed on wooden pallets in a horizontal position so as not to put them directly on the floor. The gap of 0.07 m between the ground and the upper surface of the pallet allowed for air circulation and natural drying.

The first measurement of moisture took place a day after the cut, and next measurements at regular monthly intervals for six months. Plants were cut into section of 0.100 ± 0.002 m along the shoot (Fig. 1), then each section was cut and divided into three averaged samples and placed in measurement containers (Fig. 2).

Moisture was determined by dried-weight method according to the standard S358.2 ASABE [28]. For this purpose, three averaged samples of material from each section of the plant were weighed on the scales RADWAG WPS 600/C with an accuracy of 0.01 g and then dried to constant weight at 103 ± 2 C using a laboratory dryer SLW 115 for 24 h. Because in each measuring monthly cycle the 270 samples were prepared, weighing the moist material was carried out on one day and the drying of the whole lot of material of one cycle took about a week.

The characteristics of the changes in the dynamics of moisture content of plant shoots of *Spartina*, *Miscanthus* and willow along their height during storage are presented in graphs, and a statistical analysis was performed using a standard statistical package Statistica ver 10.

3. Results and Discussion

During the harvest, grasses had a high moisture, ranging from 45% to 68% (Figs. 3a and 3b), and willow shoots 47%-52% (Fig. 3c). Prairie *Spartina* was characterized by the most equalized moisture content along the height of shoots, with a value of 60%, which is very well visible on the comparative Fig. 4a Only the initial basal part had moisture 55% and moisture content of the shoot tips gradually

Fig. 1 Cut shoots of plants: (a) prairie *Spartina*; (b) giant *Miscanthus*; (c) basket willow.

Fig. 2 Averaged samples of chopped plant material prepared for the measurement of moisture content.

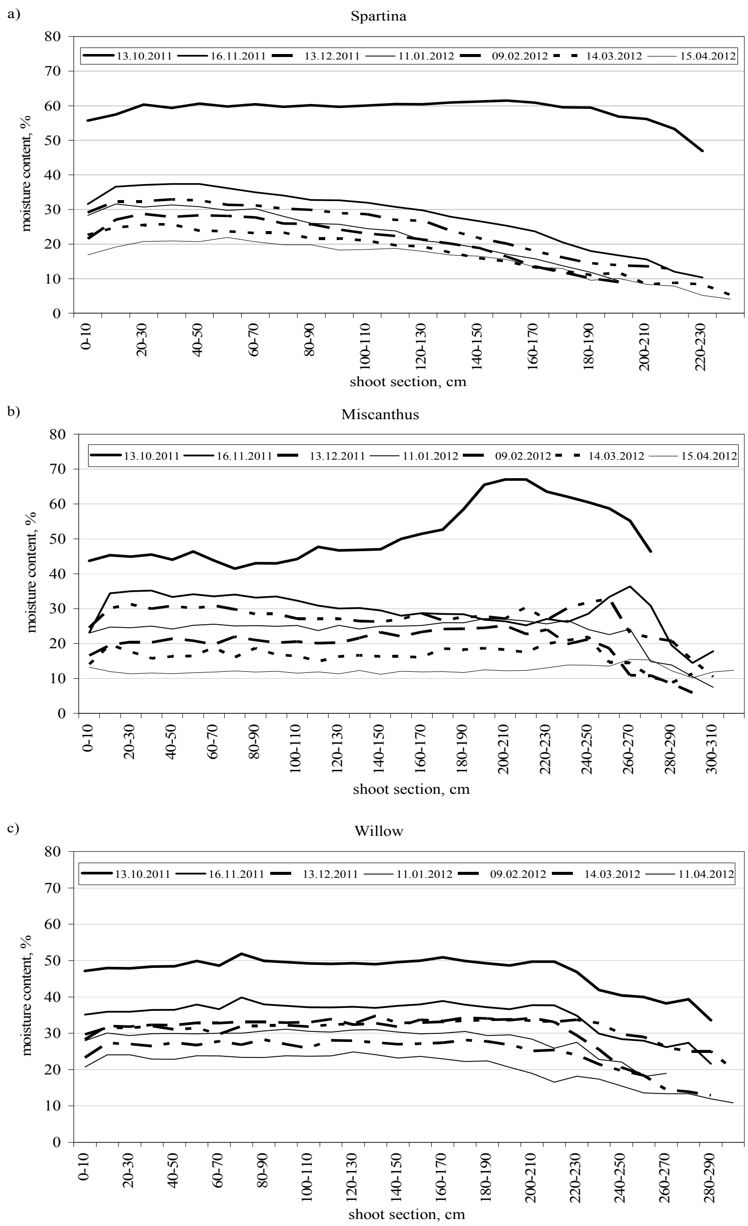

Fig. 3 Characteristics of moisture distribution along the height of shoots and the dynamics of changes in their moisture during a six-month storage in natural conditions: a) prairie spartina; b) giant miscanthus; c) basket willow.

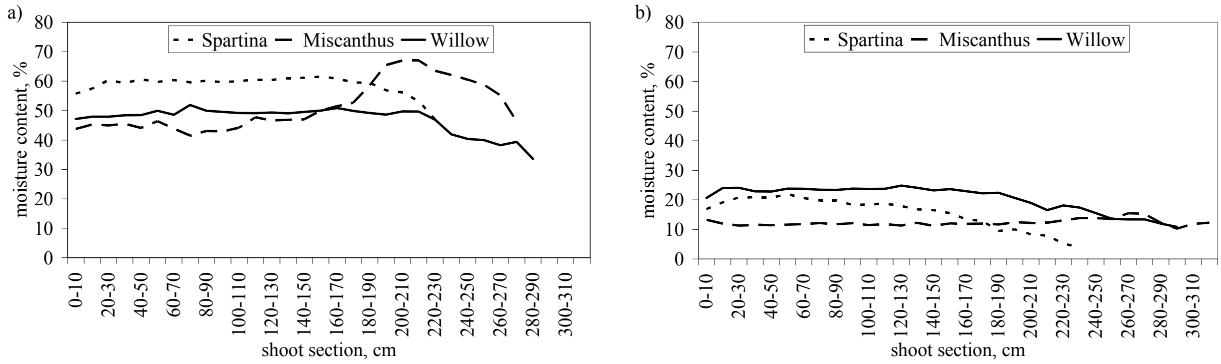

Fig. 4 Comparative characteristics of moisture distribution along the height of shoots of prairie spartina, giant miscanthus and basket willow immediately after harvest (a) and after a six-month storage in natural conditions (b).

decreased to the value of 47% over a distance of 180-230 cm.

Quite surprising changes of moisture were recorded for giant miscanthus shoots (Fig. 3b). Up to half the height of shoots (150 cm), moisture content was relatively stable (45%), and then increased to a maximum of 67% at height of 210-220 cm, then decreased quite rapidly, until the top of the panicle (290 cm) to the value of 46%.

After a month's storage of shoots, the biggest difference in moisture content occurred in *Spartina* (Fig. 3a), and then in *Miscanthus* (Fig. 3b) and the lowest in willow (Fig. 3c) by 31%, 22% and 12%, respectively. The dynamics of a decrease in the amount of moisture along the shoots was the highest for spartina, especially since the mid-height of the shoots.

The difference in moisture content in giant *Miscanthus* shoots was mainly due to base moisture content immediately after cutting, because after a month of drying in natural conditions, moisture of shoots along their height was fairly stable, between 25% and 35%, with a tendency to decrease. Only in the panicle section, at a height of 240 cm, an increase of the value moisture content was reported up to 36% at a height of 270 cm and then a sharp drop almost to the end shoots up to 15%. Willow shoots were characterized by a similar stability (Fig. 3c), with the dominant values of moisture content of 37%-38%, and its decline to a value of 22% was recorded at the height from 220 cm to 290 cm.

In almost all cases, a characteristic feature of changes in moisture of the plant material was its lower value from the side of the shoot cut. This is logical, since a large cross-sectional area of the stem permitted the movement of water along the conductive tubes of plant tissues. The exception were the willow stems (Fig. 3c), and that was due to more compact and woody tissue cells.

In subsequent monthly measurement cycles, it was found that the moisture content of plant shoots changed less intensively, especially during the storage of willow (Fig. 3c), for which the differences were approximately 1%-2%. Moisture content along the height of shoots remained fairly constant. The dynamics of drying *Spartina* were characterized by only slightly lower stability along the height of shoots (Fig. 3a), but with a systematic reduction of the moisture along their tops. Giant *Miscanthus* shoots were characterized by a greater dispersion of moisture along their height and a greater dynamics of moisture content decrease (Fig. 3b).

After six months of storage, giant miscanthus shoots had the lowest moisture content (10%-12%, Fig. 3b) and was most equalized along their height (Fig. 4b), which indicates that water is internally redistributed. *Spartina* was characterized by the most uneven moisture content along the height of shoots (Figs. 3a and 4b). At one third of the height from the bottom, the moisture of shoots was 20%, and at the top was in the range 5%-10%. The final moisture of willow shoots was about 23%, with the top part of 230-290 cm with a moisture content of 10%-20% (Fig. 3c). Similarly, Gigler et al. [9] concluded that during natural drying, within a single willow shoot in the pile, the moisture content is more or less uniform. After harvest (between November and April), during storage until August, the average willow pile moisture content was reduced to 16%-23%. The air flow through the pile of shredded willow yielded the same results after two months [12]. Frączek and Mudryk [4, 29] found that after storing spring willow shoots for 3-4 months, their moisture content decreased to 22%-23%, irrespective of the harvest period, which in the studies was conducted in autumn and spring. For this reason, the harvest period should be set based on other factors, especially weather and soil conditions of the plantation. These study findings from literature concern moisture content changes during storage, but without a detailed analysis of its distribution along the height of the energy plant shoots.

Moisture distribution along the height of shoots depended on the plant species and their construction. In general, grasses have lower moisture content at the top, but *Miscanthus*, with a spreading panicle had more moisture in this area, especially at the side shoots appearing. Grasses were characterized by very high moisture content rate of decline in the first month of storage. Increasing the rate of drying could be improved by conducting the harvest by means of a mower equipped with a conditioner or other units, such as crimping rollers or a macerator. Increasing the activation of drying, also conducted in natural conditions allows to achieve economic benefits due to a reduction or even elimination of the costly and energy intensive force drying.

The results of statistical analysis summarized in Tables 1-4 indicate a statistical significance of the impact of main factors: the section of shoot, plant species and time of measurement of the moisture content of material, because the critical level of significance is smaller than 0.0001.

Detailed statistical analysis leads to the conclusion that the differences in moisture content of plant shoots along their length does not allow to establish homogeneous groups (Table 2). Average moisture content of *Spartina* and *Miscanthus* shoots were similar and were 25.44% and 25.90%, respectively, which allowed to combine them into one homogeneous group (Table 3). The value of the average moisture content of willow shoots was significantly higher (30.35%) and constituted a separate group. The most varied moisture content was associated with the time of measurement, made in monthly cycles and therefore the mean value of each was a separate group (Table 4).

Based on the results of statistical analysis, especially in relation to two separate homogeneous groups of mean values for moisture of *Miscanthus*, *Spartina* and willow separately (Table 3) and on the significant changes in moisture content during storage of shoots (Tables 1 and 4) charts were prepared, which characterize the drying dynamics of energy plant material stored in natural conditions under roofing(Fig. 5). By examining power and exponential functions, it was found that the changes dynamics of moisture content during the six months of storing plants are

Table 1 Results of variance analysis of moisture content shoots of *Spartina, Miscanthus* and willow for the main factors: the section of shoot, plant species and time of measurement.

Source	Sum of squares	Degree of freedom	Mean square	*F*-ratio	*P*-value
Section	11,127.0	31	358.93	13.49	< 0.0001
Species	8,379.4	2	4,189.72	157.45	< 0.0001
Time of measurement	18,3420.0	6	30,570.10	1,148.85	< 0.0001
Residual	42,840.9	1,610	26.61		
Total	250,258.0	1,649			

Table 2 The results of a detailed statistical analysis of energy crops of moisture content for all sections and separate homogeneous groups of moisture content for sections of shoots.

Section	Count	Moisture content (%)	LS sigma (%)	Homogeneous groups													
295	15	17.65	1.34	X													
285	27	18.06	1.00	X													
305	8	18.60	1.83	X	X												
275	34	21.50	0.89		X	X											
265	37	23. 46	0.85			X	X										
255	42	25.04	0.80				X	X									
245	42	26.35	0.80					X	X								
235	44	26.78	0.78					X	X	X							
315	2	26.95	3.66			X	X	X	X	X	X	X	X	X	X	X	X
225	49	27.09	0.74					X	X	X	X						
215	52	27.28	0.72						X	X	X						
175	62	27.37	0.65						X	X	X						
185	58	27.40	0.68						X	X	X	X					
5	63	27.44	0.65						X	X	X	X					
195	58	27.52	0.68						X	X	X	X	X				
165	62	27.60	0.65						X	X	X	X	X				
155	62	28.02	0.65						X	X	X	X	X	X			
145	63	28.12	0.65						X	X	X	X	X	X			
205	53	28.23	0.71						X	X	X	X	X	X			
135	63	28.64	0.65							X	X	X	X	X	X		
125	63	28.97	0.65								X	X	X	X	X	X	
115	63	29.23	0.65									X	X	X	X	X	X
105	63	29.34	0.65										X	X	X	X	X
95	63	29.72	0.65											X	X	X	X
85	63	30.12	0.65												X	X	X
75	61	30.48	0.66													X	X
65	63	30.55	0.65													X	X
15	63	30.64	0.65													X	X
45	63	30.68	0.65													X	X
35	63	30.81	0.65														X
55	63	30.86	0.65														X
25	63	30.86	0.65														X

Table 3 The results of a detailed statistical analysis of energy crops moisture content for species.

Species	Count	Moisture content (%)	LS Sigma (%)	Homogeneous groups	
Miscanthus	622	25.44	0.23	X	
Spartina	439	25.90	0.29	X	
Willow	589	30.35	0.25		X

best represented by the exponential functions given in Fig. 5, for which the coefficients of determination are the biggest, and for the equations of moisture content of *Spartina* and *Miscanthus* and willow are 89.18% and 94.66%, respectively. Similar assumptions were made by Gigler et al. [9, 10] for natural drying of willow shoots. These graphs confirm the previously expressed notion that decreasing dynamics of moisture content of willow shoots was lower than for *Spartina* and *Miscanthus*. Final moisture content of plant material stored for six months was close to the equilibrium moisture content. In studies by Gigler et al. [9], willow stored in the pile also reached similar equilibrium moisture, even during a wet year.

Table 4 The results of a detailed statistical analysis of moisture content for species for time of measurements.

Time of measurement	Count	Moisture content (%)	LS Sigma (%)	Homogeneous groups						
1	236	50.57	0.36	X						
2	247	30.11	0.35		X					
3	235	27.56	0.36			X				
4	228	24.30	0.37				X			
5	223	23.20	0.37					X		
6	236	19.29	0.36						X	
7	245	15.59	0.35							X

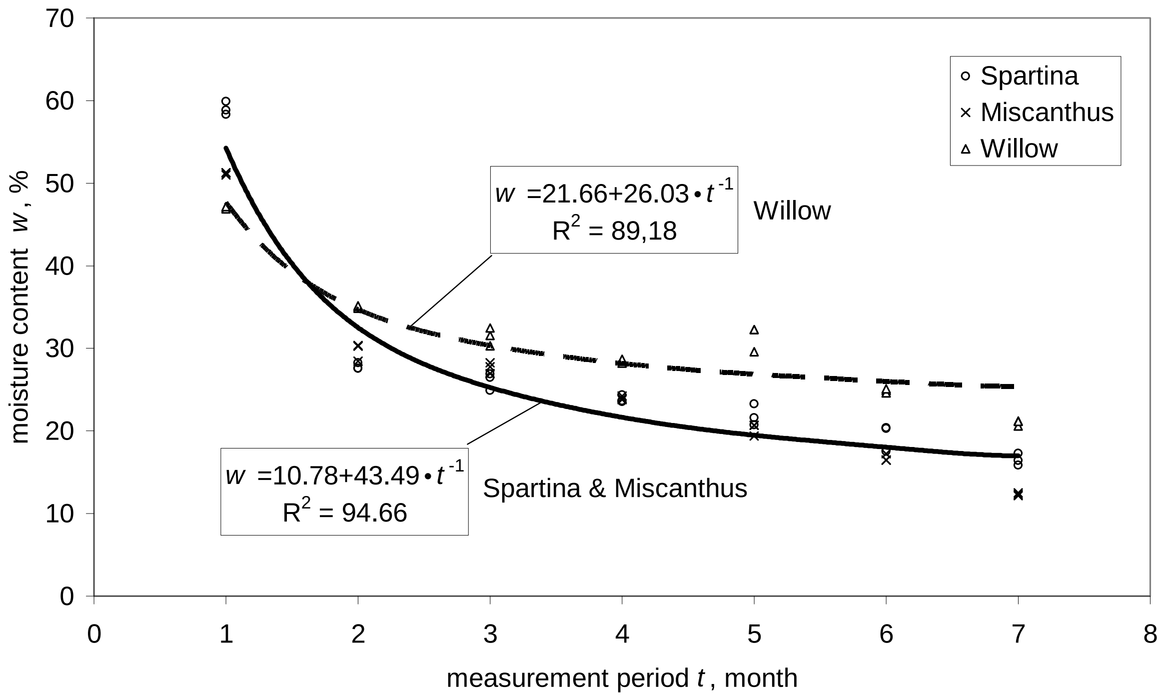

Fig. 5 Changes dynamics in moisture content of shoots of *Spartina*, *Miscanthus* and willow during the six-month storage.

Diffusion of moisture inside the shoots is a long-term process, which is governed by the relation of air humidity and ambient temperature, but it can be recommended that the time of natural drying for willow should not exceed six months, and for *Spartina* and *Miscanthus*—four months.

The estimated empirical models can be used to predict changes in moisture content for these plants stored under roofing. The decrease in the dynamics of moisture along the shoots of energy crops can be a factor for reducing the changes dynamics of load working units of machines used to harvest and convert the material for energy purposes.

4. Conclusions

Characteristics of moisture content distribution of energy plant along their height of shoots and the dynamics of changes in moisture content during a six-month storage in natural conditions differed significantly and depended on plant species.

After a month's storage of freshly cut shoots, the biggest decrease in the moisture content of shoots of prairie *Spartina* and giant *Miscanthus* were recorded, by 31% and 22%, respectively and the lowest in shoots of willow (12%).

After six months of storage of plant shoots, the giant *Miscanthus* shoots reached the lowest moisture content (10%-12%) and their moisture content was the most equalized along the height, which indicates that water is internally redistributed. The most uneven moisture content along the height was found in *Spartina* shoots and the smallest drop in moisture content was found in willow shoots, and their final moisture content was 16% and 23%, respectively.

Dynamics of moisture changes during the six-month storage of grass shoots (giant *Miscanthus*, prairie *Spartina*) in natural conditions under roofing are described by the regression equations of power function. Empirical models can be used to predict changes in moisture content of these plants in experiment conditions.

Knowledge of the dynamics of plant moisture changes along the shoots can be useful to explain the reason for the intensity of load variations on working units of machines used for harvest and converting the material for energy purposes.

References

[1] K. Dreszer, R. Michałek, A. Roszkowski, Renewable energy—The possibility of the obtaining and use in agriculture, PSAE Press, Krakow–Lublin–Warsaw, 2003. (in Polish)

[2] M. Stolarski, Production and obtaining of biomass from perennial energy crops, Prob. Agric. Eng. 3 (2004) 47-56. (in Polish)

[3] M. Stolarski, S. Szczukowski, J. Tworkowski, Biofuels from perennial energy crops, Energ. 51 (2008) 77-80. (in Polish)

[4] J. Frączek, K. Mudryk, Changes in willow shoots moisture content of *Salix viminalis* L. during seasoning, Agric. Eng. 10 (2008) 55-61. (in Polish)

[5] I. Christine, S. Volkhard, D. Werner, E. Johannes, Quality changes of chipped short rotation coppice during storage with special emphasis on human pathogen fungi, in: Proceedings of the 9th International Conference Ecological Energy Resources in Agriculture, Institute of Agricultural Engineering LUA, 2004, pp. 117-123.

[6] R. Jirjs, Effects of particle size and pile height on storage and fuel quality of comminuted *Salix viminalis*, Biomass Bioenerg. 28 (2005) 193-201.

[7] J. Paska, M. Sałek, T. Surma, The use of renewable energy resources in Denmark, Electr. Messag. 2 (2006) 6-11. (in Polish)

[8] J.K. Gigler, G. Meerdink, E.M.T. Hendrix, Willow supply strategies to energy plants, Biomass Bioenerg. 17 (1999) 185-198.

[9] J.K. Gigler, W.K.P. van Loon, J.V. van den Berg, C. Sonneveld, G. Meerdink, Natural wind drying of willow stems, Biomass Bioenerg. 19 (2000) 153-163.

[10] J.K. Gigler, W.K.P. van Loon, I. Seres, G. Meerdink, W.J. Coumans, Drying characteristics of willow chips and stems. J. Agric. Res. 77 (2000) 391-400.

[11] S. Szczukowski, J. Tworkowski, J. Piechocki, New trends in the use of biomass obtained from agricultural land for energy production, Adv. Agric. Sci. 6 (2001) 11-19. (in Polish)

[12] B. Kościk, Energy Crops, Lublin, ULS Press, 2003. (in Polish)

[13] I. Lewandowski, J.C. Clifton-Brown, J.M.O. Scurlock, W. Huisman, *Miscanthus*: European experience with a novel energy Crop, Biomass Bioenergy 19 (2000) 209-227.

[14] J.C. Clifton-Brown, M.B. Jones, J. Breuer, Yield performance of *M. giganteus* during a 10 year field trial in Ireland, Asp. Appl. Biol. 65 (2001) 153-160.

[15] J.C. Clifton-Brown, P.F. Stampfl, M.B. Jones, Miscanthus biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions, Glob. Chang. Biol. 10 (2004) 509-518.

[16] P. Nixon, M. Bullard, Optimisation of *Miscanthus* harvesting and storage strategies, Final report for DTI New and Renewable Energy Programme, 2003, pp. 1-25.

[17] P.A. Adler, M.A. Sanderson, A.A. Boateng, P.J. Weimer, H.G. Jung, Biomass yield and biofuel quality of switchgrass harvested in fall and spring, Agron. J. 98 (2006) 1518-1525.

[18] F. Jonkanski, *Miscanthus*: The future biomass crop for energy and industry, biomass for energy, environment, agriculture and industry, in: Proc. of the 8th European Biomass Conference 3-5 Oct., 1994 Vienna, Austria. Elsevier Science Ltd., Oxford, UK1, pp. 372-379.

[19] R. Samson, S. Bailey-Stamler, C. Ho Lem, Optimization of switchgrass management for commercial fuel pellet production, Final report to Ontario Ministry of Agriculture, Food and Rural Affairs under Alternative Renewable Fuels R&D Fund, 2008, pp. 1-40.

[20] D.J. Hayes, M.H.B. Hayes, The role that lignocellulosic feedstocks and various biorefining technologies can play in meeting Ireland's biofuel targets, Biofuels Bioprod. Biorefin. 3 (2009) 500-520.

[21] G. Hadders, R. Olsson, Harvest of grass for combustion in late summer and in spring, Biomass Bioenerg. 12 (1997) 171-175.

[22] L. Sevel, T. Nord-Larsenb, K. Raulund-Rasmussen, Biomass production of four willow clones grown as short rotation coppice on two soil types in Denmark, Biomass Bioenerg. 46 (2012) 664-672.

[23] S. Landstrom, L. Lomakka, S. Andersson, Harvest in spring improves yield and quality of reed canary grass as a bioenergy crop, Biomass Bioenerg. 11 (1996) 333-341.

[24] M.A. Sanderson, P.R. Adler, A.A. Boateng, M.D. Casler, G. Sarath, Switchgrass as a biofuels feedstock in the USA, Can. J. Plant Sci. 86 (2006) 1315-1325.

[25] E.A. Heaton, F.G. Dohleman, S.P. Long, Seasonal nitrogen dynamics of *Miscanthus giganteus* and *Panicum virgatum*, Glob. Chang. Biol. 1 (2009) 297-307.

[26] C. Igathinathane, A.R. Womac, S. Skohansanj, L.O. Pordesimo, Mass and moisture distribution in aboveground components of standing corn plants, T. ASABE 49 (2006) 97-106.

[27] A. Lisowski, J. Chlebowski, J. Klonowski, T. Nowakowski, A. Strużyk, M. Sypuła, Energy plant harvesting technologies, WULS Press, Warsaw, 2010. (in Polish)

[28] ASABE Standards, Moisture measurement—Forages ASABE S358.2 (R2008), in: ASABE Standards 2011, ASABE, St. Joseph, MI, USA, 2011, pp. 780-781.

[29] J. Frączek, K. Mudryk, Measurement of moisture content of shoots and energy willow chips, Agric. Eng. 12 (2006) 137-144. (in Polish)

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Estimation of Some Plant Secondary Products in *Urtica dioica* L., *Viola odorata* L. and *Melissa officinalis* L. Naturally Grown in Hawraman-Kurdistan Region of Iraq

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Abstract: The plant aerial parts of three species, *Urtica dioica* L., *Viola odorata* L. and *Melissa officinalis* L. were collected at randomly-different locations, according to altitudes in May 2010. The aerial parts of *Urtica* were collected within three replications from different locations: Biare 1,090 masl (meters above sea level), Tawile 1,450 masl and Awiser 1,680 masl. The aerial parts of *Viola* and *Melissa* were collected randomly within three replications at different locations: Biare 1,090 masl, Degashikhan 1,250 masl and Tawile 1,450 masl. The extracts of the aerial parts of these species were purified by filtrations for several times in preparation for HPLC analyses. The chromatograms of *Urtica* indicated the presence of five major important alkaloid components (fragrine, benzylisoquinoline, scopoletin, glucoquinone and dotriacotaine) and ten major important phenolic compounds (formic acid, tannin, chlorogenic acid, caffeoylmalic acid, anthocyanine, quercetin, zeaxanthin, luetin epoxide, coumarine and vanillin). All the concentrations of alkaloid and phenolic compounds were increased significantly due to higher altitudes, except that of alkaloid dotriacotaine. The chromatograms of *Viola* indicated the presence of four major important alkaloid components (violine, isoquinoline, cycloviolacin and luteolin-3-glucoronide) and ten major important phenolic compounds (formic acid, tannin, chlorogenic acid, caffeoylmalic acid, anthocyanine, quercetin, zeaxanthin, luetin epoxide, coumarine and vanillin). The results of the influence of altitudes showed that the concentrations of all alkaloids and phenolic compounds were increased significantly due to higher altitudes, except that of the alkaloid luteolin-3-glucoronide and the phenolic compounds zeaxanthin and luetin epoxide. The chromatograms of *Melissa* indicated the presence of five major essential oils (pinene, linalool, citronellol, geraniol and rosmarinic acid). Their quantitative evaluations were influenced by altitudes indicating that the concentrations of all oils were increased significantly due to the higher altitude, except that of the pinene.

Key words: *Urtica dioica* L., *Viola odorata* L., *Melissa officinalis* L., altitudes, secondary products, medicinal plants.

1. Introduction

Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavors, fragrances and medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide humankind with new remedies [1]. The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition. Eventually, information regarding medicinal plants was recorded in pharmacopoeias [2, 3]. Plants produce an array of chemicals that are known as secondary metabolites which have been utilized by human beings for various purposes, especially for making medicines and as healing agents [4].

Plants growing at high elevation often exhibit many morphological and physiological features, which differ from the same kinds of plant growing at low altitude. In addition, altitude plays a major role in determining the micro-climate at any location. The main features differences between altitudes are due to

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the differences in the pressure, temperature, relative humidity, precipitation and radiation. The variation of topography and climate of Kurdistan Region of IRAQ have created a great diversity in the phytogeography and the distribution of the flora in the region, in addition to their influence on the type of constituents, distribution, qualitative and quantitative presence in each species [5-10]. The components of Kurdistan Region of IRAQ flora seems to be a virgin field for new phytochemical biodynamic constituents, which needs to be exploited as a source of new natural products. Out of the flora, a three distinct species (*Urtica dioica* L., Family *Urticaceae*, Stinging Nettle; *Viola odorata* L., Family *Violaceae* Sweet violet or Banafsaj and *Melissa officinalis* L., Family *Lamiaceae*, Balm or Lemon Balm) were studied in this investigation due to their special habitat where they form a wide spread colony habit typically dominated by their species, their use in traditional Kurdish ethno medicinal and ethno botanical culture and remediation [8]. The aim of this study was to put the fingerprint of the secondary products and quantitatively determination of these products in the natural flora of the region, which can be considered as a national wealth and to evaluate the altitudes and their influence on the production of these constituents during different stages of plant growth, since these types of investigations had not been conducted previously, and most of the available literatures concerning these natural flora which are the species related to our interest, are growing elsewhere.

2. Materials and Methods

2.1 Samples Collection

Leaves of *Urtica dioica* L. were collected during May, just before flowering, at 50% flowering in June and post flowering during October, 2010. The samples were air dried, mixed together and stored for chemical analyses at three locations: Biare, Tawile, and Awiser at Sulaimani North of IRAQ, at elevations of 1,090, 1,450 and 1,680 masl, respectively. *Viola odorata* L. plant leaves were collected at post flowering in May, 2010 at Biare, Degashikhan and Tawile in Sulaimani, at elevations of 1,090, 1,250 and 1,450 masl, respectively.

Leaves of *Melissa officinalis* L. were collected at pre-flowering in May, at 50% flowering in June, and at post flowering at October, 2010. The samples were air dried, mixed together and stored for chemical analyses at three locations; Biare, Degashikhan, and Tawile with elevations of 1,090, 1,250 and 1,450 masl, respectively. All above samples were randomly collected within three replications, in each location and for each stage.

2.2 Extraction and Separation

To isolate alkaloids from *Urtica dioica* L. (1 g) of the dried leaves were extracted with 50 mL hexane to removes fat, oils, terpenes and waxes, this extract is discarded. The remains material is then subjected to an alcohol extraction, with 20 mL ethanol 96%, and evaporated to 1 mL crude alkaloids mixture [11], the extracts were qualitatively and quantitatively analyzed by High Performance Liquid Chromatography (HPLC) Research Laboratory of the Green Field Company in Baghdad using a model shimadzu corporation, Kyoto Japan, LC-10 AV double delivery pump model LC-10 A Shimadzu.

Separation of alkaloid mixture was done using HPLC, on reversed phase C-8 (50 mm × 2.6 mm ID) column, 3 μm particle size, mobile phase was methanol-water (80:20 v/v), the flow rate was 1 mL/min. The separated peaks were monitored by UV detector set at 254 nm, and quantitatively analyzed by comparing the area of well known standard (Fragrine, Benzylisoquinoline, Scopoletin, Glucoquinone and Dotriacotaine) with the area of the sample under the same separation conditions [12]:

$$Conc.\,of\ sample\,(mg/mL) = \frac{Area\ of\ sample}{Area\ of\ standard} \times Conc.\,of\ standard \times Dilution\ factor$$

To isolate phenols from *Urtica dioica* L. 0.5 g of grounded dry *Urtica* leaves has been dissolved in 5 mL hot water for 2 h, using ultrasonic bath for 20 min to get all extract dissolved in hot water at 60 °C, then the extracts were filtered on filter paper No. 1, 0.5 mm to separate the fiber, and then prepared for analysis [13], the extracts were separated on HPLC system, on reversed phase C-18 (50 mm × 4.6 mm ID) column, 3 μm particle size, mobile phase was 0.1% acetic acid-methanol (40:60 v/v), the flow rate was 1.2 mL/min, the separated peaks were monitored by UV detector set at 275 nm, and the quantitatively analyzed by comparing the area of well known standard (formic acid, tannin, chlorogenic acid, caffeoylmalic acid, anthocyanine, quercetin, zeaxanthin, luetin epoxide, coumarine and vanillin) with the area of the sample under the same separation conditions [12].

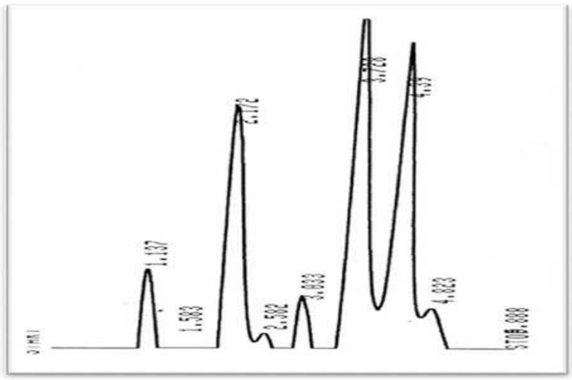
To isolate alkaloids and phenols from *Viola odorata* L., extraction was done as for *Urtica dioica* L., to isolate essential oils from *Melissa officinalis* L., 20 g of dry grounded *Melissa* leaves were distilled by steam distillation with 300 mL of water, with a distillation rate of 2-3 mL/min and a total distillation time of 2 h for each sample. Essential oils were extracted from the aqueous phase with 100 mL of hexane in a separation funnel and then evaporated in rotary evaporator [11], the extracts were separated on HPLC system as mentioned in phenols analyses, and the same equation of concentration of sample were measured.

2.3 Statistics

HPLC data were statistically analyzed through the method of analysis of variance to test the effect of elevations, while for comparisons among means, Duncan's multiple range tests were used at 1% significant level [14].

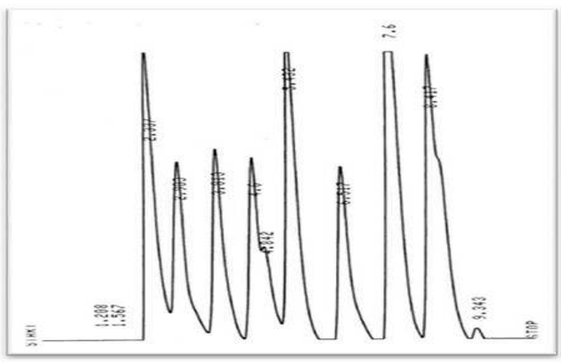
3. Results and Discussion

Figs. 1-5 represent the chromatograms of *U. dioica* L. (alkaloids and phenols), *V. odorata* L. (alkaloids and phenols) and *M. officinalis* L. (essential oils), respectively. The chromatogram in Fig. 1 shows five main peaks for *Urtica*, representing alkaloids (fragrine, benzylisoquinoline, scopoletin, glucoquinone and dotriacotaine), while the chromatogram in Fig. 2 shows the phenols in *Urtica*, representing phenols (formic acid, tannin, chlorogenic acid, caffeoylmalic acid, anthocyanine, quercetin, zeaxanthin, luetin epoxide, coumarine and vanillin).



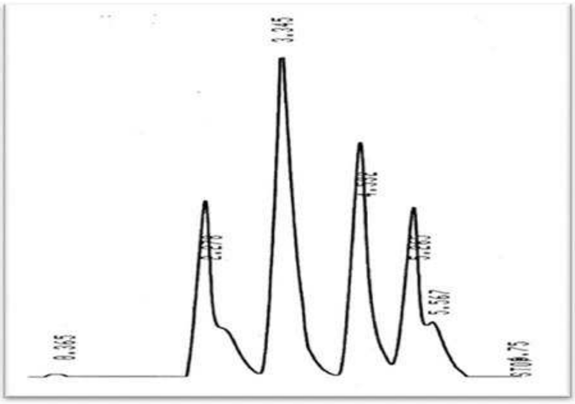
Alkaloids	Rt. (min.)
Fragnine	1.137
Benzylisoquinoline	1.583
Scopoletin	3.033
Glucoquinone	3.728
Dotriacotaine	4.390

Fig. 1 Chromatogram of different alkaloids in *U. dioica* L..



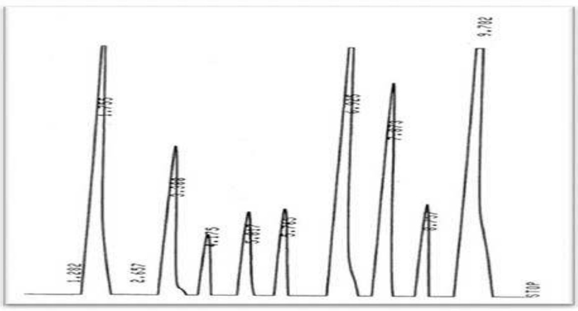
Phenols	Rt. (min.)
Formic acid	1.208
Tannin	2.337
Chlorogenic acid	2.983
Caffeoylmalic acid	3.813
Anthocyanine	4.600
Quercetin	5.432
Zeaxanthin	6.517
Luetin epoxide	7.600
Coumarine	8.417
Vanillin	9.343

Fig. 2 Chromatogram of different phenols in *U. dioica* L..



Alkaloids	Rt. (min.)
Violine	2.278
Isoquinoline	3.345
Cycloviolacin	4.532
Luteolin-3-glucoronide	5.265

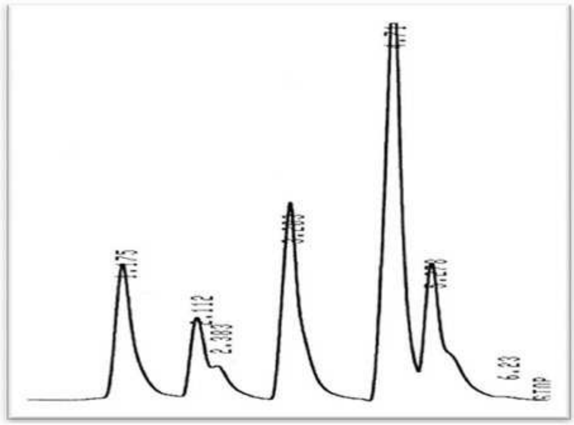
Fig. 3 Chromatogram of different alkaloids in *V. odorata* L..



Alkaloids	Rt. (min.)
Formic acid	1.282
Tannin	1.775
Chlorogenic acid	2.657
Caffeoylmalic acid	3.388
Anthosyanine	4.175
Quercetin	5.017
Zeaxanthin	6.925
Luetin epoxide	7.873
Coumarine	8.757
Vanillin	9.702

Fig. 4 Chromatogram of different phenols in *V. odorata* L..

Figs. 3-5 represent the chromatograms of *V. odorata* L. (alkaloids and phenols), respectively. The chromatograms in Figs. 3 and 4 show *Viola* alkaloids (violine, isoquinoline, cycloviolacin and luteolin-3-glucoronide) and phenols (formic acid, tannin, chlorogenic acid, caffeoylmalic acid, anthocyanine, quercetin, zeaxanthin, luetin epoxide, coumarine and vanillin), respectively. However, the



Essential oils	Rt. (min.)
α-pinene	1.175
Linalool	2.112
Citronellol	3.283
Geraniol	4.710
Rosmarinic acid	5.278

Fig. 5 Chromatogram of different essential oils in *M. officinalis* L..

chromatogram in Fig. 5 shows five main peaks for *Melissa*, representing essential oils (α-pinene, linalool, citronellol, geraniol and rosmarinic acid). In addition, some small minor peaks which could not be identified were also shown in all chromatograms due to the lack of standard that equivalent to their retention times. Similar results of detecting the same components of alkaloids, phenols, and essential oils were obtained in other locations which confirmed by Patrizia et al. [15-19].

Table 1 shows the average concentrations of alkaloids components in *U. dioica* L., which were sampled at different altitudes as an average of different growth stages. It was observed that the location Awiser showed the maximum significant average concentrations of fragrine, benzylisoquinoline, scopoletin and glucoquinone which were 0.398, 1.349, 1.042 and 1.216 mg/g, respectively, while the location Biare gave the maximum significant value of 1.470 mg/g dotriacotaine. Higher altitudes companied lower temperature especially at night compared to lower altitudes where the plants grow, hence the respiration

Table 1 Average concentration of some chemical components of alkaloids in *Urtica dioica* L. grown at different altitudes (mg/g).

Alt. masl	Fragrine	Benzylisoquinoline	Scopoletin	Glucoquinone	Dotriacotaine
Biare 1,090	0.270 c	0.766 b	0.458 c	1.000 b	1.470 a
Tawile 1,450	0.289 b	0.804 b	0.934 b	1.148 a	1.070 b
Awiser 1,680	0.398 a	1.349 a	1.042 a	1.216 a	0.776 c

Means followed by the same letters within a column are not significant by DMRT ($P \leq 0.01$).

Table 2 Average concentrations of some chemical components of phenols in *Urtica dioica* L. grown at different altitudes (mg/g).

Alt. masl	Formic acid	Tannin	Chloroge acid	Caffeoyl acid	Antho-cyanine	Quer-cetin	Zeaxa-nthin	Lepoxide	Coumane	Vanillin
Biare 1,090	0.042 c	0.736 c	0.814 c	1.434 c	0.839 c	1.166 c	1.183 b	0.696 c	0.824 c	0.929 b
Tawile 1,450	0.269 b	1.561 b	1.455 b	1.559 b	1.234 b	1.589 b	1.049 c	1.057 b	1.597 a	0.526 c
Awiser 1,680	0.430 a	1.694 a	1.841 a	1.626 a	1.376 a	1.924 a	1.347 a	1.831 a	1.059 b	1.021 a

Means followed by the same letters within a column are not significant by DMRT ($P \leq 0.01$).

Table 3 Average concentrations of some chemical components of alkaloids in *Viola odorata* L. grown at different altitudes (mg/g).

Alt. masl	Violine	Isoquinoline	Cycloviolacin	Luteolin-3-glucoronide
Biare 1,090	0.025 c	1.560 c	2.201 b	0.802 b
Degashikhan 1,250	0.226 b	1.962 b	2.112 b	1.030a
Tawile 1,450	0.861 a	2.808 a	2.993 a	0.705 c

Means followed by the same letters within a column are not significant by DMRT ($P \leq 0.01$).

rates of the plant reduced and accumulated photosynthetic products, mainly acetyl CoA and phosphenolpyruvate (PEP) tend to produce secondary metabolites such as alkaloids, phenols and essential oils through different pathways which previously mentioned by Kofidis et al. [20, 21]. The average concentrations of some phenols in *U. dioica* L. as averages of different growth stages shown in Table 2, which showed the significant differences due to different altitudes where plants were grown. The maximum value of formic acid, tannin, chlorogenic acid, caffeoylmalic acid, anthocyanine, quercetin, luetin epoxide, zeaxanthin and vanillin were given by the plants at the locations of Awiser (1,680 masl) with the values of 0.430, 1.694, 1.841, 1.626, 1.376, 1.924, 1.347, 1.831 and 1.021 mg/g, respectively. However, the phenols coumarine gave the maximum significant value of 1.597 mg/g in Tawile only. The increasing of phenols components may be due to the temperature decreases in higher altitudes of Awiser location which previously confirmed by Zidorn et al. [22, 23].

Table 3 shows the average concentrations of some alkaloids of *Viola odorata* L. grown at different altitudes. These data showed that Tawile (1,450 masl) location gave the highest values of alkaloids concentrations (0.861, 2.808 and 2.993 mg/g) in violine, isoquinoline and cycloviolacin, respectively. While the altitude Degashikhan (1,250 masl) gave the maximum significant value of (1.030 mg/g) of luteolin-3-glucoronide.

Table 4 shows the concentrations of different phenols components of *V. odorata* L. collected from different altitudes. The results showed that formic acid, tannin, chlorogenic acid, caffeoylmalic acid, anthocyanine, quercetin, coumarine and vanillin concentrations at Tawile (1,450 masl) gave the highest values of 3.094, 0.271, 1.647, 1.921, 1.121, 1.464, 1.391 mg/g and 2.996 mg/g, respectively. Zeaxanthin and luetin epoxide also showed significantly higher values of 2.142 and 1.708 mg/g, respectively at Biare (1,090 masl) altitudes. Quercetin and coumarine did not show any significant differences in the values of their

Table 4 Average concentrations of some chemical components of phenols in *Viola odorata* L. grown at different altitudes (mg/g).

Alt. masl	Formic acid	Tannin	Chloro-genic acid	Caffeoyl-malic acid	Anthocy-anine	Quer-cetin	Zeaxa-nthin	Luetin-epoxide	Coum-arine	Van-Illin
Biare 1,090	0.131 b	0.006 b	0.616 c	0.733 c	0.663 b	1.436 a	2.142 a	1.708 a	1.354 a	0.878 b
Degashikhan 1,250	0.006 c	0.014 b	1.405 b	1.840b	0.467 c	1.192 b	1.744 b	0.294 c	1.026 b	0.427 c
Tawile 1,450	3.094 a	0.271 a	1.647 a	1.921 a	1.121 a	1.464 a	0.720 c	0.705 b	1.391 a	2.996 a

Means followed by the same letters within a column are not significant by DMRT ($P \leq 0.01$).

Table 5 Average concentrations of some chemical components of essential oil in *Melissa officinalis* L. grown at different altitudes (mg/g).

Alt. masl	∝-pinene	Linalool	Citronellol	Geraniol	Rosmarinic acid
Biare 1,090	1.390 a	0.507 c	0.809 c	2.019 c	0.475 c
Degashikhan 1,250	0.742 b	0.881 b	0.867 b	2.137 b	0.939 b
Tawile 1,450	0.428 c	2.169 a	2.056 a	3.159 a	1.082 a

Means followed by the same letters within a column are not significant by DMRT ($P \leq 0.01$).

concentrations between Tawile and Biare locations. The above results indicated that geographical and climatic conditions in different regions could lead to significant differences in phenols components, these results in accordance with Zhou et al. [24], which mentioned that the maximum polyphenole and flavonoids contents recorded in plants, sampled at higher altitude locations. Table 5 shows the values of some chemical components of essential oils in *M. officinalis* L. as averages of different growth stages, sampled at different altitudes. Generally, it was noticed that Tawile (1,450 masl) gave the maximum significant values of 2.169, 2.056, 3.159 and 1.082 mg/g of linalool, citronellol, geraniol and rosmarinic acid, respectively, while the component α-pinene gave the maximum significant value of 1.390 mg/g sampled at Biare (1,090 masl). The variation in essential oils content of *M. officinalis* L. depending on the locations where the plants grow mainly in favor of higher altitudes which recorded by Milos et al. [25].

4. Conclusions

Out of the flora, the three distinct species, *U. dioica* L., *V. odorata* L. and *M. officinalis* L. due to their special habitat are growing successfully in different locations at Biare (1,090 masl), Degashikhan (1,250 masl), Tawile (1,450 masl) and Awiser (1,680 masl), where these locations were not recorded in the flora of IRAQ.

Altitudes influenced the secondary metabolites products of the three plant species; plant grown at high altitudes produced highest concentrations of the three chemical contents (alkaloids, phenols, and essential oils).

References

[1] G.M. Cragg, D.J. Newman, Biodiversity: A counting source of novel drug leads, Pure Appl. Chem. 77 (2005) 7-24.

[2] M.J. Balunas, D.A. Kinghorn, Drug discovery from medicinal plants, review article, Life Sci. 78 (2005) 431-441.

[3] B. Patwardhan, D. Warude, P. Pushpangadan, N. Bhatt, Ayruveda and traditional Chinese medicine: A comparative over view, E.C.A.M. 2 (2005) 465-473.

[4] M. Ozturk, S. Gucel, A. Celik, T. Mert, E. Akcicek, E. Altundag, Genetic Resources, Chromosome Engineering and Crop Improvement: Medicinal Crops, Chapter 8, Vol. 6, CRC Press, 2011.

[5] E. Guest, Flora of Iraq, Ministry of Agriculture, Baghdad, Iraq, 1966, pp. 58-106.

[6] C.C. Townsend, E. Guest, Flora of Iraq, Ministry of Agriculture, Baghdad, Iraq, 1966, Vol. 1, pp. 1-47.

[7] A. Al Rawi, H.L. Chakravarty, Medical Plant of Iraq, 2nd ed., Ministry of Agriculture, Baghdad, Iraq, 1988, pp. 1-29.

[8] H.L. Chakravarty, Plant Wealth of Iraq, Ministry of Agriculture, Baghdad, Iraq, 1976, pp. 52-152.

[9] P.H. Davis, R.R. Mill, K. Tan, Flora of Turkey and the East Aegean Islands, The Univ. Press, Edinburgh, Vol. 8,

1984, p. 632.

[10] K.H. Rechinger, Labiatae, Flora of Iran Co., Akad. Or.u. Ver., Gra., 150, 1982, p. 597.

[11] S. Blated, H. Wagner, Plant Drug Analysis, Springer, NY, USA, 1996, pp. 1-78.

[12] G. Bisignano, K. Sanogo, A. Masino, R. Aquino, U.D. Angelo, M.P. Germano, et al., Phytochemistry 30 (2000) 105-108.

[13] R. Nicolas, P. Morris, K. Judith, Phenolic Compounds: Extraction and Analysis, *Lotus japonicus* Handbook, Chapter 7, 2005, pp. 349-355.

[14] Addinsoft, XLSTAT Pro. Version 7.5.3 [Online], 2005, http://www. xlstat.com/en/ho.

[15] P. Patrizia, I. Francesca, V. Pamela, B. Laura, B. Silvia, R. Annalisa, Extraction and HPLC analysis of phenolic compounds in leaves, stalks, and textile fibers of *Urtica dioica* L., J. Agric. Food Chem. 56 (2008) 9127-9132.

[16] K. Koll, E. Reich, A. Blatter, M. Veit, A new HPTLC-method for identification of stinging nettle leaves, Z. Arzn. Gew. Pfl. 8 (2003) 175-180.

[17] B. Samara, G. Dastagir, F. Hussain, P. Sanaullah, Elemental composition of *Viola odorata*, Pak. J. Sci. 12 (2006) 141-143.

[18] L. Ancatoiu, I. Oniga, M. Ttamas, Quantitative analysis of some phenolic compounds from Viola species tinctures, Farmacia 4 (2008) 440-447.

[19] B.B. Reyhan, The essential oil of lemon balm (*Melissa officinalis* L.), its components and using fields, J. of Fac. of Agric. OMU. 21 (2006) 116-121.

[20] G. Kofidis, A.M. Bosabalidis, M. Moustakas, Combined effect of altitude and season on leaf characteristics of *Urtica dioica* L., Environmental and Experimental Botany 60 (2007) 60-69.

[21] N. Krishnan, A. Jeyachandran, N. Nagendran, Effect of seasonal and altitudinal variation on growth performance of *Urtica dioica*, in Alagar Hill, South India, Tropical Ecology 41 (2000) 41-45.

[22] C. Zidorn, B. Schubert, H. Stuppner, Altitudinal differences in the contents of phenolics in aerial parts of *Urtica dioica* in New Zealand, Biochemical Systematics and Ecology 33 (2005) 855-872.

[23] I. Schonhof, H. Klaring, A. Krumbein, W. Chuben, M. Schrerer, Effect of temperature increase under radiation condition on phytochemicals and ascorbic acid in greenhouse grown stinging nuttle, Agriculture, Ecosystems and Environment 119 (2006) 103-111.

[24] M. Zhou, Y. Chen, Q. Ouyang, S.X. Liu, Z.J. Pang, Influence of environmental factors on antioxidant activity, phenol and alkaloids contents of *V. odorata* L. and *B. hyrcana* L., Am. J. Chin. Med. 28 (2000) 239-249.

[25] M. Milos, A. Radonic, N. Bezic, V. Dunkic, Localities and seasonal variations in the chemical composition of essential oils of *Melissa officinalis* L. and *S. cuneifolia* Ten., Flavour Frag. J. 16 (2001) 157-160.

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Effect of Mercury on the Proximate Composition of Maize (*Zea mays* L.)

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Abstract: Plants can be exposed to mercury either by direct administration as antifungal agents, mainly to crop plants through seed treatment or foliar spray, or by accident. Mercury poisoning has become a problem of interest on a global scale. Natural emissions of mercury form two-thirds of the input; man-made releases form about one-third. Significant amounts of mercury may be added to agricultural land with sludge, fertilizers, lime and manures. Total mercury levels were determined in six breeds of quality protein maize using cold vapor atomic absorption spectrometry (CVAAS). The breeds analyzed were Aziga, Abeleehi, Akposoe, Golden Jubilee, Etubi and Obaatanpa. Proximate composition of these breeds was also determined. The objective of this study was therefore to assess the effect of mercury on the proximate composition of quality protein maize. The proximate composition of the maize varieties analyzed showed that the moisture content ranged from 11.57% ± 0.205% to 12.76% ± 0.042%, ash from 1.11% ± 0.064% to 1.58% ± 0.021%, protein, 6.51% ± 0.307% to 10.39% ± 0.306%, fiber 1.44% ± 0.071% to 1.87% ± 0.057%, fat 1.84% ± 0.078% to 2.75% ± 0.092% and carbohydrate 71.77% ± 0.035% to 76.54% ± 0.216%. The total mercury levels in the maize breeds analyzed ranged from 0.0010 ± 1.17E-05 µg/g to 0.0079 ± 1.00E-05 µg/g. The mercury levels detected were lower than the WHO limit for mercury in food of 0.5 µg/g in all the maize breeds. The low levels of mercury in the maize samples show they are safe for consumption.

Key words: Mercury, toxicity, maize, proximate composition, CVAAS.

1. Introduction

Mercury poisoning has become a problem of current interest as a result of environmental pollution on a global scale. Natural emissions of mercury form two-thirds of the input and man-made releases form about one-third [1]. Significant amounts of mercury may be added to the soil with the introduction of sludge, fertilizers, lime and manures [1]. The most important sources of contaminating agricultural soil have been the use of organic mercury as a seed-coat dressing to prevent fungal diseases in seeds [1]. The availability of soil mercury to plants is low, but, mercury has the tendency to accumulate in the roots, indicating that the roots serve as a barrier to mercury uptake. Mercury concentration in above ground parts of plants appears to depend largely on foliar uptake of Hg volatilized from the soil [1]. Uptake of mercury has been found to be plant specific in bryophytes, lichens, wetland plants, woody plants and crop plants. Maize ranks third in the global cereal production and is utilized as food, feed and fodder [2]. Maize is high yielding, easy to process, readily digested and cheaper than other cereals. Maize is also a versatile crop, growing across a wide range of agro-ecological zones [3]. Quality protein maize has high concentration of lysine and trytophan content and therefore reduces malnutrition, diseases and death among low income maize consumers in the developing countries [4-8]. Mercury cations have a high affinity for sulphydryl

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(-SH). Almost all proteins contain sulphydryl groups or disulphide bridges, hence, mercury can disturb any function in which critical or non-protected proteins are involved [9]. There is not much literature on the effect of mercury on maize. The objective of this study was therefore to assess the effect of mercury on the proximate composition of quality protein maize.

2. Materials and Methods

2.1 Sample Collection

Six different breeds of quality protein maize namely Aziga, Abeleehi, Akposoe, Golden Jubilee, Etubi and Obaatanpa, were collected from store houses of the Crops Research Institute (Council for Scientific and Industrial research), Ghana. The samples were stored in well labeled transparent plastic containers and kept at room temperature prior to analysis.

2.2 Reagents

All reagents were of analytical reagent grade. HCl, H_2SO_4 and HNO_3 were purchased from BDH (London, UK). Titrasol (1,000 mg/L) stock solution of mercury was purchased from Merck. (Stuttgart, Germany). DORM-2 certified reference material for Hg was obtained from the National Research Council of Canada (NRCC).

2.3 Instrumentation

A Cold Vapor Atomic Absorption Spectrometer equipped with an automatic Direct Mercury Analyzer Model HG-5000 (Sanso Seisakusho Co., Ltd., Japan) was used for Hg analysis. Signal outputs were captured on a Yokogawa Model 3021 strip chartrec order.

2.4 Method Description

All glassware was soaked overnight in 10% (v/v) nitric acid followed by washing with 10% (v/v) hydrochloric acid. Acid-washed glassware was rinsed with double distilled water and oven dried before use. Double distilled water was used wherever water is specified.

2.5 Preparation of Solutions

Standard stock solution of mercury was prepared from Titrasol (1,000 mg/L) by dilution to the desired concentration with water. Working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions with the acid mixture of 1 M HCl and 5% H_2SO_4. Stannous chloride solution (10% w/v) was prepared by dissolving 10 g of the salt in 100 mL, 1 M HCl.

2.6 Sample Preparation

The maize samples were air dried for 72 h and milled into fine powder.

2.7 Proximate Analysis

The chemical composition of the various maize species was determined using standard methods described in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) [10]. The parameters under investigation were moisture, ash, protein, fat, fiber and total carbohydrate contents. All the parameters analyzed were expressed in percentages.

2.8 Determination of Moisture Content

The method used involved measurement of weight loss due to evaporation of water. Labeled crucibles according to the various maize brands were washed and dried in an oven at 110 °C for 10 min and cooled in a desiccator. A 2.0 g weight of each ground sample was put in their respective crucibles and placed in a hot oven and dried to a constant weight at 105 °C for 72 h. The difference in weight was estimated as the moisture content.

2.9 Determination of Ash Content

Organic matter in the samples was burnt away leaving the in organic residue. Well labeled ceramic crucibles were heated in a muffle furnace at 500 °C for 1 h, cooled in a desiccator and weighed. A 2.0 g weight of each of the ground samples was put into their respective crucibles. These were then ignited in the furnace at 500 °C for 3 h. The crucibles and their content were then cooled in a desiccator to room temperature and weighed. The difference in weight was estimated as the ash content.

2.10 Determination of Protein Content

Nitrogen content was determined by the micro-Kjeldahl technique and protein content of each sample was estimated ($N \times 6.25$), where N is the nitrogen content, following the method of AOAC [10]. A 1.0 g portion of maize powder of each variety was weighed into a Kjeldahl digestion flask and a catalyst mixture (2.0 g of potassium sulfate, 1.0 g of copper sulfate and 0.1 g selenium powder) was added and followed by 10 mL concentrated H_2SO_4. The flask was then heated cautiously under fume hood until a greenish clear solution appeared. After clearing, heating continued for more than 30 min and the mixture allowed cooling. A 10 mL aliquot of distilled water was added into the digest and shaken thoroughly. Steam was passed through a Markham distillation. The digest was transferred into 100 mL measuring cylinder and diluted with distilled water to the 100 mL mark. Under the condenser of the distillation apparatus, a receiver flask containing 10 mL of 2% boric acid as indicator was placed, 10 mL of the diluted digest was introduced into the distillation unit and 10 mL of 40% NaOH solution was slowly added. The distillation continued for 5 min and was stopped by closing the inlet stop cork first and then opening the steam bypass. The distillate was titrated with 0.01 M HCl to a pink colored endpoint.

2.11 Determination of Fiber Content

In determining the fiber content of the sample, a 2.0 g portion of each sample was weighed into a 1 L preheated Erlenmeyer flask and 0.128 M H_2SO_4 added. The solution was boiled for 30 min using a water pressure filter system. The solution was filtered and residue washed three times with hot water. The residue was collected and 150 mL preheated 0.22 M KOH was added and boiled for another 30 min, the mixture was filtered and residue washed on the water pressure system three times with acetone. The residue was collected in crucible, dried at 130 °C for 1 h and weighed. It was ignited in muffled furnace for 3 h at 500 °C, cooled and weighed. The loss in weight was estimated as the fiber content.

2.12 Determination of Fat Content

Fat content was determined by the soxhlet extraction method [10]. A 250 mL fit round bottom flask was washed and dried in an oven at 100 °C and cooled in a desiccator and weighed. A 1.0 g portion of each sample was placed in an extraction thimble. This was placed in the extraction column with the condenser connection. About 150 mL petroleum ether (boilingrange 40-60 °C) was poured into the round bottom flask and fitted into the extraction unit. The extraction unit was then heated in a heating mantle at 60 °C for 2 h under reflux. After the extraction, the thimble was removed from the column and the solvent distilled from the flask. The flask was disconnected and placed in an oven at 100 °C for 2 h and later weighed after cooling. The weight of empty flask was subtracted from the final weight. The estimated weight was expressed as the fat content.

2.13 Determination of Carbohydrate Content

This was determined by difference after adding the percent crude protein, moisture, ash, crude fiber and fat content and subtracted from100%.

2.14 Determination of Mercury

A 0.5 g weight of each powdered sample was weighed into a 0.5 L glass digestion tube, and 10 mL of concentrated $HNO_3/HClO_4$ (1:1) and 5 mL of concentrated H_2SO_4 were slowly added. The tube was then placed on top of a steam bath unit and samples were allowed to dissolve completely. The digest was removed from the steam bath, cooled and filtered. A

10 mL solution of stannous chloride (10% w/v) was added to the filtered solution before carefully transferring it into a 50 mL volumetric flask. The flask was diluted to the mark with distilled water.

Mercury levels were determined directly on each final solution. A 5 mL aliquot of each sample was injected into the direct mercury analyzer on the cold vapor atomic absorption spectrophotometer and signals recorded. Calibration standard solutions, reagent blanks and reagent blank spikes for Hg were analyzed in the same way as the sample.

2.15 Limit of Detection

The limit of detection (LOD) for the solution was initially estimated for each analytical batch as three times the standard deviation of the three reagent blanks. Sample LODs were then calculated for each metal by multiplying the solution limit of detection by the dilution volume and dividing by the weight of the actual sample. The solution LODs (µg/mL) for mercury is 0.001.

2.16 Quality Assurance and Quality Control Measures

To ensure the validity of results, the following measures were taken. Prior to sample analysis, certified reference samples (DORM-2) for all metals were analyzed in order to verify adequate system performance. Agreement of spectrometer readings with DORM-2 certified reference standards analyzed prior to sample analysis and in between 10 sample runs were satisfactory (Table 1). Each batch of sample analysis was prepared to include three reagent blanks in duplicates to control for background contamination and two reagent blank spikes in duplicate to confirm satisfactory metal recovery greater than 90%. In addition, the correlation coefficient (r^2) for the calibration curve was required to be greater than 0.995. All samples were analyzed in duplicate.

2.17 Statistical Analysis

The mean and standard deviation were calculated using Microsoft Excel software.

3. Results and Discussion

The proximate composition of the various maize breeds has been summarized in Table 2.

The moisture content of the various maize breeds spanned over a range of 11.57% ± 0.205% to 12.76% ± 0.042%. The highest moisture content found in Abeleehi and Aziga had the least. The differences in moisture content of the samples may be due to varietal differences. High moisture content in maize increases fungal growth and reduces storage time as grains go bad. The moisture content obtained in all samples compared favorably with literature [11, 12].

Etubi had the highest ash content of 1.58% ± 0.021% with Abeleehi having the least of 1.11% ± 0.064%.

Table 1 Validation of automatic mercury analyzer performance with NRCC DORM-2 standards.

Element	DORM-2 standards	
	Certified value (mg/kg)	Measured value (mg/kg)
Hg	4.64 ± 0.26	4.43 ± 0.17

Table 2 Proximate composition of maize (mean ± standard deviation).

Sample	Moisture (%)	Ash (%)	Protein (%)	Fiber (%)	Fat (%)	Carbohydrate (%)
Aziga	11.57 ± 0.205	1.56 ± 0.064	9.29 ± 0.278	1.87 ± 0.057	2.36 ± 0.078	73.36 ± 0.525
Abeelehi	12.76 ± 0.042	1.11 ± 0.064	8.34 ± 0.549	1.77 ± 0.113	2.18 ± 0.099	73.84 ± 0.358
Akposoe	11.68 ± 0.205	1.50 ± 0.064	10.39 ± 0.306	1.69 ± 0.064	2.04 ± 0.120	72.72 ± 0.019
Golden Jubilee	12.00 ± 0.495	1.36 ± 0.057	6.51 ± 0.307	1.76 ± 0.106	1.84 ± 0.078	76.54 ± 0.216
Etubi	12.63 ± 0.297	1.58 ± 0.021	9.84 ± 0.332	1.44 ± 0.071	2.75 ± 0.092	71.77 ± 0.035
Obaatanpa	12.42 ± 0.064	1.41 ± 0.014	7.56 ± 0.297	1.73 ± 0.071	2.10 ± 0.007	74.79 ± 0.410

These values compared favorably with literature [11, 12], but was generally lower than the reported values by Purseglove et al. [13]. The ash content gives an indication of the metals content in the grain sample. Low ash content therefore indicates low metals content.

The protein content ranged from 6.51% ± 0.307% to 10.39% ± 0.306%. Akposoe showed the highest percentage crude protein. This implies that Akposoe can be used as a highly nutritious cereal in malnourished human beings and livestock. These high protein levels are due to genetic improvement of the maize breeds. Golden Jubilee on the other hand, had the least protein content. Quality Protein Maize is known to have average levels of protein of about 9% [13]. The protein content obtained in the maize breeds in most cases agreed with this value.

The fiber content ranged from 1.44% ± 0.071% to 1.87% ± 0.057%. Aziga had the highest fiber content and the least was found in Etubi. High crude fiber content aids in the digestion process of humans and livestock and prevents constipation [14]. The values obtained for fiber in the maize breeds were lower than reported values in literature [11-13].

The fat content of the maize breeds ranged from 1.84% ± 0.078% to 2.75% ± 0.092%. Etubi had the highest fat content with Golden Jubilee having the least. The fat content of the maize gives an indication about the amount of oil it contains and whether it is suitable for oil extraction. Fat content in the maize varieties were generally low as compared with literature values of other quality protein maize varieties studied [11, 13].

Mercury concentrations of the various maize varieties determined showed the highest level in Obaatanpa, recording 0.0079 ± 1.00E-05 µg/g and least in Abeelehi recording 0.0010 ± 1.17E-05 µg/g. Mercury is toxic even in minute quantities. The WHO tolerable limit for mercury in food is 0.5 µg/g. Mercury exposure through food often occurs when seafood containing mercury is eaten, or when mercury containing plants are consumed. The mercury levels in the maize breeds are summarized in Table 3.

Table 3 Levels of mercury in maize (mean and standard deviation).

Sample	Concentration (µg/g)
Aziga	0.0012 ± 5.08E-05
Abeleehi	0.0010 ± 1.17E-05
Akposoe	0.0058 ± 1.00E-05
Golden Jubilee	0.0056 ± 1.00E-05
Etubi	0.0076 ± 1.00E-05
Obaatanpa	0.0079 ± 1.00E-05

Phytotoxic effects of mercury compounds have been reported in several plants, including Triticumaestivum [15], Oryza sativa [16] and several other grain crops [17, 18]. In general, the degree of impact depends on the concentration, the formulation, the mode of application and the cultivar. The seed injury caused by organic mercurial to cereals has been characterized by abnormal germination. A mercury ion may bind to two sites of a protein molecule without deforming the chain, or it may bind two neighboring chains together, or a sufficiently high concentration of mercury may lead to protein precipitation [1, 9].

4. Conclusions

The mercury levels detected were lower than the WHO limit for mercury in food of 0.5 µg/g in all the maize breeds. The low levels of mercury show they are safe for consumption. The effect of the mercury content on the proximate composition was not significantly evident in this study due to very low levels obtained, however, an increase in the mercury levels generally showed a corresponding increase in the ash content. There were some exceptions due to varietal changes. The low protein content in Obaatanpa may also be due to the level of mercury found in it. There was no significant correlation between the mercury concentration and the proximate composition of the various maize breeds analyzed.

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References

[1] M. Patra, A. Sharma, Mercury toxicity in plants, The Bot. Rev. 66 (2000) 379-422.

[2] J. Romana, S. Saeed, S. Mughal, K. Ashraf, N. Arshad, K. Hussain, et al., Venomous effects of $CuSO_4$ on maize (*Zea mays* L.), Bot. Res. Intl. 5 (2012) 40-43.

[3] O.A. Akinbode, Evaluation of antifungal efficacy of some plant extracts on *Curvularialunata*, the causal organism of maize leaf spot, Afr. J. Env. Sci. Tech. 4 (2010) 797-800.

[4] F.A. Showemimo, Analysis of divergence for agronomic and nutritional determinants of quality protein maize, Trop. Subtr. Agroecosyst. 4 (2004) 145-148.

[5] S.A. Olakojo, O. Omueti, K. Ajomale, B.A. Ogunbodede, Development of quality protein maize: Biochemical and agronomice valuation, Trop. Subtr. Agroecosyst. 7 (2007) 97-104.

[6] A.F. Krivanek, D.G. Hugo, S.G. Nilupa, O.D. Alpha, F. Dennis, Breeding and disseminating quality protein maize (QPM) for Africa, Afr. J. Biotechnol. 6 (2007) 312-324.

[7] S. Upadhyay, D.B.Gurung, D.C. Paudel, K.B. Koirala, S.N. Sah, R.C. Prasad, et al., Evaluation of quality protein maize (QPM) genotypes under rain fed mid hill environments of Nepal, Nepal J. Sci. Techn. 10 (2009) 9-14.

[8] K. Mbuya, K.K. Nkongolo, A. Kalonji-Mbuyi, Nutrition analysis of quality protein maize selected for agronomic characteristics in a breeding program, Int. J. Plant Breed. Genetic 2 (2011) 325-332.

[9] T.W. Clarkson, The pharmacology of mercury compounds, Ann. Rev. Pharmacol. 12 (1972) 75-406.

[10] AOAC Standard Official Methods of Analysis of the Association of Analytical Chemists, 16th ed., Washington DC, United States, 1995.

[11] G. Olaoye, O.B. Bello, A.K. Ajani, T.K. Ademuwagun, Breeding for improved organoleptic and nutritionally acceptable green maize varieties by crossing sweet corn (*Zea mays saccharata*): Changes in quantitative and qualitative characteristics in F_1 hybrids and F_2 populations, Journal of Plant Breeding and Crop Science 1 (2009) 298-305.

[12] F.N. Nweke, Rate of water absorption and proximate analysis of different varieties of maize cultivated in Ikwo Local Government Area of Ebonyi State, Nigeria, African Journal of Biotechnology 9 (2010) 8913-8917.

[13] J.W. Purseglove, Tropical Crops, Monocotyledons, 6th ed., Longman Singapore Publishers, Singapore, 1992, p. 316.

[14] L.G. Saldanha, Fibre in diet of U.S. Children: Results of national surveys, Pediatrics 96 (1995) 994-996.

[15] H.J. Mosso, Phytotoxicity test of mercury phenyl acetate in wheat seeds (*Triticumaestivum*), Universidade Buenos Aires, Facultadde Agronomia, Buenos Aires, 1979.

[16] T. Shimizu, M. Maeda, T. Nakamura, Effect of lead and mercury on the rice plant and some vegetables, Bull. Osaka Agric. Res. Center 12 (1975) 117-129.

[17] L.H. Blomqvist, Influence on six-rowed barley by disinfection compounds: A comparison between mercury containing and mercury disinfectants [imazalil], Meddelandefraan Statens Utsae-deskontroll 60 (1985) 62-69. (in Swedish)

[18] S. Karlberg, The phytotoxic effects of some disinfectants (mercury and non-mercury compounds on barley, oats, rye, wheat), Meddeland, Statens Centrala Froekontrollanstalt, Solna 51 (1976) 43-51. (in Swedish)

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Comparative Life-cycle Assessment of Sheet Molding Compound Reinforced by Natural Fiber vs. Glass Fiber

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Abstract: We present comparative life-cycle assessments of three fiber-reinforced sheet molding compounds (SMCs) using kenaf fiber, glass fiber and soy protein resin. Sheet molding compounds for automotive applications are typically made of unsaturated polyester and glass fibers. Replacing these with kenaf fiber or soy protein offers potential environmental benefits. A soy-based resin, maleated acrylated epoxidized soy oil (MAESO), was synthesized from refined soybean oil. Kenaf fiber and polyester resins were used to make SMC1 composites, while SMC2 composites were made from kenaf fiber and a resin blend of 20% MASEO and 80% unsaturated polyester. Both exhibited good physical and mechanical properties, though neither was as strong as glass fiber reinforced polyester SMC. The functional unit was defined as mass to achieve equal stiffness and stability for the manufacture of interior parts for automobiles. The life-cycle assessments were done on SMC1, SMC2 and glass fiber reinforced SMC. The material and energy balances from producing one functional unit of three composites were collected from lab experiments and the literature. Key environmental measures were computed using SimaPro software. Kenaf fiber-reinforced SMC composites (SMC1 and SMC2) performed better than glass fiber-reinforced SMC in every environmental category. The global warming potentials of kenaf fiber-reinforced SMC (SMC1) and kenaf soy resin-based SMC (SMC2) were 45% and 58%, respectively, of glass fiber-reinforced SMC. Thus, we have demonstrated significant ecological benefit from replacing glass fiber reinforced SMC with soy-based resin and natural fiber.

Key words: Natural fiber, reinforced composites, sheet molding compound, life-cycle assessment.

1. Introduction

Sheet molding compounds (SMCs) are mixture of molding resin, fiber, filler and additives. Traditional SMC resins for automotive applications consist of various unsaturated polyester resins (UPR) and vinyl esters. Reinforcements are usually made of short chopped fiberglass and carbon fibers. The morphology of bast fibers, such as kenaf, is similar to that of glass fibers. Further, the tensile strength and modulus of bast fibers is very attractive. Research is underway to determine whether these renewable natural fibers could replace the non-renewable glass and plastic fibers. Various natural fiber-reinforced polymer composites have been investigated using natural fibers such as kenaf, hemp, jute and coir and commodity polymers such as polyethylene, polypropylene and unsaturated polyester resins [1, 2]. However, no natural fiber reinforced composites have achieved physical and mechanical properties comparable to glass fiber-reinforced composites. This is the case even in the laboratory where process parameters are controlled. The water resistance and impact toughness of natural fiber-reinforced polymer composites are far inferior to that of glass fiber-reinforced composites [3]. Natural fibers are hydrophilic and tend to intertangle, making it more difficult to disperse the fibers uniformly into the resin matrices in a scalable production. Although, there is need for improved technology to enable natural fiber-reinforced

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composites for automotive applications, this paper focuses on the environmental impacts of using natural fiber-reinforced composites using current technology.

Natural fiber-reinforced composites have been generally perceived as renewable, biodegradable and environmentally friendly. Clearly, utilizing natural fibers to make composite materials reduces global dependency on petroleum, however, quantitative measurements of this characteristic have not been fully conducted. It is not self-evident that using natural fibers will reduce global carbon dioxide emissions given that agriculture itself generates greenhouse gases through the use of fertilizers, herbicides, pesticides and land clearing. While there is often an intuitively appealing sense about the renewability, biodegradability and environmental friendliness of a product, process or service, the claims do not always stand up to objective analysis. In addition, there are very little relevant data on the end-of-life of these bio-based materials. In a landfill, for example, off-gassing, primarily of carbon dioxide and methane, occurs with natural materials. Methane is both a greenhouse gas and an important energy source. Conventional plastics degrade very slowly (on a timeframe of hundreds of years) in landfills. While this is not a positive quality from a landfill capacity perspective, it does mean that carbon is sequestered, minimizing air and water pollution [4]. In this sense, biodegradability is not a desirable product feature.

A landfill, however, with a gas collection system, can use off-gas to generate electricity for distribution to the municipal electric grid. In Denton, TX (population 113,383), for example, a landfill gas generation system produces 1.6 megawatts, powering the equivalent of approximately 1,600 homes per year (www.cityofdenton.com). In this sense, biodegradability and rate of degradation determine rates of methane gas production and landfill capacity recovery. This unique effort in Denton to utilize landfill emissions provides significant economic and environmental benefits. In this case, biodegradability is a desirable product feature.

Life-cycle assessment (LCA) is a standardized process for quantifying the life-cycle environmental impacts of materials, processes or services. Environmental impact indicators may include global warming potential, embodied energy and embodied water. The LCA consists of goal definition and scoping to define the product, process or activity, inventory analysis (which identifies material usage and environmental releases), impact analysis (which assesses the human and ecological effects of energy, water and material usage) and interpretation, which evaluates the results of each analysis. Integration of life-cycle analysis elements into the early stages of material development and product and construction designs will avoid development of short-lived, costly, and resource-intensive products and structures with negative environmental impacts. With a clear understanding of the main causes of the environmental load in various life-cycle stages, it becomes easy to set priorities in process or product improvement and optimization.

The overall goal of the current project is to substitute natural fiber reinforcement for glass fiber in thermoplastic or thermoset polymer composites to achieve cost and weight savings without sacrificing mechanical property requirements. This report investigates the environmental impacts of this substitution, concentrating on the comparison of kenaf bast fiber versus glass fiber as reinforcement in sheet molding composites. More specifically, this work will determine whether replacing glass fiber with kenaf fiber, and the use of 20% soybean oil modified resin in fabricating SMC, are advantageous from an ecological point of view.

2. Methods

A series of kenaf fiber-reinforced SMCs were fabricated in the laboratory for scoping and optimization. The LCA was conducted on three product scenarios: SMC1 (kenaf fiber SMC), SMC2 (kenaf fiber 20% soy resin SMC, a blend of 20% modified soybean oil and unsaturated polyester resin), and conventional glass fiber SMC. The LCA consisted of two steps: (1) collecting the life-cycle inventory (LCI) for material and energy inputs and emissions from SMC production processes; (2) SimaPro modeling of environmental impacts for the emissions (tabulated in (1)). The LCA data for soybean oil resin and kenaf fiber-reinforced composites were collected from the lab syntheses. Actual industrial practices are expected to be much more energy and material efficient both currently and in potential future scale-up. Data for the unsaturated polyester resin and glass fiber SMC were collected from the literature [5, 6]. Other LCA data related to the manufacturing of raw materials were obtained from the SimaPro software database (US LCI and EcoInvent databases). Catalysts and additives were not included as these materials represent less than 1% of the total material and thus have negligible environmental impact. These data include energy and material balances for the manufacturing of 1 kg of raw materials, intermediates and products, as well as emissions to air, discharges to water and solid wastes to land. These data were then entered into SimaPro V7.3. Environmental performance was measured using environmental impact indexes from the Building for Environmental and Economic Sustainability (BEES) program of the US National Institute of Standards and Technology (NIST). These included cumulative energy demand and a weighted environmental burden.

2.1 Inventory of Materials and Energy

2.1.1 Standard Unsaturated Polyester Resin Production

Life-cycle data for unsaturated polyester resin were collected from the published literature and from life-cycle databases. The commercial petroleum-based propylene glycol maleate (Aropol Q-6585) was provided by Ashland Composite Polymers Company. Both electricity and natural gas were used to manufacture the resin, and this data was provided by Ashland. Onsite energy consumption, including a cooling tower, was included in Ashland's total energy figures, but material and energy inputs were not. The formulation for the standard resin is current and can be assumed to be representative of other UPR resins in the marketplace. Water as a distillate is burned through an oxidizer. The reacted resin is then diluted in styrene to produce the retail mixture. The only other process input is water for cooling, total water used is 0.01 gal/lb of resin. No air emissions, solid waste or other byproducts are produced due to the closed production system [7].

Because specific formulations are not disclosed to the public domain, this resin formulation is drawn from the literature. Fig. 1 provides an example of the reaction route for the synthesis of one kind of UPR-propylene glycol maleate [7]. The polyesterification reaction is usually carried out in a heated stainless steel reactor as a batch process. The reactor is equipped with an agitator for stirring the low viscosity contents. Glycols and molten anhydrides are used in stoichiometric quantities with 5%-10% excess glycol. The polyesterification is an equilibrium condensation reaction liberating water that is removed by an overhead fractionating condenser system. Recovered glycol is recycled back to the reactor. The overall synthesis requires 15-18 h at 190 °C to achieve the desired molecular weight in the final product. Once the reaction is complete, the product is cooled and transferred to a styrene tank. The final contents of the tank are 35% styrene by weight and are kept at room temperature to avoid copolymerization of the dissolved UPR. A small quantity of hydroquinone is

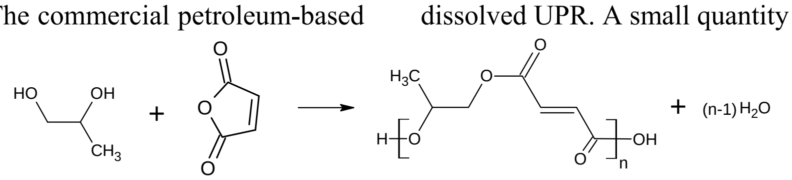

Fig. 1 Synthesis of propylene glycol maleate.

commonly used as a stabilizing agent during the dissolution of the hot UPR in the styrene monomer. Table 1 lists the life-cycle inventory for 1 kg of unsaturated polyester resin dissolved in styrene based on engineering information, process chemistry and literature [7, 8].

2.1.2 Soy Protein Resin Synthesis

Maleinated acrylated epoxidized soybean oil (MAESO) was synthesized by reacting the acrylated epoxidized soybean oil (AESO) with the maleic anhydride. The AESO was synthesized via soybean oil composed mainly of triglyceride molecules. The triglyceride molecules were first epoxidized using formic acid, the epoxy groups are then reacted with acrylic acid to form acrylated epoxidized triglycerides. These acrylated triglycerides were further modified with maleic anhydride to further desaturate the molecules. When these functionalized triglycerides are dissolved into 35% wt% styrene, they form unsaturated polyester-like resins which can be used for SMC applications. The MAESO can also be mixed with propylene glycol maleate to increase resin bio-content. The materials and energy flows (summarized in Table 2) were estimated from the lab synthesis [5, 9].

2.1.3 Composite Fabrication

(1) Kenaf Fiber Sheet Preparation

Kenaf stems harvested by the KenGro Corporation were decorticated (removal of the core), and the bast was cut into 2 inch lengths. High quality bast fibers were obtained through a weak chemical retting process in the reactor [10, 11]. The kenaf fibers were dispersed in water by vigorous mechanical stirring. The fiber suspension was poured into a 355 mm × 355 mm deckle box and then passed through a screen (mesh 35), on which the fiber sheets were formed as the water flowed down gravitationally. The fiber sheets were dried in an oven at 80 °C.

(2) Kenaf SMC Fabrication

The composites were fabricated with a sheet molding compound process. The UPR resin was unsaturated polyester AROPOL Q 6585 from Ashland.

The UPR contained 65 parts propylene glycol maleate and 35 parts styrene by weight. MAESO were added to the UPR resin paste to copolymerize with unsaturated polyester. The free radical initiator was

Table 1 Materials and energy input and output of 1 kg unsaturated polyester resin (UPR) dissolved in styrene.

Standard unsaturated polyester resin		
Material-energy/kg	Quantity	
Propylene glycol	0.29	kg
Maleic anhydride	0.19	kg
Styrene	0.35	kg
Diethylene glycol	0.031	kg
Hydroquinone	0.0002	kg
Water	15.93	kg
Cooling water	106	kg
Electricity	0.379	MJ
Natural gas	0.063	m^3
Total UPR	1	kg

Table 2 Materials and energy input and output of 1 kg MAESO.

Soy resin production		
Material/energy	Quantity	
Refined soybean oil	0.54	kg
Formic acid	0.1	kg
Acylic acid	0.15	kg
Maleic anhydride	0.21	kg
Water	0.022	Gal
Electricity	0.258	kWh
Natural gas	0.091	m^3
Total soy resin	1	kg

Table 3 Kenaf sheet molding compound formulations (wt%).

Kenaf SMC formulations	SMC (0% soy resin)	SMC2 (20% soy resin)
Standard UPR resin	60.5	48.5
Soy resin	0	8
Styrene	0	4
Catalyst (TBP)	1.3	1.3
Inhibitor	0.1	0.1
Kenaf fiber	38	38
Kenaf SMC properties		
Density (g/cm^3)	0.95	1.04
Flexural modulus (GPa)	7.38	7.29
Flexural strength (MPa)	65.7	72.0
Tensile modulus (GPa)	6.37	6.39
Tensile strength (MPa)	43.0	39.0

t-butyl peroxy benzoate (TBP) from Akzo Nobel Polymer Chemicals LLC (Chicago, IL). Butylated hydroxytoluene (BHT) was used as an inhibitor to improve the shelf life of the resin matrix. Kenaf fiber was controlled at 38 wt%. Two kinds of panels were fabricated as shown in Table 3. The standard UPR composite without added MAESO was SMC1; in the SMC2 20 wt% MAESO replaced the unsaturated polyester in AROPOL Q 6585 [5].

Unsaturated polyester AROPOL Q 6585 resin, MAESO and styrene were thoroughly mixed together. The catalyst TBP was then added and thoroughly dispersed into the resin mixture. Inhibitor was then added. The resin paste mixture was evenly poured onto the surface of a single kenaf fiber sheet. Another kenaf fiber sheet was placed on top to form a sandwich with resin paste in the core. The sample was then transferred to a stainless steel mold to be placed into a hot press. The resin was cured at 100 °C and 100 psi for 2 h. The resin was then post-cured at 150 °C for another 2 h. During the hot pressing, the polyester resin emits styrene which can be smelled at a concentration of about 0.15 ppm. These styrene emissions are suspected of causing central nervous system damage. Styrene emissions were estimated to be around 1% of the styrene weight at the pressing of SMCs. Under an average styrene of 35% in the UPR, styrene emissions are approximately 0.35% of the resin applied in the SMC mass [12].

Table 4 Glass fiber sheet molding compound formulation (wt%).

Glass [6] SMC Formulations	(%)
Standard UPR resin	32.3
Release Zink stearate	0.8
Catalyst (TBP)	0.3
Thicker Mg hydroxide	0.5
Glass fiber (1 chopped)	50
Calcium carbonate (Filler)	16.1
Glass fiber SMC properties	
Density (g/cm^3)	1.87
Flexural modulus (GPa)	14.0
Flexural strength (MPa)	314
Tensile modulus (GPa)	15.8
Tensile strength (MPa)	164

The density of the composites was determined by averaging the mass/volume measurements of six specimens in accordance with the ASTM D1622-03 standard. Flexural strength and moduli of the panels were tested on six specimens using an Instron universal testing machine (model 5869, Canton, MA) in accordance with the procedures described in the ASTM D 790-07 standard.

Tensile strength testing was performed in accordance with the procedures described in ASTM D 638-03. Five replicates were used and averaged. The specimen dimension was 165 × 19 × 3.2 mm and the cross-section of the narrow section was 57 mm × 13 mm (length × width). The crosshead speed was 5 mm/min.

(3) Glass Fiber SMC Formulation

The formulation and properties of standard glass fiber reinforced SMC are summarized in Table 4 [6].

2.2 Functional Unit

Environmental performance of materials is usually compared based on a functional unit, that is, an entity capable of accomplishing a specific purpose. The developed SMCs are supposed to be used for automotive manufacture. Interior parts in automobiles are still designed for stiffness. For automotive applications, optimum design requires a material that minimizes the component weight for the desired component stiffness. The elastic deflection, δ, of a structural or automotive component under a force, *F*, is given as [8]:

$$\frac{F}{\delta}=\frac{Ebt^3}{cl^3} \quad (1)$$

where, *t* is the sheet thickness, *l* and *b* are component length and width, respectively, *E* is the elastic modulus, and cis a constant depending on the orientation of the component. The component mass can also be written as $m = \rho btl$, where ρ is the material density:

$$\frac{F}{\delta}=\frac{Em^3}{cb^2l^6\rho^3} \quad (2)$$

To achieve equal stiffness (Eq. 1) to a reference material (r subscript) the mass (m) and thickness (t) are calculated by Eqs. 3 and 4.

$$m = \left(\frac{E_r}{E}\right)^{\frac{1}{3}} \frac{\rho}{\rho_r} m_r \tag{3}$$

$$t = \left(\frac{E_r}{E}\right)^{\frac{1}{3}} t_r \tag{4}$$

Using glass fiber SMC as the reference material, the masses required to achieve compatible stiffness were calculated as shown in Table 5. The running capacity during the use phase was assumed as: 175,000 km (12 years). The coefficient for fuel reduction achieved from using lighter materials in gasoline powered vehicles is 0.34 L/(100 kg × 100 km) [13]. The gasoline saved was thus calculated (Table 5).

2.3 End-of-Life Disposal

An additional benefit of replacing glass fiber with natural fiber in reinforced fiber composites is that they can be combusted at the end of their product life. Incineration of discarded glass fiber-reinforced composites generates a lot of black smoke and odors. Because glass fibers fuse to the incinerator it is often damaged in the process. Natural fiber composites were assumed to be incinerated at the end of life without any adverse effect on an incinerator. The energy of incineration was: Heat: 0.24 MJ/kg, Electricity: 0.36 MJ/kg; bonus of incineration of plasticsenergy recovery (LHV = 30.5 MJ/kg), Heat: 26%, Electricity: 10%; bonus of incineration of kenaf energy recovery (LHV =14 MJ/kg), Heat: 26%, Electricity: 10% [14].

During incineration, usable energy in the form of heat and electricity can be recovered. It is possible to account for these valuable products by allocating them an environmental load. In this study, the allocation was avoided by system extension, that the heat and the produced electricity replaced a quantity of heat and electricity, which did not have to be manufactured in the economic background system. In a practical way, energy consumption and environmental impacts would be reduced. To model the useful application of waste or byproducts, SimaPro has an "avoided products" option. The energy resources saved were entered in this option and SimaPro subtracted the emissions and resources associated with the production of natural gas.

2.4 System Boundaries

The product system considered the entire life-cycle of the functional unit, that is, the raw material production with agricultural pre-chains, semi-finished part production, bus component production, use phase and disposal (Fig. 2). Processes that are the same for all product systems were neglected in the comparative LCA. The scope included raw material inputs and emissions from production of all reagents and ancillary materials, as well as the extraction, conversion and delivery of energy inputs.

2.5 Environmental Impact Assessment

Life-cycle impact assessment methods describe environmental impacts in terms of characterization factors. For a wide assessment of environmental impact, the BEES impact set and Eco-indicator 99 were used. Life-cycle inventory for the product comparisons are classified into impact categories, that is, categories in which a set of related flows may contribute to the impacts on human or environmental health. Three types of environmental damages were considered: human health, ecosystem quality and resource depletion. These damages are quantified by damage models. The eco-indicator 99 points were calculated by normalization and weighting of the damage factors. The eco-indicator single score is helpful in the search for more environmentally friendly design alternatives and is intended for internal use within an organization. One point on the eco-indicator scale represents one thousandth of the annual environmental load from one average European person [15]. Energy resource efficiency was assessed using cumulative energy demand. The cumulative energy demand considered the entire

Table 5 Functional unit with equal stiffness.

	Glass fiber SMC	SMC1 (Kenaf SMC)	SMC2 (Kenaf 20% Soy Resin)
Density (g/cm^3)	1.87	0.95	1.04
Flexural strength (GPa)	14	7.38	7.29
Mass (kg) with equal stiffness	1	0.63	0.69
Save gasoline at use phase (L)	Ref.	2.2	1.8

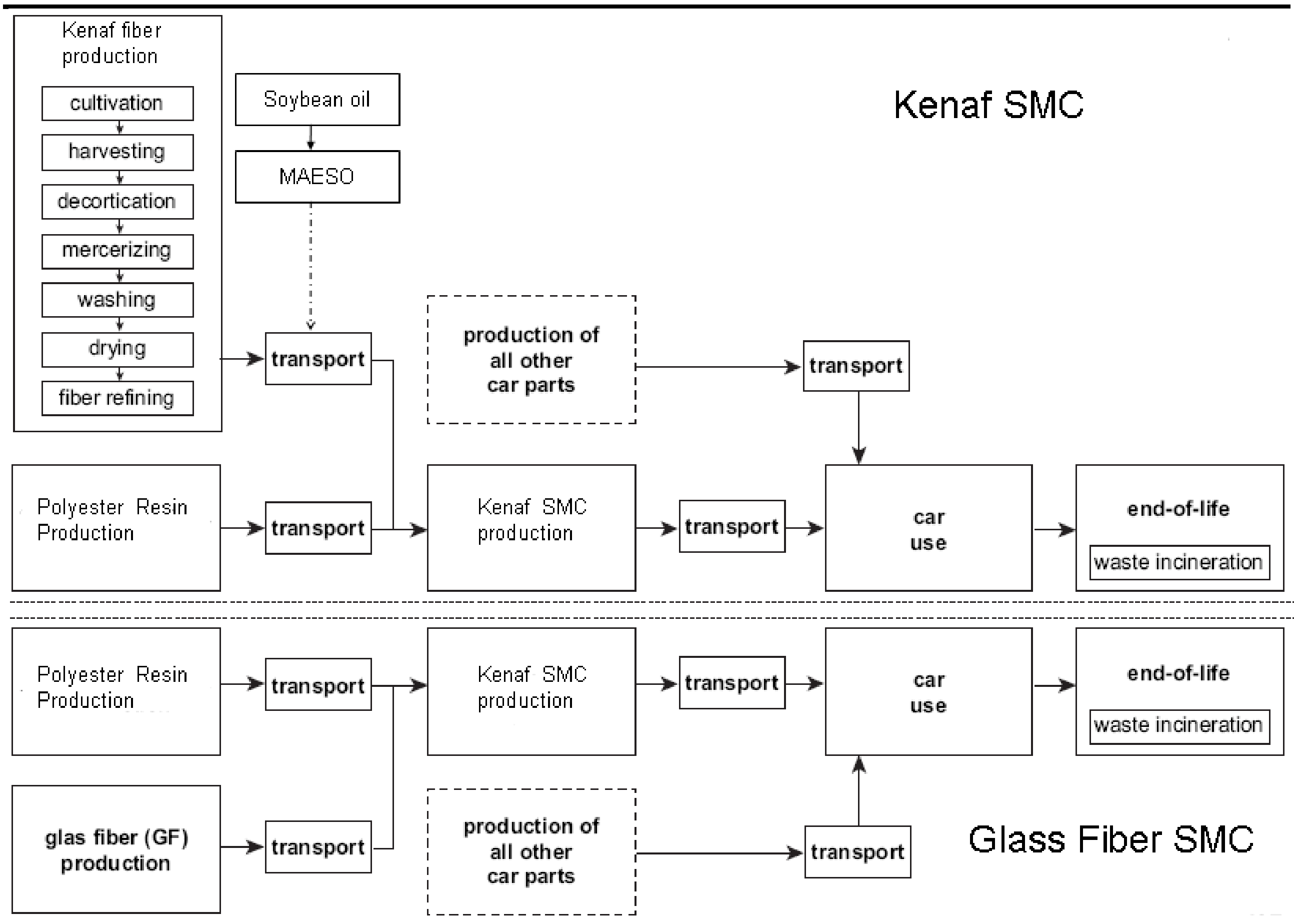

Fig. 2 Steps in the production of kenaf SMC and glass fiber SMC for this study.

demand of primary energy which flowed into the product system per functional unit [16].

BEES has a recognized and accepted methodology to ensure a level playing field. All midpoint scores are expressed in units of a reference substance and related to the four damage categories of human health, ecosystem quality, climate change and resources as shown in Table 6 [17]. The global warming potential (GWP) used by BEES was developed in 2001 by the International Panel on climate change. The one hundred year GWP are: fossil carbon dioxide, 1; methane, 23; nitrous oxide, 296; CFC/HCFCs, 1700; methylene chlorine, 10; and HCFC22, 1700. Biogenic CO_2 uptake is considered to have a negative impact.

3. Results and Discussion

The cradle to gate LCA for fibers and resins, and the comparative LCA of SMCs are summarized in Figs. 3-9. The products with the highest environmental impact will be shown as 100%, while the impact of the other products is shown as a percentage of that value.

Table 6 Environmental impact indices.

	Impact category	Units
1	Global warming	CO_2 equivalents
2	Acidification	H+ equivalents
4	Human health-cancer	C_6H_6 equivalents
5	Human health-noncancer	C_7H_7 equivalents
6	HH criteria air pollutants	microDALYs
7	Eutrophication	N equivalents
8	Ecological toxicity	2,4-D equivalents
9	Smog	NOx equivalents
10	Natural resource depletion	MJ surplus energy
11	Indoor air quality	TVOC equivalents
12	Habitat alteration	T & E count
13	Water intake	liters of water
14	Ozone depletion	CFC-11 equivalents

3.1 Fiber LCA

Fig. 3 indicates that kenaf fiber has fewer negative environmental impacts than glass fiber throughout all stages—from raw material extraction to fiber manufacturing (cradle to gate). Fig. 4 shows that bast fibers (jute and kenaf) consume less energy than other fibers in manufacturing 1 kg fibers; most of the consumed energy is from renewable sources. The CED is the most meaningful parameter in judging the energy efficiency of systems because losses due to transformation and transport are fully accounted for. In addition to the cumulative process and transportation energy, it also contains the "feedstock energy", which is the primary energy equivalent of the materials produced from oil, coal, wood and so on.

Fig. 5 shows the environmental burdens of different fibers from cradle to gate. Onepoint represents one thousandth of the annual environmental load fromone average European inhabitant. Overall, fibers have a greater environmental impact on respiration mainly due to releases of inorganic particles, such as sulphites and nitrates. Another aspect worthy of mention is the consumption of fossil fuels to produce petroleum-based fibers. Glass fiber production produces a significant amount of carcinogens. Land use, on the other hand, is the biggest factor in the production of natural fibers. Fig. 5 shows the lower overall environmental burden from natural fibers.

3.2 Resin LCA

Figs. 6-8 show the comparisons of three resins in commulative energy demand, BEES emvironmental impact indices, and Eco-indicator 99 Points. The use of fossil fuel in manufacturing the resins contributes a large portion of the environmental impacts.

3.3 LCA of SMC

The key environmental measures for three composite scenarios were computed with SimaPro

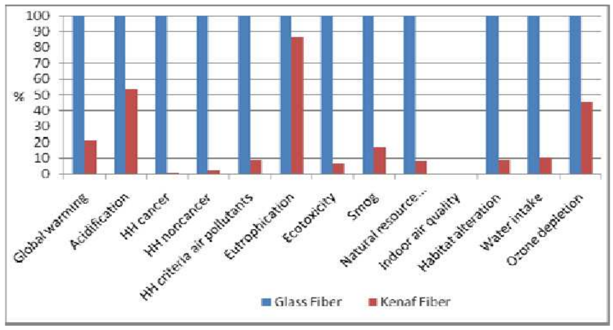

Fig. 3 Comparison of environmental impacts of glass and kenaf fibers in BEES impact indices. Functional unit: 1 kg fiber, cradle to gate. Data: kenaf fiber, India; glass fiber, Europe.

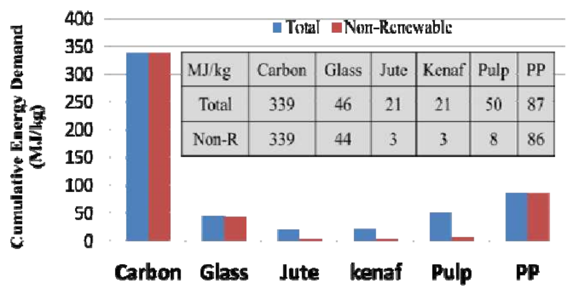

Fig. 4 Cumulative (primary) energy demand (CED) per 1 kg fiber.

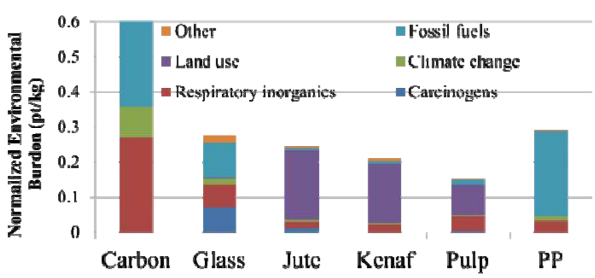

Fig. 5 Comparison of environmental impacts of fibers in Eco-indicator 99 Points per 1 kg fiber.

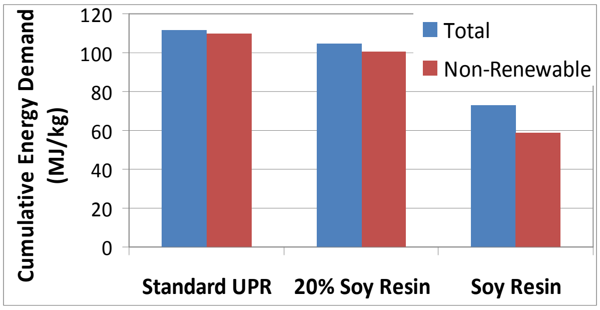

Fig. 6 The cumulative (primary) energy demand (CED) of resins per 1 kg resin.

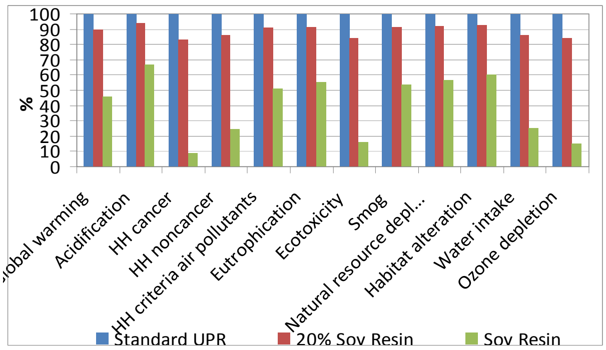

Fig. 7 Comparison of environmental impacts of three resins in BEES impact indices.

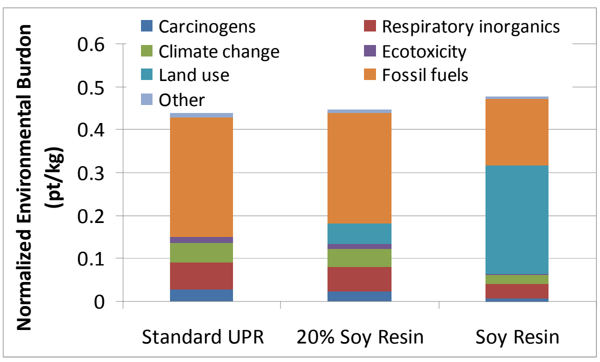

Fig. 8 Comparison of environmental impacts of resins in Eco-indicator 99 Points per 1 kg resin.

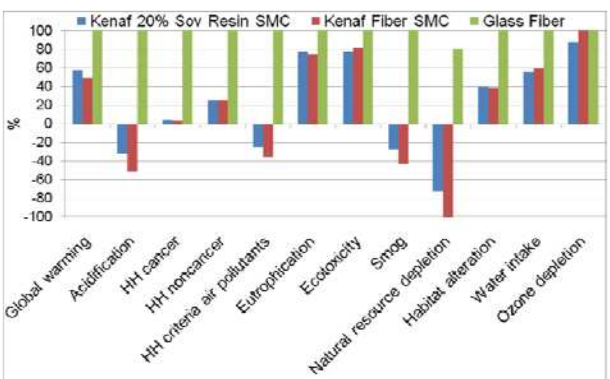

Fig. 9 Relative contribution of three SMCs per 1 functional unit.

software (Fig. 9). Both of the kenaf-fiber reinforced SMCs (SMC1 and SMC2) performed better than glass fiber SMC in every environmental category. The global warming potential of the kenaf fiber SMC (SMC1) was only about 45% of that for the glass fiber SMC. The global warming potential of the kenaf fiber soy-resin composites (SMC2) was slightly higher than that of the kenaf fiber SMC due to environmental effects from agricultural production of soybeans.

A negative value as indicated in Fig. 9 means carbon credits—saving non-renewable resources due to end-of-life energy recovery. Generally, at end-of-life, the kenaf and soy resin composites generated additional energy. In the SimaPro program, this additional energy is treated as a substitute of fossil resources to significantly reduce the impacts of acidification and air pollution. Kenaf and soy are annual crops that, if farmed sustainably, do not contribute to natural resource depletion.

4. Conclusions

This preliminary result demonstrated the potential for reduction of environmental impacts by substituting modified soybean oil and natural fiber for glass fiber in sheet molding composite production. Life-cycle assessment is an effective tool to analyze the environmental impacts of materials and products. Life-cycle thinking and assessment can also be a great educational tool to promote renewable bio-products.

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References

[1] J. Holbery, D. Houston, Natural-fiber-reinforced polymer composites applications in automotive, Jom 58 (2006)

80-86.

[2] S. Kalia, A. Dufresne, B.M. Cherian, B.S. Kaith, L. Averous, J. Njuguna, Cellulose based bio-and nanocomposites, a review, 2011, DOI: 10.1155/2011/837875.

[3] M.J. John, R.D. Anandjiwala, Recent developments in chemical modification and characterization of natural fiber reinforced composites, Polymer Composites 29 (2008) 187-207.

[4] Franklin Associates, Life cycle inventory of five products produced from polylactide (pla) and petroleum-based resins, Prepared for Athena Institute International [Online] 2006, http://www.diamondpackaging.com/green/pdf/Athena_Plastic_Products_LCA_Summary_Report.pdf.

[5] K. Liang, S.Q. Shi. Resins from soybean oil-based additives for natural-fiber sheet molding compound (SMC) composites: Synthesis and characterization, Pages 91-96 in Proceedings, 2009 International Conference on Wood Adhesives, 2010.

[6] G.S. Springer, Effects of temperature and moisture on sheet molding compounds, Journal of Reinforced Plastics and Composites 2 (1983) 70.

[7] The United Soybean Board, Omni Tech International, Life Cycle Impact of Soybean Production and Soy Industrial Products, 2010.

[8] V. Khanna, B.R. Bakshi, Carbon nanofiber polymer composites: Evaluation of life cycle energy use, Environmental Science and Technology 43 (2009) 2078-2084.

[9] J. Lu, S. Khot, R.P. Wool, New sheet molding compound resins from soybean oil, I. Synthesis and characterization, Polymer 46 (2005) 71-80.

[10] K. Liang, S.Q. Shi, D. Nicolas, L. Sites, Acceleratehid weathering test of kenaf fiber unsaturated polyester sheet molding compounds, Wood and Fiber Science 45 (2013) 1-7.

[11] J. Shi, S.Q. Shi, H.M. Barnes, M. Horstemeyer, J. Wang, E.B.M. Hassan, Kenaf bast fibers—Part I: Hermetical alkali digestion, International Journal of Polymer Science, 2011.

[12] M. Schmehl, J. Mussig, U. Schoneld, H.B. von Buttlar, Life cycle assessment on a bus body component based on hemp fiber and PTP (R), Journal of Polymers and the Environment 16 (2008) 51-60.

[13] S.V. Joshi, L.T. Drzal, A.K. Mohanty, S. Arora, Are natural fiber composites environmentally superior to glass fiber reinforced composites?, Composites Part A-Applied Science and Manufacturing 35 (2004) 371-376.

[14] T. Corbiere-Nicollier, B.G. Laban, L. Lundquist, Y. Leterrier, J.A.E. Mσnson, O. Jolliet, Life cycle assessment of biofibres replacing glass fibres as reinforcement in plastics, Resources, Conservation and Recycling 33 (2001) 267-287.

[15] M. Goedkoop, R. Spriensma, M. van Volkshuisvesting, R.O. en Milieubeheer, C.D. Communicatie, The Eco-indicator 99: A damage oriented method for life cycle impact assessment, Ministerie van Volkshuisvesting, Ruimtleijke Ordening en Milieubeheer, 1999.

[16] VDI, VDI-Richtlinie 4600: 1997-06: Kumulierter Energieaufwand-Begriffe, Definitionen, Berechnungsmethoden (VDI guideline 4600: 1997-06: Cumulative energy demand-terms, definitions, calculation methods), VDI Verlag, Düsseldorf, Germany, 1997.

[17] B.C. Lippiatt, BEESRG 4.0: Building for Environmental and Economic Sustainability Technical Manual and User Guide, 2007.

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Studies on Mode of Expression of Apple (*Malus* × *domestica* Borkh.) Bud Dormancy under Tropical and Temperate Climatic Conditions

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Abstract: A study was conducted to describe the progression of bud dormancy in 1-year-old apple (*Malus* × *domestica* Borkh) shoots grown at two contrasting climatic conditions (Belgium, temperate and Ethiopia, tropics). The experiment was carried out on “Golden” and “Gala” cultivars for two consecutive years (2010/2011 and 2011/2012). Moreover, a validation experiment was conducted on “MM106” apple rootstock during 2010/2011 only in Ethiopia. Variations in inverse of time to 50% budburst were interpreted in terms of evolution of growth capacity of the buds. Despite differences observed with chilling accumulation later in winter or early in spring, depending on environments, depth of endodormancy intensity during winter can be summarized as follows: buds from pruned shoots were less endodormant than terminal buds of the intact shoots and terminal buds were more endodormant than the dormancy intensity of upper buds of the disbudded shoots, suggesting proximal buds can grow more readily than does terminal ones. Our results provide evidence for the existence of a considerably strong paradormancy inhibition by distal shoot parts and buds, which was more pronounced in Ethiopia than in Belgium, highlighting the importance of designing and applying appropriate pruning and dormancy avoidance strategies in mild-winter climates. Finally, as still there is knowledge gap on bud dormancy progression and its control mechanism especially under mild climates, our study highlights the need for further in-depth research using biological and biochemical tests.

Key words: Bud dormancy, basitony, acrotony, *Malus* × *domestica*, Ethiopia, Belgium.

1. Introduction

Growth in most temperate-zone deciduous fruit trees is characterized by a cyclic pattern of activity and inactivity [1], which is an adaptive response to survive unfavorable winter conditions [2-4]. During the inactivity, buds are in a quiescent status called bud dormancy. A precise definition of bud dormancy is difficult to achieve mainly due to the gradual transition and overlapping between the phases of active growth and inactivity. In an attempt to establish a universal terminology for bud dormancy, Lang et al. [5] designated three successive and sometimes overlapping types of bud dormancy based on the controlling factors: para-, endo- and eco-dormancy. Paradormancy is regulated by physiological factors

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within the plant but outside the dormant structure. Endodormancy is regulated by physiological factors within the affected bud itself and is released by chilling temperatures [3, 5, 6]. According to Faust et al. [4], endodormancy is further divided to d-enododrmancy and s-endodormancy, where the latter is the stage during which dormancy breaking chemicals can substitute for chilling while in the first no substitution for chilling is possible. Finally, ecodormancy, an inhibition imposed by unfavorable environmental factors and growth, is resumed when the conditions again become favorable. Though the initial triggers for these three types of dormancy could be different, their outcome "temporary suspension of visible growth" would be the same [5, 7]. This does not mean that activity within the bud cease completely as slow differentiation of internal organs may still continue, which in turn affects future potential development and growth of the bud [8].

Generally, bud endodormancy is a complex, dynamic and multi-faceted phenomenon [7], which has puzzled researchers for more than 200 years and is still not fully understood [8]. It is believed that its depth is related to the chilling requirement (CR) needed; the deeper the dormancy, the higher are the number of chilling hours required to break it [3]. Thus, chilling temperature has been considered the main environmental factor responsible for the initial induction and subsequent release of bud dormancy in temperate woody plants [3, 9]. According to Fennell [10], knowledge of the dormancy depth of a cultivar has practical and economic implications for temperate fruit trees husbandry. It helps to optimize cultivar selection to specific climatic conditions and to design and apply appropriate agronomic practices at orchard level. However, apple buds differ in depth and course of endodormancy depending on cultivar [11], type of bud, its position on the shoot and stage of rest [3], and climate [12], which led to the conclusion that the CR of buds is not a constant factor [13]. Although cultivar's CR is determined by its genetic makeup, environmental factors during the endodormancy onset can affect the value as its chilling units to satisfy that requirement are accumulated by the environment [3].

Under temperate climates with sufficient winter chilling, a synchronized start of bud activity and an acrotonic budburst gradient is the norm in apple shoots during spring [14, 15]. However, in mild-winter climates, winter chilling is always insufficient, resulting in delayed budburst, reduced and uneven budburst in spring, a condition commonly referred to as delayed foliation [3]. This in turn has a negative impact on branching, tree architecture and the economic viability of apple orchards under conditions of inadequate winter chilling [16]. Furthermore, progression of dormancy in these climates differ from temperate climates (an acrotonic budburst) to which apples are well adapted, where in mild-winter climates a basitonic budburst gradient is experienced [16-18]. Cook and Jacobs [17] explained that paradormancy and lack of an extended dormant period impedes the development of acrotony in apple shoots in warm areas.

Despite the plasticity expressed by the apple species, the major apple cultivars show delayed and uneven budburst when grown in the tropical climatic conditions like the northern highlands of Ethiopia [19, 20]. Its current cultivation is constrained by a number of factors of which lack of winter chilling resulting in symptoms of prolonged dormancy, less foliation, irregularity of growth, and production of fewer fruits situated mostly at the terminal positions among others. Nevertheless, systematic characterization of dormancy evolution in apple in the Ethiopia highlands was not carried out so far except the study of Ashebir et al. [19] who evaluated the response of different apple cultivars (Golden Delicious, Gala, Fuji, Granny Smith and Jonagold) grafted on M9 rootstock to different dormancy management practices. It is imperative, therefore, to describe the progression of bud dormancy as an entry point for designing and applying appropriate dormancy-breaking strategies at orchard level.

The purpose of our work was to describe bud dormancy progression in apple under contrasting climatic conditions (temperate—Belgium and tropics—Ethiopia) using biological test expressed as inverse of days to 50% budburst which measures the rate of bud development [15, 21]. This method has been considered as better criterion when compared with either the percentage budburst or bud developmental stages recorded within a fixed time interval [21, 22].

2. Materials and Methods

2.1 Plant Material, Experimental Sites and Sampling

The experiment was done on two apple cultivars: "Gala" and "Golden Delicious" on "M9" apple rootstock grown at the experimental orchards in Northern Ethiopia (Latitude, 13°28′47″; Longitude, 39°29′10″; Altitude, 2,150 m) and in Belgium (Latitude, 50°47′31″; Longitude, 5°12′6″; Altitude, 75 m). It was conducted for two successive seasons of 2010/2011 and 2011/2012. The trees in Ethiopia were 8 years old during the study period whereas in Belgium they were about 15 years. Long term (2001-2012) monthly average temperatures and rainfall prevailing in the areas are given in Figs. 1a and 1b, respectively. The overall monthly average temperature in Belgium was 11.0 °C while in Ethiopia, it was 18.7 °C. In Ethiopia, not only temperatures were high all year round but also their seasonal variations are less pronounced unlike those in Belgium where a clear variation between summer and winter was evident. The yearly average amount of rainfall at Belgium (704.4 mm) was not only higher than that of Ethiopia (590.1 mm) but also it was uniformly distributed across months. In Ethiopia, most of the rainfall occurred within July and August. The predominant soil type at the orchard in Ethiopia comprised 30% sand, 30.6% silt and 39.4% clay then likely classified as clayey Vertisol while in Belgium, it was a loam-clayey Luvisol.

The orchard in Ethiopia was irrigated with tap water using hose manually and fertilized with urea and diammonium phosphate (DAP) fertilizers. Though spraying with rest-breaking agent was the common practice in Ethiopia, no rest-breaking agent was applied for the trees used in the experiments during the study period. During the two successive seasons, one-year-old, unbranched shoots were sampled randomly from different trees in Ethiopia and Belgium at 2-3 weeks interval. Dates of sampling are given in Table 1. The number of shoots per sampling date and per cultivar was 15 and 20 in Ethiopia and 27 and 36 in Belgium during 2010/2011 and 2011/2012 respectively. In times where shoots harvested before natural leaf fall, most often in Ethiopia and at the start of the

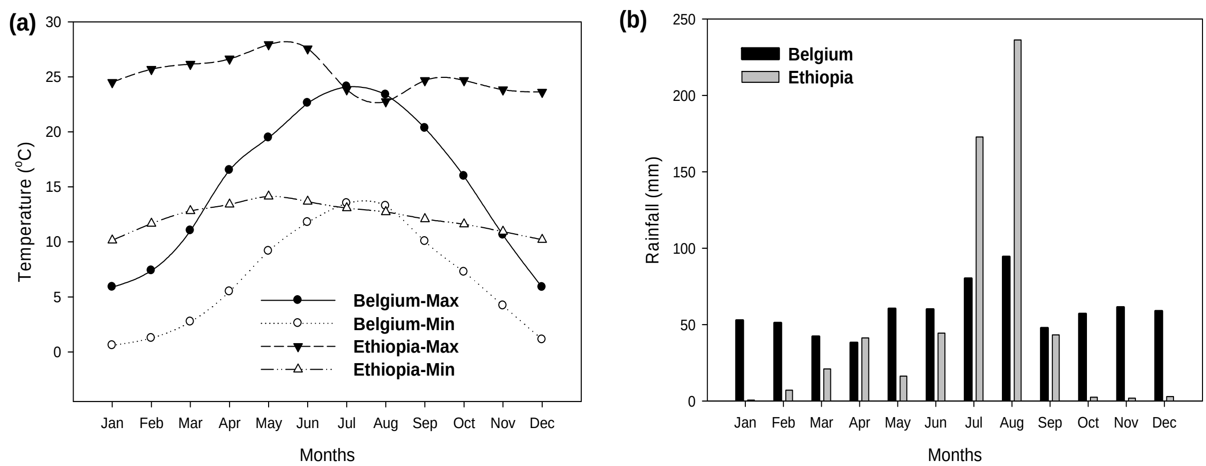

Fig. 1 Long term (2001-2012) average monthly temperatures (a) and rainfall (b) recorded at the Belgian and Ethiopian experimental sites.

Table 1 Dates of harvesting shoot samples for the dormancy progression study in Belgium and Ethiopia.

Samples	2010/2011		2011/2012	
	Belgium	Ethiopia	Belgium	Ethiopia
1	06/10/10	15/09/10	08/09/11	01/09/11
2	22/10/10	30/09/10	22/09/11	22/09/11
3	08/11/10	14/10/10	13/10/11	13/10/11
4	29/11/10	29/10/10	03/11/11	03/11/11
5	13/12/10	12/11/10	15/12/11	24/11/11
6	10/01/11	10/12/10	05/01/12	15/12/11
7	02/02/11	14/01/11	26/01/12	05/01/12
8	22/02/11	29/01/11	16/02/12	26/01/12
9	-	11/02/11	08/03/12	16/02/12

experiment in Belgium, they were defoliated manually at harvest. The average length of the shoots was 21.1 cm with about 10 buds each in Ethiopia and 39.4 cm with 14 buds each in Belgium.

Another experiment, which was meant as "validation" for the previous experiment, was carried out on "MM106" apple rootstock shoots grown at the same orchard where "Gala" and "Golden" were grown in Ethiopia. A total of 35, one-year-old, unbranched, ca. 55 cm long shoots were collected randomly from at least 10 different stools of "MM106" at monthly interval starting from August 5, 2010 to January 5, 2011. As there were not enough shoots, the sampling was not continued after January. Similarly, as shoots did not shed their leaves naturally, they were defoliated manually at harvest.

2.2 Experimental Procedure

During each sampling period, sampled shoots were immediately delivered to the laboratories at Mekelle University, Ethiopia and KU Leuven, Belgium. Then after, the sampled shoots were split into groups with five shoots (Ethiopia) and nine shoots (Belgium) each and were treated as follows: intact shoot, 1/3 of the upper shoot pruned, 2/3 of the upper shoot pruned and 1/3 of the upper buds removed. In 2010/2011, the treatment with 2/3 of the upper shoot pruned was not included. These treatments allowed estimating endodormancy and paradormancy simultaneously. The uppermost bud on these shoots was defined as terminal bud, upper bud, lower bud and "disbudded" respectively. Furthermore, a bud in the intact shoot found at a comparable position as that of the upper bud in the 1/3 pruned shoot was used to measure the influence of paradormancy and endodormancy. Finally, shoots were labeled and placed in buckets with tap water and forced in a room at a temperature of about 20 °C. The water was changed every three days, and approximately 0.5 cm of the basal shoot section was cut off weekly to ensure that the vascular system remained functional. Budburst for both vegetative and reproductive buds was recorded every three to four days for about 40 days. Vegetative budburst was considered when a green tip of the emerging leaves was visible, which corresponds to De Wit's bud developmental stage 1 [23] and similarly for the reproductive buds, budburst was considered when a central green tip within the bud scales was visible, which also corresponds to Chapman and Catlin's growth stages for apple reproductive stage 3 [24]. Time to budburst was calculated as the time in days needed for budburst to occur on three and five per treatment for Ethiopia and Belgium, respectively, i.e., days to budburst in 60% and 55% of the shoots in that order. The higher days required to budburst, the more dormant the buds were considered. To use the same percentile in both environments, they were changed into days to 50% budburst and then the inverse value of days to 50% budburst was calculated.

For the "MM106" apple rootstock experiment in Ethiopia, the 35 shoots sampled during each sampling period were divided into seven treatments of five shoots, described as: (1) intact shoot; (2) 1/3 of the upper shoots pruned; (3) 2/3 of the upper shoot pruned; (4) 1/3 of the upper buds removed manually; (5) 2/3 of the upper buds removed manually; (6) 1/3 of the upper buds removed with a knife and (7) 2/3 of the upper buds removed with a knife. The uppermost bud on these treatments was also defined in a similar fashion as the previous ones.

Shoots were labeled and placed in pots inside a room, where temperature was measured with the use of a data logger. Average room temperature recorded throughout the study period was 19.6 °C. Water was refreshed every four days to avoid possible fungi infection, lime deposit and to make sure that shoots were able to take up water efficiently. For the same reason, approximately 0.5 cm of the base of the shoot was cut off weekly. Budburst recording and time to budburst estimation was made in a similar manner with that of vegetative buds of “Gala” and “Golden”.

2.3 Temperature and Chilling Accumulation

Hourly temperatures were recorded in each environment with automatic data-loggers: Escort Junior (Escort data logging systems). Chilling accumulation was assessed by chill units of the Utah model, measuring “Richardson Chill Units-RCU” [25] for Belgium and Infruitec model, measuring “Infruitec Chill Units-ICU” [26] for Ethiopia, starting from September 1 to March 31 in each year. Both models accumulate the same way, with the exception that the Infruitec model assumes that temperatures greater than 16 °C do not have an effect on the accumulation of chilling units.

3. Results

3.1 Maximum and Minimum Daily Temperatures

Daily maximum and minimum temperatures recorded during the periods studied are presented in Figs. 2a-2d. The pattern of temperatures recorded during 2010/2011 in Ethiopia was the same to that of 2011/2012. However, in terms of magnitude, both the lowest and highest temperatures were recorded during 2011/2012. In 2010/2011, the minimum temperature was 5.1 °C, registered on December 18, 2010 while in 2011/2012, it was 3.7 °C registered on January 23, 2012. The maximum temperatures were 28.3 °C and 29 °C recorded on March 4, 2011, and February 3 and March 29 of 2012, respectively. In both years, the daily temperature amplitude is greater than the average seasonal variations (Figs. 1a, 2a and 2b).

In Belgium, the winter of 2010/2011 started earlier and was colder than in the winter of 2011/2012. In 2010/2011, the lowest temperatures were recorded during December and January (Fig. 2c) while in 2011/2012 it was during February 2012. Temperatures less than -10 °C were recorded on numerous days during February 2012 (Fig. 2d).

3.2 Chilling Accumulation

Figs. 3a and 3b indicate, the chilling accumulations in both environments during the periods studied. In Ethiopia, the chilling accumulation was relatively higher during 2011/2012 (ca. 764.5 ICU) than 2010/2011 (ca. 651.5 ICU). The break-down curve of the chilling amount from end of February onwards shows that the cold period was short and principally the coldest months were December, January and February with an average of 177.25, 136 and 114 ICU, respectively (Fig. 3a). In Belgium, the accumulated chill varied between months except for the month of December in both years (Fig. 3b). The lowest chill (ca. 78 RCU) was registered in December 2010 with temperatures on numerous days below 0 °C. In 2010/2011, the total accumulated chill was a bit higher (ca. 867.5 RCU) till November than in 2011/2012 (ca. 677.0 RCU); while after January, this was reversed. Finally, the total chill accumulated till March 2010/2011 and 2011/2012 was 2,193.5 and 2,361 RCU, respectively.

3.3 Dormancy Progression in Ethiopia

“Gala” and “Golden”: Though budburst during

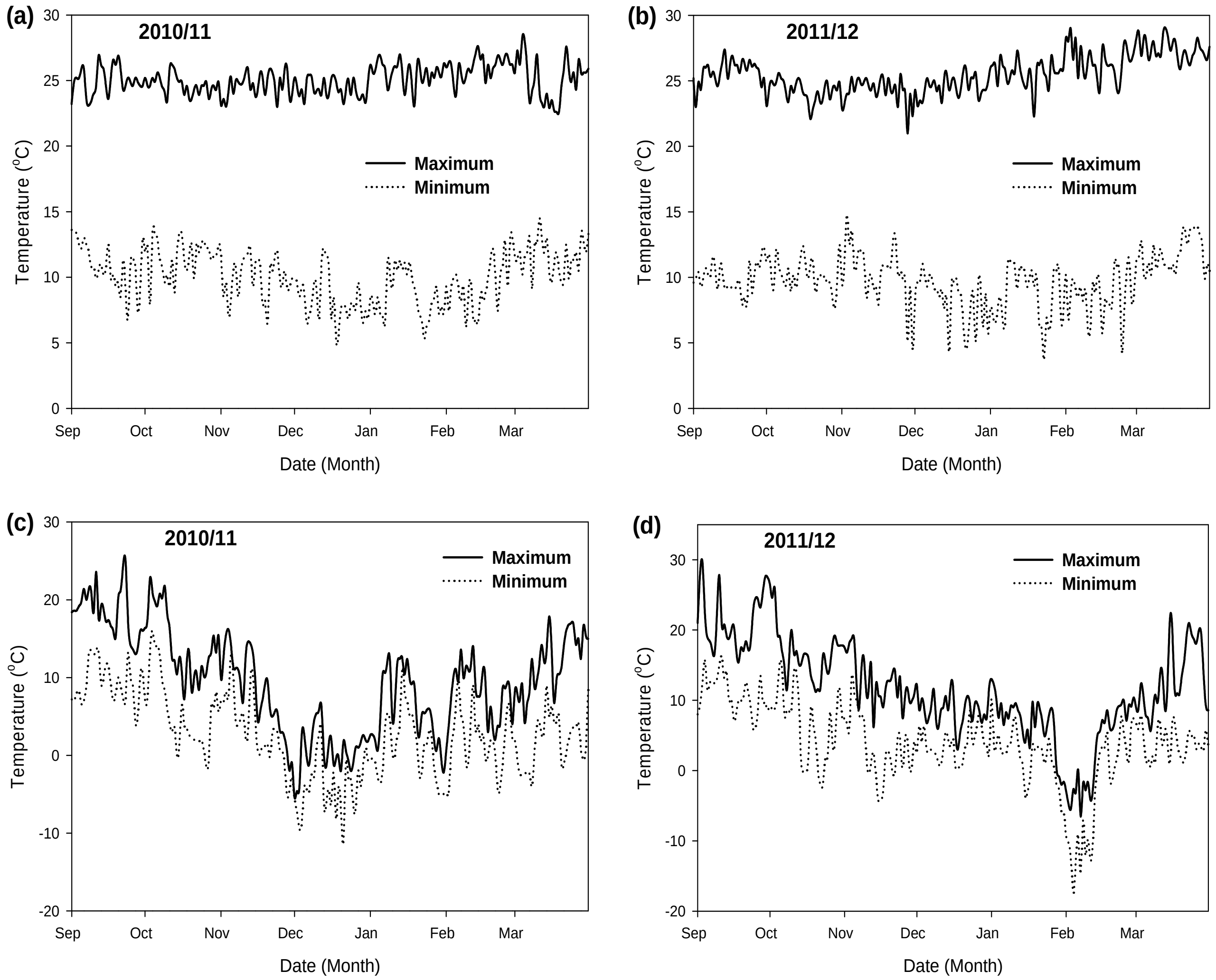

Fig. 2 Maximum and minimum daily temperatures recorded in 2010/2011 and 2011/2012 in Ethiopia (a, b) and Belgium (c, d).

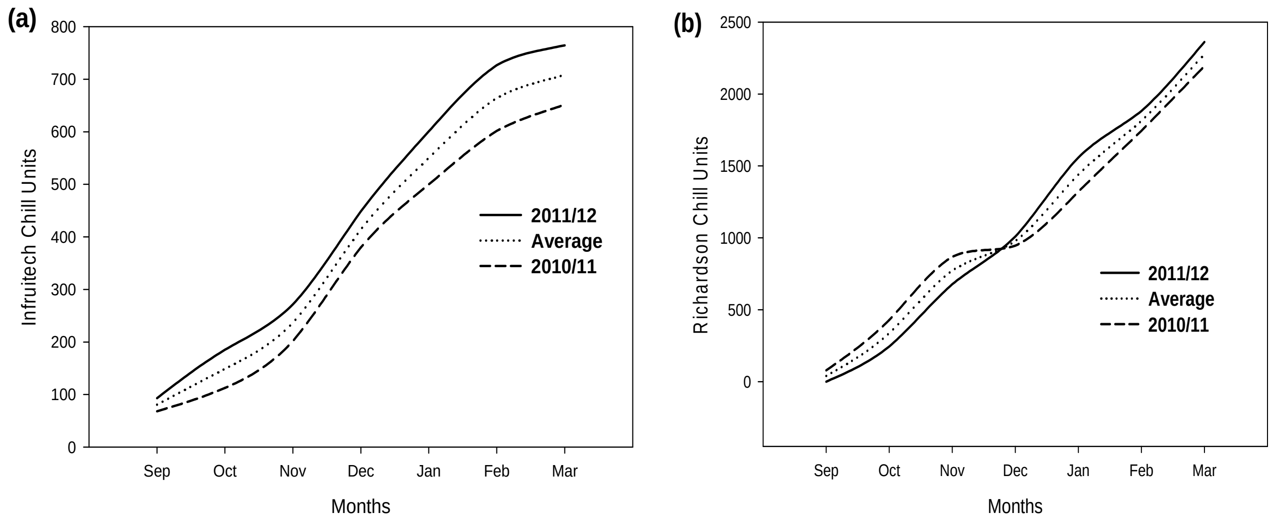

Fig. 3 Cumulative chill accumulated in 2010/11 and 2011/12 in Ethiopia, following the Infruitec model (a) and Belgium using the Utah model (b).

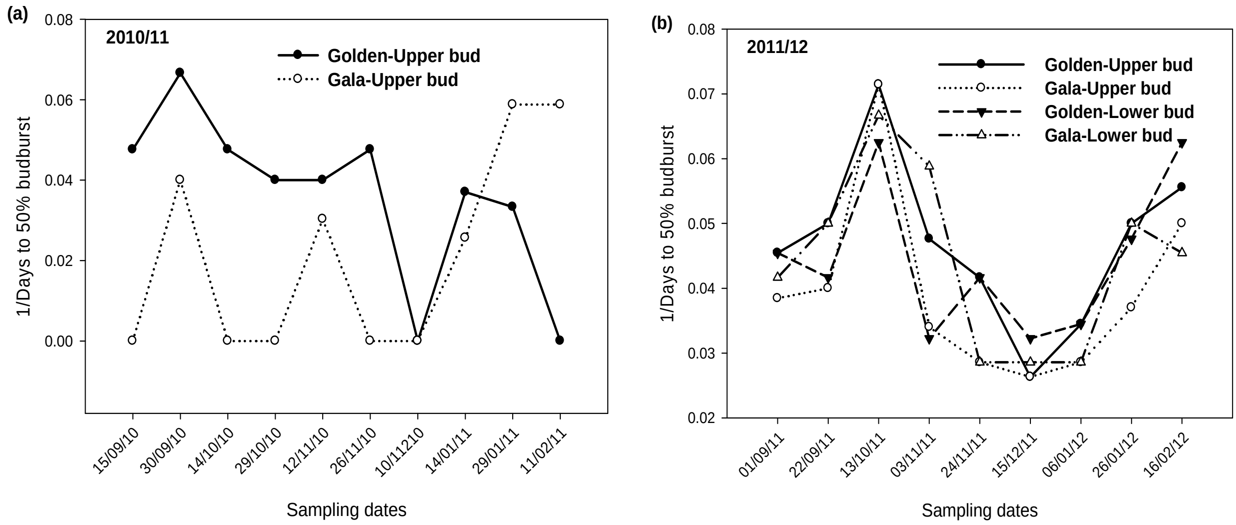

Fig. 4 Budburst rate progression, expressed as inverse of days required to 50% budburst in "Golden" and "Gala" one-year-old shoots during the period 2010/2011 and 2011/2012 in Ethiopia (upper bud = 1/3 of the upper shoots pruned; lower bud = 2/3 of the upper shoot pruned). No bud burst in intact and disbudded shoots. In 2010/11, lower buds (2/3 of the upper shoot pruned was not part of the treatments). Data points at 0.00 imply less than 50% budburst.

2010-2011 (Fig. 4a) was more erratic compared to 2011-2012 (Fig. 4b), generally an oscillating pattern of budburst progression was observed during the two studied periods. During both years, better budburst was observed in upper buds of "Golden" compared to "Gala" (Figs. 4a and 4b), though there was a slight shift from the end of January 2011 on. During 2010-2011, both cultivars had entered a state of deep dormancy (Fig. 4a), in which a pause in dormancy release phase occurred from the last week of December to mid of January. From the first sampled shoots (September 15, 2010), 80% budburst was observed in the upper buds of the intact shoots of "Golden" at a position similar to the pruned and disbudded shoots (data not shown). Thereafter, such buds did not burst anymore. To verify causes of paradormancy, these buds of "Golden" were compared with their equivalent buds from the disbudded shoots and found that budburst from the disbudded was 40% (data not presented). On the other hand, terminal buds of both cultivars and buds from the intact and disbudded shoots of "Gala" at similar positions did not show any budburst in all the sampling dates.

During 2011-2012, difference among treatments was unclear, with a fluctuating trend (Fig. 4b). However, the final bud developmental stage of the lower buds, buds from the 2/3 pruned shoots, increased to a maximum (data not shown). A general increase in budburst rate was observed from the first sampling date (Sep. 1) up to the third sampling date (Oct. 13, 2011). This was followed by a general decrease in budburst, and reaching a pause for upper buds of "Gala" on the November 3 and lowest rate for the other treatments by mid of December. Budburst from the intact and disbudded shoots were either below 50% or no burst at all during the whole experimental period, and hence not included in the graph.

"MM106" apple rootstock: Dormancy progression curve for "MM106" apple rootstock in Ethiopia, in 2010, is shown in Fig. 5. In this figure, only results obtained from the pruned treatments (treatments 2 and 3) are included since the other treatments either did not reach 50% budburst (terminal buds of the intact shoot treatment) or did not burst at all (all disbudded treatments). As it can be seen in Fig. 5, both pruned treatments had similar dormancy progression patterns. Specifically, the upper buds of the 1/3 pruned were able to burst quicker than the lower buds of the 2/3 pruned in most of the sampling dates. However, when

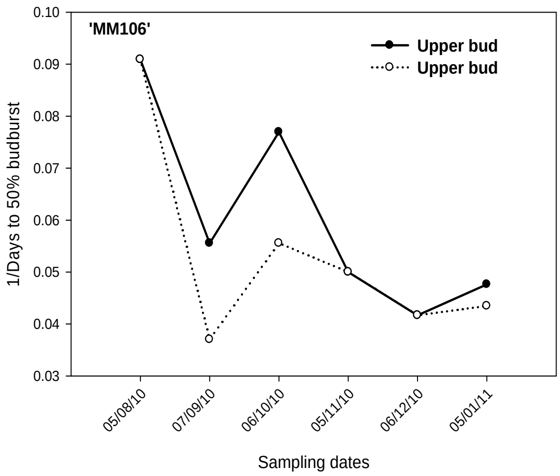

Fig. 5 Budburst progression, expressed as inverse of days to 50% budburst in "MM 106" apple rootstock shoots during 2010/2011 in Ethiopia (upper bud = 1/3 of the upper shoots pruned; lower bud = 2/3 of the upper shoot pruned). No bud burst in intact and disbudded shoots.

we compared the final bud developmental stages to which burst buds developed further, the lower buds developed more than the upper buds (data not presented). The shallowest dormancy was achieved in the first sampling period (August 5, 2010) for both treatments. While from the beginning of October until the beginning of December, a steady decrease in budburst rate was observed. In January, they started a slightly ascending curve in their budburst rate, though the sampling was not continued after January due to lack of enough shoots in the field. In practice, since matured shoots are harvested in the beginning of December for propagation purposes and normal re-growth starts within one-to-two week time.

3.4 Dormancy Progression in Belgium

The progression of bud dormancy over the two study years varied greatly, as shown in Figs. 6a and 6b. In 2010/11, all types of buds of both cultivars entered dormancy as early as October and no budburst was observed till the end of November 2010 (Fig. 6a). Thereafter, a general increase in budburst rate was observed from the upper buds of the pruned shoots. Initially upper buds of "Gala" had a higher rate of budburst than "Golden" though later, as of the beginning of February, it was the reverse. From the first week of January onwards, terminal buds of "Golden" had a higher rate of budburst than terminal buds of "Gala". During the last two sampling dates (February 2 and 22, 2011), terminal buds of each cultivar burst more quickly than their respective upper buds. By the last date of the sampling (February 22, 2011), the first "natural" budburst observed on the trees at the orchard, all types of buds from both

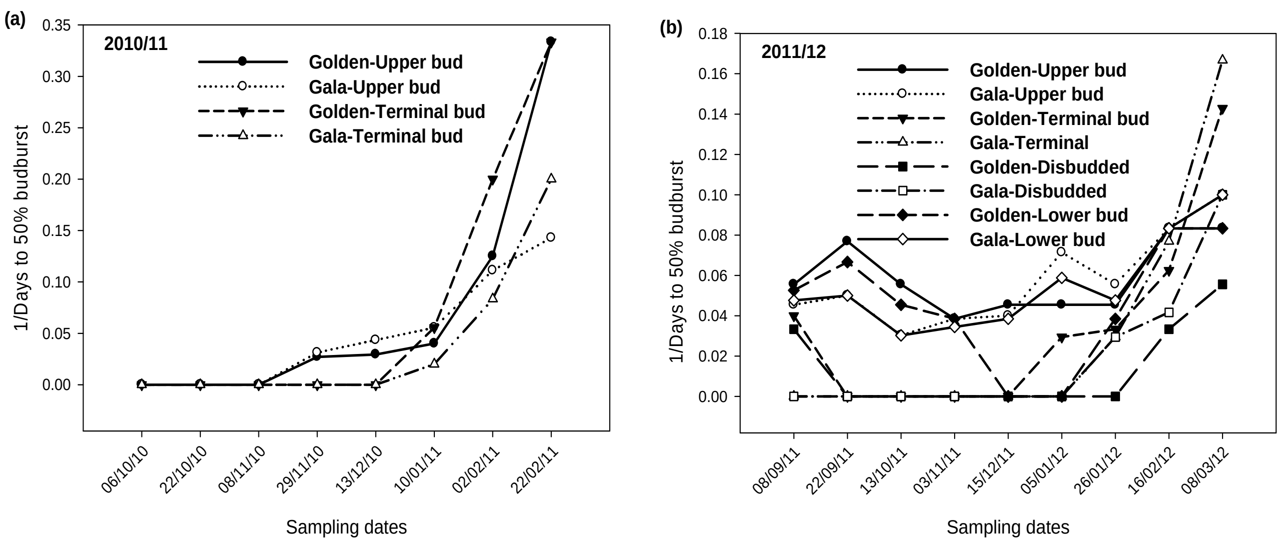

Fig. 6 Budburst progression, expressed as inverse of days to 50% budburst in "Golden" and "Gala" one-year-old shoots during the period 2010/2011 and 2011/2012 in Belgium (upper bud = 1/3 of the upper shoots pruned; lower bud = 2/3 of the upper shoot pruned). In 2010/2011, lower buds (2/3 of the upper shoot pruned was not part of the treatments). Data points at 0.00 imply less than 50% budburst.

cultivars burst in fewer than 7 days in the growth room, with an earliest burst from “Golden” (3 days). Paradoxically, buds from the disbudded shoots and upper buds of the intact shoots at a comparable position to that of the 1/3 pruned shoots from both cultivars did not reach 50% budburst at any time during the observation periods.

Unlike 2010/2011, in 2011/2012 pruned buds of both cultivars had not entered to the state of deep endodormancy throughout the study period and followed an oscillating budburst pattern, with a slightly increasing budburst trend during end of January 2012 (Fig. 6b). During the first sampling date (Sep. 8, 2011), budburst was observed from the disbudded and terminal buds of “Golden”. This was followed by a long pause in budburst and then later increase in budburst rate. The disbudded and terminal buds of “Gala” were in deep dormancy till the last week of January 2012. Moreover, upper buds of “Golden” had a higher budburst rate till mid of December 2011 though the trend was fluctuating after that time. As in the previous year, terminal buds of “Golden” were the first to burst though later the budburst rate was higher for “Gala”. The difference in budburst rate between upper and lower buds of both cultivars was not pronounced. However, upper buds of the intact shoots of both cultivars situated at similar positions with those of the disbudded shoots did not show any budburst in all the sampling dates, hence they are not included in the graphs.

4. Discussion

4.1 Temperature and Chilling Accumulation

The variation in temperature registered in the two environments is related to their geographic location (see Materials and Methods). Thus, high thermal amplitude between the minimum and maximum temperatures was observed in Ethiopia, which is situated in the tropics with a cool tropical semi-arid climate. Such greater variation in maximum and minimum temperatures between consecutive days observed in Ethiopia demands due attention, especially with the concern in climate change. In Ethiopia, temperatures are rarely ever sufficiently low to accumulate the chill units necessary to terminate endodormancy though it varies with elevation. The chilling accumulation in 2011/2012 (ca. 764 ICU) was a bit higher than in 2010/2011 (ca. 651 ICU) (Fig. 3a). Had it not been the South African chill unit model, ICU, used for the chill accumulation calculation, but the Utah model in RCU, it would have resulted to nil chill units. Over the two years, there was an average of 46 h at less than or equal to 7 °C, which is less than that of Ashebir et al. [19] reported for 2004-2006 (55 h) for the same orchard, suggesting fluctuation in temperature. Not only temperatures are too high all year round but also their seasonal variations are not pronounced (Figs. 1a, 2a and 2b). Hence, the major limiting factor for apple fruit production in Ethiopia is lack of adequate winter chilling, which varies from 200 (cv “Anna”) to 1,500 (cv “Wright #1”) h [27, 28]. This lack of chilling affects the behavior of apple and disrupts the dormancy progression [3, 16-18] and consequently altered tree architecture [16-18] and its cropping potential [16, 21] in mild-winter climates.

In Belgium, on the other hand, the cold autumns and winters were more than sufficient (Fig. 3b), with > 2,000 RCU at the end of March in both years to satisfy chilling requirement for majority of the commercial apple cultivars, that is between 600 h and 800 h below 7 °C of chilling units [29]. Hence, lack of chilling is not actually a problem but the spring frost, which is closely related to bud phenology, could be the problem which can induce an important loss of yield [30].

4.2 Dormancy Progression in Ethiopia

In general, the pattern of dormancy progression observed for “Gala” and “Golden” was similar with that of “MM106” apple rootstock (Figs. 4a, 4b and 5). Similarly, the dormancy progression pattern in 2010-2011 was similar to the one observed in

2011-2012 (Figs. 4a and 4b) though the chilling accumulation in 2011/2012 was relatively higher than in 2010/2011 (113 ICU more in 2011/2012). In all the cases, buds of the pruned shoots were the first to show budburst and continually burst during most of the sampling dates, regardless of the chilling accumulation. This is in agreement with the common understanding that decapitation of dormant buds prior to forcing conditions appears to remove paradormancy [31]. This was explained by the stimulating effect of the cut on overcoming of paradormancy and with the fact that lateral buds have lower endodormancy than terminal buds during the dormant period [9, 22]. Similarly, in the present study, terminal buds, and upper buds of the intact and disbudded shoots of both "Gala" and "Golden" as well as "MM106" rootstock did not burst at all or only achieved less than 50% budburst during the evaluation period. This indicates that the terminal buds were in deep endodormancy and/or seem to receive extra inhibitions from the late season water shortage [9] while a strong paradormancy accounts for the inhibition of budburst of the upper buds of both the intact and disbudded shoots [17, 18]. It was only in 2010/2011 that all the treatments for "Gala" and "Golden" reached the deepest level of endodormancy from end of November till Mid of January, as indicated by the pause of budburst within that period (Fig. 4a). By the end of November 2010, the accumulated chilling was about 200 ICU (Fig. 3a). Though this agrees with the common belief that buds reach endodormancy with the onset of chilling [3, 9], it did not happen in the same way in November 2011 (ca. 280 ICU). Similarly, Cook and Jacobs [18] reported contrasting results from South Africa: in a moderately cold region, apple cultivars of "Granny Smith" and "Golden Delicious" reached a maximum endodormancy before any considerable amount of chilling accumulated (< 100 chill units) whereas in a warmer region, ca. 600 chill units accumulated before the same cultivars reached to their maximum endodormancy.

Moreover, maximum bud developmental stage was achieved from the lower buds (data not shown), although the upper buds were the first to burst in both the scion and rootstock treatments. Thus, the physiological state of lower buds is probably different from that of the upper buds since their respective bud developmental growth, once their buds burst, was not identical. Such maximum bud developmental stage of the lower buds could be associated with minimal endodormancy [21]. According to Cook and Jacobs [17], once the proximal lateral buds are released from paradormancy, they exhibit a growth potential similar to the terminal bud and possess a greater expression of autonomy between shoots which lead to basal dominance. They further explained that the lack of an extended dormant period associated with mild-winter climates impeded the predominantly acrotonic branching behavior of apple observed in temperate climates and subsequently apical control, resulting in basitonic branching. Similarly such basitonic branching tendency was observed in some genotypes of a two-year-old "Telamon-Braeburn" progeny growing in the same orchard where the shoot cuttings for the present study were collected (personal observation). Furthermore, well developed and greater number of flower buds were observed in a basitonic gradient from a preliminary examination of buds sampled at one time point (October 15, 2011), using light and scanning electron microscopy (data not shown). Hence, the more budburst towards the proximal or basitonic gradient observed could be further explained by the basitonic flower buds recorded, as the chilling requirement for flower buds is lower than those needed for vegetative buds [3, 13]. Thus, one important point is worth mentioning here: development of appropriate pruning strategy to know when and where to prune as implicated by the difference in bud developmental growth between the upper and lower buds of pruned shoots and the position of the flower buds.

In contrast to endodormancy which concerns with the bud itself and its chilling requirement, paradormancy results from the action of one or many other organs (stem, leaves, roots, other buds) within the plant but outside the dormant bud [5, 6, 31]. With this concept and as the upper buds of the disbudded shoots and that of the intact shoots found at a similar position with the disbudded did not burst during the study, it gives evidence for the existence of a considerably strong inhibition by the distal shoot parts and/or buds. This also agrees with the findings by Cook and Jacobs [17, 18] in apple and Campoy et al. [32] in apricot that paradormancy accounts largely for the inhibition of budburst of the upper lateral buds under conditions of sub-optimal chilling. Such distal inhibition within a shoot or paradormancy is believed to be mainly associated with the apical dominance mechanism, and most probably with the effects of polar auxin transport [4]. Though our results are not entirely conclusive about the main source of paradormancy, Cook and Jacobs [17] reported that the main causes may reside more in the shoot piece than the buds themselves.

The relative budburst rate also depended on the cultivar and was higher for "Gala" than "Golden" in most of the sampling dates of the two years studied (Figs. 4a and 4b). This variation could be due to the genetic variation in chilling requirement in apple cultivars [12, 28]. According to Bernardi [33], the cultivar "Gala" is categorized as low-chill while the cultivar "Golden" as high-chill under the sub-tropical region of Santa Catarina, Brazil. However, this is contrary to the findings of Herter et al. [34], who stated that "Gala" shows deeper dormancy than "Golden Delicious" under the climatic conditions of South Brazil. This indicates that in addition to chilling requirement, there might be other important interactions among cultivars and environmental factors that are responsible for budburst [27]. In any case, both cultivars are among the top recommended apple cultivars for production in warm climates with the application of rest-breaking agents [11, 33]. Similarly, Ashebir et al. [19] and our experiences show that both cultivars perform well upon the application of "Dormex" and "winter-oil" in the tropical highlands of Northern Ethiopia.

4.3 Dormancy Progression in Belgium

Despite variations existing between years, our results in general agree with the established understanding of bud dormancy progression under temperate climatic conditions [5, 9]. As shown in Figs. 6a and 6b, the entry to endodormancy over the two study years varied greatly. This variability in endodormancy induction over years might be due to the different temperatures recorded during the end of summer and beginning of autumn. Before the first sampling (October 6, 2010), average temperatures were already around 10 °C (Fig. 2c) which resulted in accumulation of about 80 RCU under field conditions (Fig. 3b). However, at the same date during 2011/2012, the average temperature was around 15 °C (Fig. 2d) and consequently no chilling was accumulated (Fig. 3b). Thus, dormancy induction could have been started in the field earlier in 2010/2011 than 2011/2012, which might signify the importance of summer/autumn temperatures for bud dormancy progression in apple. This agrees with Cook et al. [14] and De Wit et al. [15] who found that apple rootstocks in Belgium to be dormant already in the beginning of October. Moreover, De Wit et al. [15] reported year to year variation in the induction, depth and cessation of endodormancy.

Irrespective of years or cultivars, terminal buds of the intact shoots achieved a deeper endodormancy until January than the lateral ones (Figs. 6a and 6b). Thereafter, the trend was reversed; a generalized abrupt increase in growth rate and an earlier budburst of the terminal buds than the lateral was observed. By this time, about 980 RCU of chilling had accumulated (Fig. 3b). This agrees with previous research [9, 14, 15, 24], which concluded that budburst rate increases with chilling accumulation later in winter or early in spring mostly in the terminal buds followed by distally situated lateral buds. Hauagge and Cummins [12] and De Wit et al. [15] also noted that lateral buds appeared less endodormant than terminal buds, even they were in doubt whether the lateral buds become endodormant every year. These findings are in agreement with our present results, which we show a gradual but year dependent bud progression of laterals. In 2010/2011, the laterals seem deep endodormant until the end of November followed by slow and steady increase in budburst till the first week of January; then afterwards, a sharp increase in budburst (comparable to or higher than that of the terminal buds) is achieved (Fig. 6a). This corresponds with the first natural budburst in the field. The exceptions were the buds from the disbudded shoots and upper buds of the intact shoots situated at an equal position with that of the disbudded, which did not reach 50% budburst at any time during the observation period. We suspect that either the paradormancy inhibition from the stem part was strong or there might be a problem of bud manipulation for the disbudded ones and a problem during the data recording for both types of shoots.

In 2011/2012, on the other hand, lateral buds were less endodormant throughout the sampling period, as continuous budburst in an oscillating pattern followed by a rapid increase towards the end of January was observed irrespective of cultivars (Fig. 6b). This pattern may be influenced by the relatively higher temperatures during summer/autumn of 2011/2012. Thus, the lateral buds seem to be inhibited largely by the stem, buds or both, which were situated above (correlative inhibition) before the manipulation [9] while their endodormancy component was shallow. Furthermore, Cook et al. [14] and De Wit et al. [15] stated that a proximal shoot-forming or a basitonic gradient exists during the end of autumn till early winter, while as spring approaches, this tendency shifts to a distal shoot-forming or acrotonic gradient. In general terms, our results are in agreement with these findings. Finally, lateral buds of the intact shoots of both cultivars situated at similar positions with those of the disbudded shoots did not show any budburst in all the sampling dates while budburst was recorded from the disbudded shoots as spring approaches. This indicates that the total inhibition in the lateral buds of the intact shoots was considerably greater than those of the disbudded ones, which is in agreement with previous works [14, 15].

Regarding the cultivar difference in budburst rate, our results show different trends, depending on years, seasons within a year and type of buds. In 2010/11, a more rapid increase in budburst rate was observed in terminal buds of “Golden” than terminal buds of “Gala”; whereas, lateral budburst was earlier in “Gala” than in “Golden” though the trend was reversed later in the winter season and afterwards. Similarly in 2011/12, terminal budburst was earlier in “Golden” despite the trend was shifted to terminal buds of “Gala” at the end of January. The lateral buds behave differently. Both the upper and lower lateral buds of “Golden” were the first to burst during autumn while in the winter season upper buds of “Gala” showed a higher budburst rate value than those of “Golden”. This suggests that budburst of different buds within a cultivar reacts differently, depending on the climate (year or season) irrespective of the cultivar’s chilling requirement [12, 27].

5. Conclusions

The present study provides a systematic analysis of budburst characteristics of apple cultivars over two years under contrasting chilling conditions (Belgium—temperate climate and Northern Ethiopia—semi-arid tropical climate). In summarizing our results, the following points are worth mentioning:

(1) Differences in the budburst rate values (which could explain depth of dormancy) are not exclusively related to the chilling requirements of the cultivars, as cultivar × environment (location, year and season within the year) interactions are evident.

(2) Irrespective of the environment or cultivar,

terminal buds are more dormant than laterals (a basitonic budburst gradient achieved) throughout the winter season though this relation is reversed during spring in Belgium (terminal buds burst earlier than lateral buds—acrotonic gradient). However, as expected, the mild-winter in Ethiopia impedes the acrotonic budburst pattern throughout the observation period.

(3) Although rate of budburst under forcing conditions is determined by the sum of the endodoramnt and paradormant components, results give evidence for the existence of a considerably strong paradormancy inhibition by the distal shoot parts and/or buds, which was more pronounced in mild-winter climates (Ethiopia) than in "normal" winter climates (Belgium).

(4) Despite the progress made on dormancy research so far, still gaps remain in our knowledge of dormancy evolution and its control mechanism especially under mild-winter climates. Thus, our study highlights the need for further in-depth research in this area with a full range of cultivars (low to high chill requirements) growing under different locations of warm climates using biological and biochemical tests simultaneously.

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References

[1] Z. Calle, B.O. Schlumpberger, L. Piedrahita, A. Leftin, S.A. Hammer, A. Tye, et al., Seasonal variation in daily insolation induces synchronous bud break and flowering in the tropics, Trees 24 (2010) 865-877.

[2] J.V. Anderson, D.P. Horvath, W.S. Chao, M.E. Foley, Bud dormancy in perennial plants: A mechanism for survival, in: E. Lubzens (Eds.), Dormancy and Resistance in Harsh Environments, Topics in Current Genetics 21 (2010) 69-90.

[3] A. Erez, Bud dormancy, phenomenon, problems and solutions in the tropics and subtropics, in: A. Erez (Ed.), Temperate Fruit Crops in Warm Climates, Kluwer Academic Publishers, The Netherlands, 2000, pp. 17-48.

[4] M. Faust, A. Erez, L.J. Rowland, S.Y. Wang, H.A. Norman, Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance, and release, Hort Sci. 32 (1997) 623-629.

[5] G.A. Lang, J.D. Early, G.C. Martin, R.L. Darnell, Endo-, para-, and ecodrmancy: Physiological terminology and classification for dormancy research, Hort. Sci. 22 (1987) 371-377.

[6] D. Horvath, Bud dormancy and growth, in: E.C. Pua, M.R. Davey (Eds.), Plant Developmental Biology—Biotechnological Perspectives, Springer-Verlag, Berlin Heidelberg, 2010, pp. 53-70.

[7] C. van der Schoot, Dormancy and symplasmic networking at the shoot apical meristem, in: G.A. Lang (Ed.), Plant Dormancy: Physiology, Biochemistry and Molecular Biology, CAB International, Wallingford, UK, 1996, pp. 59-81.

[8] J.A. Campoy, D. Ruiz, J. Egea, Dormancy in temperate fruit trees in a global warming context: A review, Scientia Horticulturae 130 (2011) 357-372.

[9] J. Crabbé, P. Barnola, A new conceptual approach to bud dormancy in woody plants, in: G.A. Lang (Ed.), Plant Dormancy: Physiology, Biochemistry and Molecular Biology, CAB International, Wallingford, UK, 1996, pp. 83-113.

[10] A. Fennel, Systems and approaches to studying dormancy: Introduction to the workshop, Hort Sci. 34 (1999) 1172-1173.

[11] R. Hauagge, J.N. Cummins, Pome fruit genetic pool for production in warm climates, in: A. Erez (Ed.), Temperate Fruit Crops in Warm Climates, Kluwer Academic Publishers, The Netherlands, 2000, pp. 267-303.

[12] R. Hauagge, J.N. Cummins, Seasonal variation in intensity of bud dormancy in apple cultivars and related *Malus* species, J. Amer. Soc. Hort. Sci. 116 (1991) 107-115.

[13] J. Tromp, J.A.D. Webster, S.J. Wertheim, Fundamentals of Temperate Zone Tree Fruit Production, Backhuys Publishers BV, The Netherlands, 2005, p. 400.

[14] N.C. Cook, E. Rabe, J. Keulemans, G. Jacobs, The expression of acrotony in deciduous fruit trees: A study of the apple rootstock M9, J. Amer. Soc. Hort. Sci. 123 (1998) 30-34.

[15] I. De Wit, A. Duponcheel, J. Keulemans, Evolution of endodormancy in apple rootstock M9, Acta Hort. 517 (2000) 131-142.

[16] N.C. Cook, Apple production under conditions of sub-optimal winter chilling in South Africa, Acta Hort. 872 (2010) 199-204.

[17] N.C. Cook, G. Jacobs, Sub-optimal winter chilling impedes development of acrotony in apple shoots, Hort Sci. 34 (1999) 1213-1216.

[18] N.C. Cook, G. Jacobs, Progression of apple (*Malus* × *domestica* Borkh.) bud dormancy in two mild winter climates, J. of Hort. Sci. and Biotech. 75 (2000) 233-236.

[19] D. Ashebir, T. Deckers, J. Nyssen, W. Bihon, A. Tsegay, H. Tekie, et al., Growing apple (*Malus* × *domestica*) under tropical mountain climate conditions in Northern Ethiopia, Expl. Agric. 46 (2010) 53-65.

[20] G. Hruy, N. Aregay, Y. TekeleBirhan, T. Deckers, H. Bauer, K. Amare, et al., Apples in tropical highlands of Northern Ethiopia: Potentials and challenges, Chronica Horticulturae 52 (2012) 16-21.

[21] R. Hauagge, J.N. Cummins, Relationships among indices for the end of bud dormancy in apple cultivars and related *Malus* species under cold winter conditions, J. Amer. Soc. Hort. Sci. 116 (1991) 95-99.

[22] F.G. Dennis, Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants, Hort. Sci. 38 (2003) 347-350.

[23] I. De Wit, Branching dynamics and tree architecture in apple trees: Involvement of dormancy and correlative effects, Ph.D. Dissertation, Katholieke Universiteit Leuven, Belgium, 2002, p. 162.

[24] P.J. Chapman, G.A. Catlin, Growth stages in fruit trees from dormant to fruit set, New York's Food and Life Sciences Bulletin 58 (1976) 11.

[25] E.A. Richardson, S.D. Seeley, D.R. Walker, A model for estimating the completion of rest for Redhaven and Elberta peach trees, Hort Sci. 9 (1974) 331-332.

[26] A. Hacking, An overview of the South African low chill fruit industry, Netpro Update, 2006.

[27] R. Hauagge, J.N. Cummins, Phenotypic variation of length of bud dormancy in apple cultivars and related *Malus* species, J. Amer. Soc. Hort. Sci. 116 (1991) 100-106.

[28] I.F. Labuschagne, J.H. Louw, K. Schmidt, A. Sadie, Genetic variation in chilling requirement in apple progeny, J. Amer. Soc. Hort. Sci. 127 (2002) 663-672.

[29] C.R. Hampson, H. Kemp, Characteristics of important commercial apple cultivars, in: D.C. Ferree, I.J. Warrington (Eds.), Apple, Botany, Production and Uses, CABI Publishing, Wallingford, UK, 2003, pp. 61-89.

[30] E. Eccel, R. Rea, A. Caffarra, A. Crisci, Risk of spring frost to apple production under future climate scenarios: The role of phenological acclimation, Int. J. Biometeorol. 53 (2009) 273-286.

[31] P. Champagnat, Bud dormancy, correlation between organs, and morphogenesis in woody plants, Soviet. Plant Physiol. 30 (1983) 458-471.

[32] J.A. Campoy, D. Ruiz, N. Cook, L. Allderman, J. Egea, Clinal variation of dormancy progression in apricot, South African Journal of Botany 77 (2011) 618-630.

[33] J. Bernardi, Behaviour of some apple cultivars in the subtropical region of Santa Catarina, Brazil, Acta Hort. 232 (1988) 46-50.

[34] F.G. Herter, N.L. Finardi, J.C. Mauget, Dormancy development in apple trees cvs. Gala, Golden and Fuji, in Pelotas, RS, Acta Hort. 232 (1988) 109-115.

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Chlorocholine Chloride Induces Cacao Reproductive Development Leading to Improved Fruitlets Productivity of Cacao Trees in the Field

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Abstract: Fruitlet abscission (cherelle wilt) is a major factor contributing to lower productivity of cacao plantation in Indonesia. An attempt was made to reduce such cherelle wilt by spraying plant growth retardants (PGRs) on cacao trees at about 10 weeks before flowering session. The treatment was repeated every three weeks. Both the flowering initiation time and the number of flowers on the treated trees were improved significantly. With the most effective composition, flowers were initiated at 24 days after spraying (DAS), where as the flowering initiation was 11-day later in the controls. At the 35 DAS, the chlorocholine chloride (CCC) 2,000 treatment induced about 20-80 folds more flowers than the controls. Extended observation was made to assess the treatment effect on the fruiting capacity. The treatments were found to improve fruiting ability by increasing the number of fruits set on the trees and shortening the time for fruit setting. At 20 weeks after the first spraying (WAS) there were on average 12.57 fruits per CCC 2,000-treated tree, as opposed to only 4.14 fruits per untreated tree. Analyses of the metabolites content in the flower cushions of the trees indicated that the reproductive growth is significantly correlated with the increased metabolites particularly the reduced sugar. Exogenous addition of sucrose to the PGR treatment gave greater improvement mainly in the fruiting. In conclusion, foliar spray of CCC induces flowering of cacao tress in the field that leads to improve the fruitlets productivity.

Key words: Plant growth retardant, induced flowering, *Theobroma cacao* L..

1. Introduction

Productivity of cacao plantations in Indonesia are varied from 300 kg to 1,580 kg dried bean/ha annually [1]. The potency of cacao productivity was reported 3,375 kg dried bean/ha per annum [2]. Thus, the average, about 900 kg dried bean/ha, is less than 30% of its potential productivity. One of the factors contributing to this poor productivity is low fruit density on the trees.

A healthy mature cacao tree produces 5,000-10,000 flowers annually. However, only 500-1,000 of them set up fruits, 70% to 90% of which are wilted early and consequently only 50-100 fruits are harvestable from every cacao tree [3]. In most Indonesia cacao plantations, the density of harvestable fruits was even less, with 33-40 fruits per tree [4]. Besides, cacao trees mostly only flower once a year, in the beginning of rainy session.

With this regard, an attempt to induce reproductive development in cacao plantation had been done in the non-flowering session. Inductions were conducted using plant growth retardants (PGRs) combined with some other components. PGRs have been used to improve productivity of some crops [5-8]. This research was aimed at studying how PGRs alter reproductive development of cacao trees in the field. Furthermore, it is of our interest to see whether

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alteration of cacao flowering could correspondingly alter fruiting. The PGR used in this experiment was chlorocholine chloride (CCC) which inhibits the biosynthesis of gibberellins directly before *ent*-Kaurene [9]. In most cases, Paclobutrazol (PBZ), a PGR inhibiting GA biosynthesis at the oxidative steps from *ent*-kaurene to *ent*-kaurenoic acid and proven effective to induce flowering of mango trees during off session [10], was used for comparison. The flowering initiation time was recorded. The physiological parameters including the flower numbers, fruit setting and fruit numbers, were investigated. Some pertinent biochemical parameters were also analyzed.

2. Materials and Methods

Cacao trees of about 15 years old used for the experiments to induce reproductive development belong to PTPN VIII located in Bandung, West Java in Indonesia. CCC powder with 98% purity or greater was purchased from Sigma Chem. Co or imported from China. PBZ was obtained from local supplier.

2.1 In vitro Test of the PGR Activity

To confirm the PGR activity of CCC, an *in vitro* assay was performed using tobacco plantlets grown on MS solid media supplemented with 0 ppm to 50 ppm of CCC. Tobacco plantlet cuttings with two leaves were planted on the media and cultured as previously described [11]. Data recording was made for the vegetative growth, the stem length and the leave numbers of the plantlets for one month.

2.2 Selection for an Effective CCC Application Technique

With several possible methods of application, 400 ppm CCC working solution pH 5.0 was used to treat cacao trees. The methods include direct spray to the fruits, foliar spray, spread on the cambium or pouring 50 cm around the trees. The working solution was applied early in the morning. Several hours after the treatment, some leaves were detached from the treated trees. Before analysis for the PGR content using HPLC [12], the leaves were rinsed three times with clean water to remove contaminant.

2.3 PGR Treatments in the Field

The solutions containing 1,000 ppm CCC, 2,000 ppm CCC, 500 ppm PBZ or 1,000 ppm PBZ were prepared just before application. Sucrose was added to the same working solutions for final concentration of 1%. Two months before flowering session, the solutions were applied onto cacao trees early in the morning, with seven replicates of tree. In most cases applications were conducted by foliar spray [13]. Flower initiation was observed once a week. The time of flower initiation and the number of flowers were recorded for each tree, within areas of 2 m above soil. In the mean time, cacao tissues were harvested for analyses of metabolic contents.

Morphological changes that were recorded include time of flower initiation, number of flower induced, small fruits (fruitlets) developed and fruitlets wilted. Again, the counting was restricted to only those on the trunks and the branches 2 m above the soil. C/N ratio, sucrose, and total N content in the xylem or the flower cushions were analyzed several weeks following the applications on cacao trees. The metabolites content were determined using the reported method [14]. The levels of the PGR in the cacao tissues were assayed using HPLC with proper condition [12].

3. Results and Discussion

3.1 Application of Vegetative Growth Retardant

To confirm the retarding activity of CCC on vegetative growth of plant, an *in vitro* assay was conducted using tobacco plantlet grown on MS media as described in the materials and method. The result showed that CCC greatly reduces the vegetative growth of the plantlets as the retardant concentrations increased (Fig. 1). At the level of 50 ppm, CCC retarded the stem elongation by 82%, averaging 11

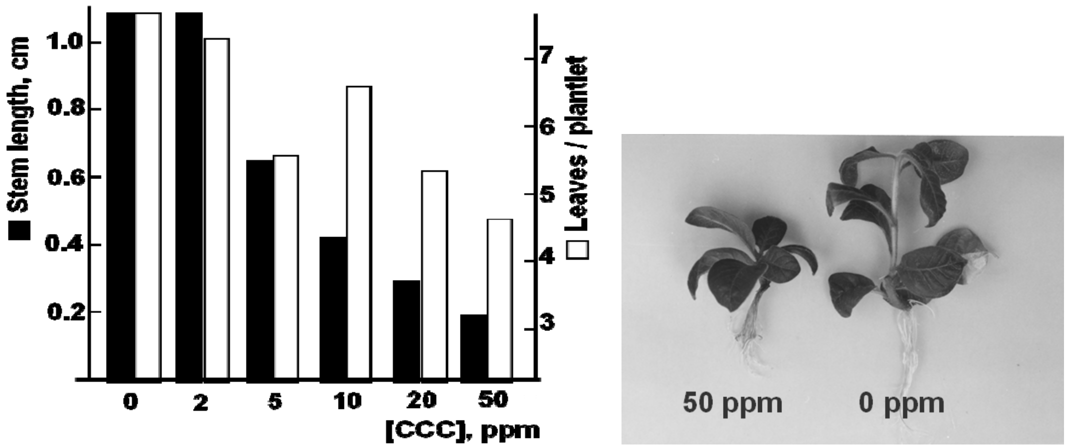

Fig. 1 The growth retarding effect of CCC on tobacco plantlets *in vitro.*

mm in the control plantlets grown on MS media with no CCC, and 2 mm in the 50 ppm CCC-treated plantlets. This result confirmed that the CCC has strong plant growth retarding activity as reported previously [15, 16]. However, a contradictive response was reported on vegetative growth of ornamental plant of *Lilium* [8].

There are several ways to apply exogenous phytohormone to plant tissues for influencing plant development. Widely used methods include incorporation with growth, spraying onto the leaves or fruit, and smear onto cambium of the trunk [12, 17, 18]. To find the most effective ways, several application techniques were tested by analyzing the CCC intake by the leaves of treated plants. The result demonstrated that applying CCC through upper foliar spray was the most effective way, with 4.66 ppm CCC in the leaves, followed by lower foliar spray that leads to 1.00 ppm CCC in the leaves (Table 1). CCC intake was undetectable in the leaves applied by cambium spreading, although the cambium spreading has been shown effective in rubber trees [18]. Ineffectiveness of the cambium spreading on cacao trees was possibly due to the presence of mucilage formed when cacao tissues were wounded [19].

3.2 Flower Induction of Cacao on the Field

The experiments of cacao flower induction on the field were conducted during off session. Therefore,

Table 1 Effectiveness of application techniques for CCC intake by cacao leaves in the field.

No.	Application techniques	CCC in leaves (ppm)
1	Upper foliar spray	4.66
2	Lower foliar spray	1.00
3	Fruit spray	0.18
4	Cambium spread	0.00
5	50 cm around soil water	0.91

any data of flowering collected during the time period should mostly represent the present of the exogenous inducer instead of seasonal or internal factors. The data collected at the time when the first flowering was induced are presented in Table 2. Those data also recorded the observation until 35 days after spraying (DAS) when all the tested treatments had initiated flowering. The observation on the effects of the inducer application lasted for five weeks and continued until the fruit setting has been developed which is about eight to ten weeks after application.

The experimental data from application of flowering inducers demonstrate that the flower-inducing treatments are mostly effective. Two out of five treatments, control and P-0.5, failed to initiate flowering until 28 DAS. PBZ 1 ppm and CCC 2,000 ppm are the two treatments inducing the most flowers, averaging 3.86 and 10.86 flowers per tree, respectively. At 35 DAS, the control trees started to flower, at which the PBZ 1 ppm and CCC 2,000 ppm treatments were producing the most, 25.14 and 47.00 flowers per tree, indicating that treatment of PBZ 1 ppm

Table 2 Flowering initiation and number of PGR-induced flowers (Flo) of cacao.

Treatment	Average flowering time (DAS)	#/tree at 28 DAS		#/tree at 35 DAS	
		Flo bunch	Flower	Flo bunch	Flower
Control, H_2O	32	0.00	0.00	0.43	0.43
PBZ 500 ppm	28	0.14	0.14	12.00	15.57
PBZ 1,000 ppm	24	3.57	3.86	18.86	25.14
CCC 1,000 ppm	26	0.57	0.57	10.00	13.14
CCC 2,000 ppm	24	9.57	10.86	31.29	47.00

or CCC 2,000 ppm increased flowering more than four folds within one week. Compared to the controls, the two best inducers made around 100 folds more flowers. If these numbers can be extrapolated timely until the stages of fruit development, the productivity of cacao can be expected to increase significantly. In this case attempt to reduce the incident cherelle wilt become more important.

The flower buds in cacao trees emerge mostly along the stems of the tree or branches, particularly at uneven surfaces (folded parts) (Fig. 2). At these sites, flush of shoots also usually arise. In partition, assimilate from leaf tissue is translocated to all parts of the trees and at a slower rate when it reaches the folded parts then maybe unloaded for initiation or growth of either vegetative or regenerative organs, shoots or flowers, depended on the competency of the tree. In low competency tree, the unloaded chemical energy is converted for mostly vegetative growth. In theory, assimilate partition to support generative development, assimilate from leaves is unloaded more in generative sink because the vegetative or structural competing sinks such as leaves, roots, stem are retarded so that their capacity is reduced [20]. Highly competent trees, however, will convert unloaded assimilate into development of regenerative organs. Therefore, more flowers are developed in the PGR-treated trees than in the control trees. Moreover, the untreated cacao trees developed more shoots instead (data not shown).

In addition to the flowering, the effect of induction on the reproductive development was also examined at the levels of fruit setting and its further development. The timing and number of fruit set were

Fig. 2 PGR-induced flowering at cacao flower cushions. From top to bottom are controls, PBZ and CCC treatments, respectively.

counted. Table 3 shows the timing data of fruit setting. Compared to the control trees sprayed with water only, all the PGR-treated trees set fruit earlier. The average fruit set time of seven sample trees varied between 71.14 to 74.86 DAS. Whereas control trees was 98.86 DAS, which is more than two weeks longer than that of treated trees. Variations in the number of fruit set at 8 and 20 WAS were also observed. Again, the CCC 2,000 ppm was the best treatment among those tested. The average number of developed young fruits or fruit lets at 20 WAS were 12.57 fruits/tree. The PBZ 1 ppm was the second best, 10.14 fruits/tree. The explanation for fruit setting is similar to that for the flower initiation. With prolonged influence, sprayed CCC 2,000 ppm repressed the vegetative growth. Consequently, more assimilate was directed to support fruit development. Repressing vegetating growth to support reproductive growth in cacao plantations can practically be accomplished by pruning of shoots newly emerging on cacao flower cushions during on session [21].

3.3 Biochemical Changes Caused by PGR Treatment

Knowing the physiological effects of PGR treatment on reproductive development of cacao trees, it was also our interest to know the pertinent biochemical changes of the trees caused by the treatment. For this, the targeted hormone gibberellins (GA) whose biosynthesis is inhibited by the PGRs, and primary metabolite contents of the cacao flower cushions were analyzed. The content of GA in the flower cushions is presented in Fig. 3. This data indicated that PGR treatment inhibited the GA biosynthesis. Three days after CCC spray, the GA level of flower cushions of treated cacao tree was 1.0 ppm, half of that of the water-sprayed tree. This data imply that CCC inhibits GA biosynthesis as reported before [22].

The levels of the primary metabolites including sucrose and total N, of the cacao flower cushions were analyzed. Data presented in Fig. 4 demonstrated that the PGR treatments caused reducing sugar accumulation. Sucrose level in cacao flower cushions was five folds higher than that of water spray. The sugar content was even higher when exogenous sugar was added in the PGR treatment, seven folds higher than the control. The treatments did not affect much on total N content, so that the values of C/N ratio corresponded to the sucrose contents. C/N ratio was reported to increase markedly in induced flowers of both *Sinapisalba* and *Arabidopsis thaliana* [23].

3.4 The Effects of Exogenous Sucrose

Confirming that sucrose was one of the indicators affected by PGR treatments on cacao trees, it is interesting to know the effect of exogenous sucrose to the reproductive parameters of cacao. Sucrose was added to the PGR solution so that its final concentration in the working solution was 1%. Six parameters were utilized to evaluate the effects of exogenous sucrose on the reproductive parameters of cacao. The result presented in Table 4 indicated that the exogenous sucrose improved all parameters except for the flowering time. The other five parameters investigated were improved by between 22% and 97%. The sucrose content of the flower cushions increased 24.23%. In citrus, injection of exogenous sucrose increases sucrose content of the fruits [24]. The average

Table 3 Timing and number of cacao fruit setting by PGR induction.

Treatments	Average fruit setting time (DAS)	Total fruits set at	
		8 WAS	20 WAS
Control, H_2O	98.86	0	4.14
PBZ 500 ppm	72.57	3	7.43
PBZ 1,000 ppm	71.14	3	10.14
CCC 1,000 ppm	74.86	4	9.14
CCC 2,000 ppm	72.29	2	12.57

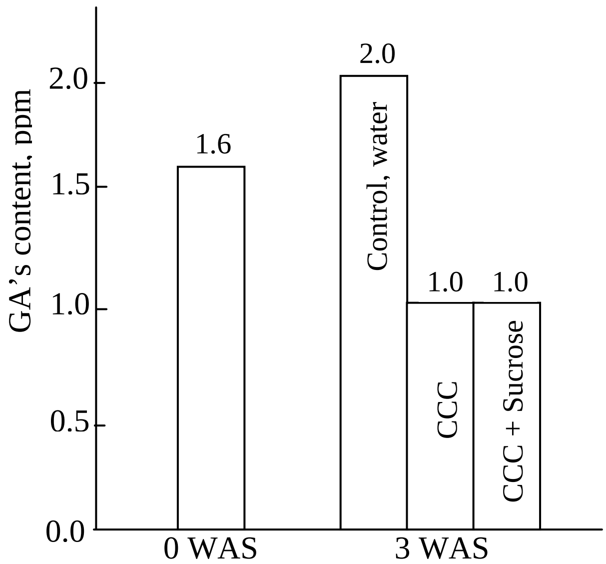

Fig. 3 Gibberellins content of cacao flower cushions before and 3 WAS of CCC.

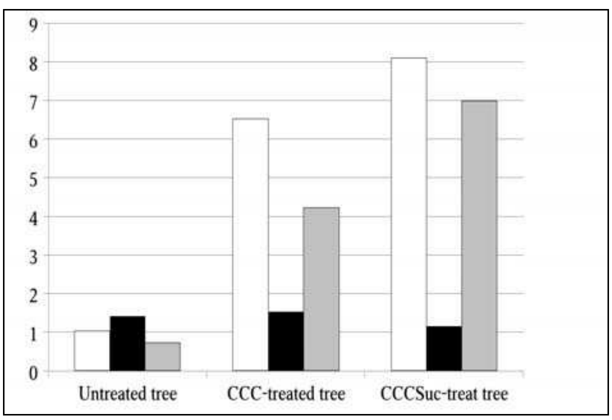

Fig. 4 Metabolite contents cacao flower cushions at 24 DAS. Open bars are sucrose (%), solid bars are total N (%), and grey bars are C/N ratio.

fruit setting time shortened 22.13%, dropping from 72.29 DAS to 56.29 DAS. In the mean time, the number of fruit set increased 97.77%, from 12.57 fruits/tree to 24.86 fruits/tree.

Significance of the role of sucrose in flowering is depicted in the report on a comparative analysis of expressed sequence tags (ESTs) from *Triticum monococcum* shoot apical meristem at vegetative and reproductive stages [25]. Sucrose synthase, an enzyme responsible for mobilizing sucrose into meristematic cells [26] was expressed 19 folds higher in reproductive SAM (shoot apical meristem) than in the vegetative SAM of *T. monococcum*. The enzyme catalyzes reversible conversion of sucrose, which is known as a signal molecule in flowering [27]. In Arabidopsis, small amount of sucrose induces expression of flowering LFY gene [28].

One of the problems in cacao plantation causing lower productivity is cherelle wilt, fruitlet abscission [12, 29]. It would be nice if the PGR treatment that could improve the cacao reproductivity can also reduce the cherelle wilt in cacao. This way of the PGR treatment was expected capable of supporting further development of cacao young fruits. To examine this possibility, an additional PGR treatment was performed and observation was extended for several weeks. The result is presented in Table 5. These data indicate that the treatments with PBZ or CCC reduced the incident of cherelle wilt more than 10%. Addition of sucrose in the treatment solutions made reduction of cherelle wilt incidents even larger, almost 20%. Significance of exogenous sucrose in supporting fruit development was also reported in citrus [30]. At molecular genetic level, some genes regulating the reproductive development of cacao have high similarity to those of citrus [31]. This approach was also applied to support the growth of vitality of live oak [32].

Table 4 The effect of exogenous sucrose on the biochemical and physiological reproductive parameters of cacao.

Parameters	CCC 2,000 ppm	CCC 2,000 ppm + sucrose	Changes (%)
Xylem sucrose (%)	6.52	8.10	24.23
C/N ratio	4.23	6.98	66.01
Flowering time (DAS)	24.00	24.00	0.00
Flower number	206.43	304.71	47.61
Fruit setting time (DAS)	72.29	56.29	-22.13
Fruit number	12.57	24.86	97.77

Table 5 Further cacao fruit development by PGR treatments.

Treatment	Number of young fruits	Cherelle wilt	
		Number	(%)
Control, water	4.14	1.29	31.03
PBZ 500 ppm	7.43	2.43	32.69
PBZ 500 + Sucrose	10.57	2.29	21.62
PBZ 1,000	10.14	2.00	19.72
PBZ 1,000 + Sucrose	13.57	1.71	12.63
CCC 1,000	9.14	1.57	17.19
CCC 1,000 + Sucrose	9.71	1.57	16.18
CCC 2,000	12.57	3.00	23.86
CCC 2,000 + Sucrose	24.86	4.57	18.39

This work has a practical importance to the cacao planters. So far, the productivity of cacao plantations in Indonesia has been considerably low. Being doubled in the fruit productivity by the CCC treatment, the cacao plantations are expected to double their cacao bean production. The productivity improvement may be even greater by addition of sucrose in the CCC composition. Indonesia is the third and sometime the second largest cacao bean producer supplying the world need. Making agriculture more productive is beneficial to the world.

4. Conclusions

Based on the experimental data, it can be concluded that foliar spray of CCC stimulates flowering of cacao trees in the field which improves significantly their fruit productivity. Addition of exogenous sucrose to the CCC spray could improve the reproductive development even higher.

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References

[1] D. Santoso, S. Wiryadiputa, T. Chaidamsari, R.A. de Maagd, Development of cacao resistant to pod borer: A prioritized application of plant genetic engineering, IBC, Yogyakarta, Oct. 23-26, 2001.

[2] J.A. Duke, *Theobroma cacao* L. Sterculiaceae: Chocolate, Cacao, Cocoa, Hand Book of Energy Crops, 1983, Unpublished update from Website, Jan. 9, 1998.

[3] A.D. McKelvie, Physiological of fruiting, Ann. Rep. West African Cacao Res. Inst., 1956-1956, 1957, pp. 71-76.

[4] The production and seed differences among cacao hybrid F1, F1 clonal and F2. Warta Puslit Kopi dan Kakao 15 (1999) 235-239. (in Indonesian)

[5] W. Rademacher, Growth retardants: Biochemical features and application in horticulture, Acta Horticulturae 394 (1995) 57-73

[6] H.P. Anosheh, Y. Emam, M. Ashraf, M.R. Foolad, Advanced Studies in Biology, Exogenous Application of Salicylic Acid and Chlormequat Chloride Alleviates Negative Effects of Drought Stress in Wheat, Vol. 4, No. 11, 2012, pp. 501-520.

[7] R. Medina, A. Burgos (*ex aequo)*, V. Difranco, L. Mroginski, P. Cenóz, Effects of chlorocholine chloride and paclobutrazol on cassava (*Manihotesculenta* Crantz cv. Rocha) plant growth and tuberous root quality, Agriscientia 29 (2012) 51-58.

[8] R. Zheng, Y. Wu, Y. Xia, Chlorocholine chloride and paclobutrazol treatments promote carbohydrate accumulation in bulbs of *Lilium*Oriental hybrids “Sorbonne”, J. Zhejiang Univ-Sci. B 13 (2012) 136-144.

[9] W. Rademacher, Growth retardants: Effects on gibberellins biosynthesis and other metabolic pathways, Annu. Rev. Plant Physiol. Plant Mol. Biol. 51 (2000) 501-531.

[10] R. Poerwanto, D. Efendi, S.S. Harjadi, Off season flower forcing of mangga gadung 21 using Paclobutrazol and dormancy breaking substances, Hayati 4 (1997) 41-46. (in Indonesian)

[11] D. Santoso, F.I. Cugito, H. Minarsih, Development of tobacco plant cells in the presence of kanamycin at various levels for transgenesis, Menara Perkebunan 68 (2000) 21-28.

[12] A.A. Prawoto, Study the phenomenon of cocoa cherelle wilt and development control efforts, in: The Symposium

on Cocoa 2000, Surabaya, Sept. 26-27, 2000. (in Indonesian)

[13] D. Santoso, A. Rachmawan, Application techniques and affectivity of VGR formulas to reduce cherelle wilt in cacao, Menara Perkebunan 70 (2000) 12-19. (in Indonesian)

[14] A. Abruzzese, I. Mignani, S.M. Cocucci, Nutritional status in apples and June drop, J. Amer. Soc. Hort. Sci. 120 (1995) 71-74.

[15] T. Tezuka, C. Takahara, Y. Yamamoto, Aspects regarding the action of CCC in hollyhock plants, J. Exp. Bot. 40 (1989) 689-692.

[16] I. Hussain, Z. Chaudhry, A. Muhammad, Effect of chlorocholine chloride, sucrose and BAP on *in vitro* tuberization in potato (*Solanumtuberosum* L. cv. Cardinal), Pak. J. Bot. 38 (2006) 275-282.

[17] S. Winasih, A.A. Prawoto, Influence cultar concentration and location of the application the yield of cocoa, Pelita Perkebunan 7 (1991) 74-78. (in Indonesian)

[18] Siswanto, Molecular markers for early detection of several important characteristics of rubber tree, in: Danimihardja (Ed.), Annual Report 1995, Biotechnology Center for Estate Crops, Bogor, 1995, p. 33. (in Indonesian)

[19] D. Santoso, Samanhudi, T. Chaidamsary, R.A. de Maagd, G. Angenent, Study on flowering induction in cacao: Identification of the LEAFY homolog in cacao, in: The Seminar of IBC, Denpasar, Dec. 1-3, 2004.

[20] M.J. Foulkes, G.A. Slafer, W.J. Davies, P.M. Berry, R. Sylvester-Bradley, P. Martre, et al., Raising yield potential of wheat, III. Optimizing partitioning to grain while maintaining lodging resistance, J. Exp. Bot. 62 (2011) 469-486.

[21] D. Santoso, Molecular and Genetic Engineering Studies toward Improvement of Cacao Bean Production, RUTi Final Report, IBRIEC, 2006, p. 133.

[22] P.J. Davies, Plant Hormones, Boston, Kluwer Academic Publisher, 1995, p. 833.

[23] L. Corbesier, G. Bernier, C. Perilleux, C:N ratio increases in the phloem sap during transition long-day plants *Sinapsis alba* and *Arabidopsis thaliana*, Plant Cell Physiol 43 (2002) 684-688.

[24] D.J. Iglesias, F.R. Tadeo, F. Legaz, E. Primo-Millo, M. Talon, *In vivo* sucrose stimulation of colour change in citrus fruit epicarps: Interactions between nutritional and hormonal signals, Physiol. Plant 112 (2001) 244-251.

[25] R. Shamloo-Dashtpagerdi, H. Razi, A. Lindlof, A. Niazi, A. Dadkhodaie, E. Ebrahimie, Comparative analysis of expressed sequence tags (ESTs) from *Triticum monococcum* shoot apical meristem at vegetative and reproductive stages, Genes Genom, 2013, DOI: 10.1007/s13258-013-0091-7.

[26] L. Corbesier, G. Coupland, The quest for florigen: A review of recent progress, J. Exp. Bot. 57 (2006) 3395-3403.

[27] M.A. Blazquez, D. Weigel, Integration of floral inductive signals in Arabidopsis, Nature 404 (2000) 889-892.

[28] M. Ohto, K. Onai, Y. Furukawa, E. Aoki, T. Araki, K. Nakamura, Effects of sugar on vegetative development and floral transition in Arabidopsis, Plant Physiol. 127 (2001) 252-261.

[29] A. Tjasadihardja, Correlation between shoot growth, fruit development and indole acetic acid content in seeds and wilted fruitlets of cacao, IPB Graduate School, Bogor, 1987, p. 124. (in Indonesian)

[30] D.J. Iglesias, F.R. Tadeo, E. Primo-Millo, M. Talon, Fruit set dependence on carbohydrate availability in citrus trees, Tree Physiology 23 (2003) 199-204.

[31] D. Santoso, Samanhudi, A. Budiani, T. Chaidamsari, R.A. de Maagd, Cloning and characterization of cacao flowering genes *Leafy*, *Apetala1* and *Agamous*, in: 15th Int'l Cocoa Research Conference, 2006.

[32] T. Martínez-Trinidad, W.T. Watson, M.A. Arnold, L. Lombardini, D.N. Appel, Carbohydrate injections as a potential option to improve growth and vitality of live oaks, Arboriculture & Urban Forestry 35 (2009) 142-147.

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Fluctuation of Mite Fauna Associated to Rice Culture (*Oryza sativa* L.: Poales, Poaceae) in Two Regions in the State of Rio Grande do Sul, Brazil

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Abstract: This survey had the aim to identify the mite fauna and mite ecologic interaction in rice culture, harvest 2010-2011, in Taquari and Cachoeirinha Counties, state of Rio Grande do Sul, Brazil. The mite population was biweekly evaluated on IRGA 424 and INTA PUITÁ CL cultivars in four areas where randomly sampled 20 plants/area. Fluctuation, ecologic indices and correlation between species and environmental factors were calculated. A total of 1,626 mites belonging to 14 species from 12 families were collected. Of the total specimens collected, 34.56% was on IRGA 424, in Taquari, 32.47% on IRGA 424-120 and 28.35% on IRGA 424-60, in Cachoeirinha and 4.61% on INTA PUITÁ CL, in Taquari. Family *Ascidae* showed great richness, with four species, *Lasioseius* sp., *Lasioseius* sp., *Proctolaelaps* sp. and *Cheiroseius* sp.. Schizotetranychus oryzae Rossi de Simons (86.65%) was the phytophagous mite more abundant, while among the predators *Neoseiulus paraibensis* (Moraes and McMurtry) (6.88%) stood out. The population peaks of *S. oryzae* and *N. paraibensis* happened on March 2011. In all evaluated areas, the correlation between *S. oryzae* and *N. paraibensis* was positive, specially on IRGA 424-120 (r = 0.93, P = 0.006).

Key words: Acari, *Schizotetranychus oryzae*, *Neoseiulus paraibensis*, biodiversity.

1. Introduction

Rice (*Oryza sativa* L.) is an annual grass belonging to *Oryza* genre, which includes 20 wild species and two tamed: *O. sativa* (Asiatic Rice) and *O. glaberrima* (Steud.) (African rice) in the world. *Oryza sativa* is adapted to aquatic environment, being grown worldly and occupies the second place as more cultivated cereal. Their domestication occurred about 10,000 years ago in Asia region [1-4].

Brazil is the ninth world producer, with irrigated system, specially in South region, responsible for 63% of national production. In Rio Grande do Sul state, the irrigated cultivated areas are distributed follows from the region: South (15.60%), Campaign (16.36%), Inner Coastal Plain (12.87%), External Coastal Plain (11.68%), Central Depression (15.36%) and West Border (28.13%). In this state, the crops use great areas and 27% of them have more than 400 ha [5]. Its production is forward to inner market, but, recently, the culture area has been increased with the exportation for other countries [6]. However, with its transformation like an international commodity enhanced the production requirement with better quality, productivity and process.

The PUITÁ INTA CL cultivar was developed by Argentina’s National Institute of Technology (INTA) and has its genetic base from IRGA 417 cultivar. It was selected because of resistance to herbicides of imidazolinone group from populations generated by induced mutations, being in this way a non-transgenic cultivar [7]. This mutagenic cultivar provides resistance to herbicide that controls the red rice.

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The IRGA 424 cultivar, especially produced in colder regions, as South Zone and Campaign, highlighted by high grains productivity and quality. This one was the first cultivar developed by IRGA genetic improvement program, in Santa Vitória do Palmar Station, where it demonstrated superior performance in the trials.

In Brazilian rice cultures phytophagous mites of families Tarsonemidae and Tetranychidae has been reported. Among the tarsonemid mites *Steneotarsonemus furcatus* (De Leon) found in Mato Grosso do Sul state [8]. The development of this species is on meristematic region of the fruit, which is covered by the perianth [9-11]. Species *Steneotarsonemus spinki* smiley, which is not reported in Brazil, stands out in world culture [12]. It causes significant damage to rice culture in China, Taiwan and Tropical Asia. In 1990 decade it was identified in Cuba and Dominican Republic rice culture [6].

Three tetranychids species are reported in Brazilian rice culture, as follows: *Aponychus schultzi* (Blanchard), *Oligonychus grypus* Baker & Pritchard and *Schizotetranychus oryzae* Rossi de Simons. *Schizotetranychus oryzae*, has been described of the specimens from Corrientes, Argentina and Rio Grande do Sul, Brazil [13]. In Brazil, there are quote from the states of São Paulo [14], Rio de Janeiro and Espírito Santo [15]. So far, there is no information about their ecology in the country and the damage intensity caused. The plants attached appear to nitrogen deficiency and show little yellowish-white areas, elongated, visible on upper surface and can be confused with viruses on leaves. These areas correspond to colonies of phytophagous mites on abaxial surface. The mites are protected by silk webbing that they produce and, in the course of time, the web coalesce producing white stripes along of the vein [15].

Phytoseiid mites are one of the important natural enemies of tetranychid mites [16]. According to feeding behavior and prey consumed, the phytoseiid mites were classified by McMurtry and Croft [17] in four categories. *Neoseiulus* species belong to type II that holds selective generalist predators. This species can survive when the main prey is low, searching to feed on other species or even by cannibalism. *Neoseiulus paraibensis* (Moraes and McMurtry) is a predator commonly associate to rice culture in several countries. It was described from females collected on *Musa* spp. L. and male on *O. sativa* in Northeast of Brazil. It was reported on rice culture in Colombia [18] and Cuba where it was associated to *S. spinki* [19]. Recently, it has been also associated with the same phytophagous species in Panamá [20].

So far, few are known about *S. oryzae* and *N. paraibensis* associations. The aim of this study is to identify the mite fauna associated to rice culture and to study the mite ecologic interaction, harvest 2010-2011, in Taquari and Cachoeirinha counties, state of Rio Grande do Sul, Brazil.

2. Materials and Methods

The mite population was biweekly evaluated between December 2010 and March 2011 in four rice areas in the municipality of Taquari and Cachoeirinha where 20 plants/area were randomly sampled. In Taquari IRGA 424 cultivar, grown with 103.5 kg urea and 126.5 kg fertilizer per hectare and INTA PUITÁ CL cultivated with 200 kg urea and 150 kg fertilizer per hectare. In Cachoeirinha, two different fields with IRGA 424, grew with 27 kg urea and 33 kg fertilizer per hectare, and other with 54 kg urea and 66 kg fertilizer per hectare.

Counting was carried out directly on the both sides of leaves by means of stereomicroscop. Mites were collected with fine paintbrush and stored in alcohol 70% for mounting and identification. All mites were mounted at Hoyer's medium.

The following dichotomic keys were used to identify the collected mites: Ascidae [21, 22], Cunaxidae [23], Histiostomidae [24], Diptilomiopidae [25], Phytoseiidae [16] and Tetranychidae [26]. The confirmation of *Tarsonemidae* species has been realized by Dr. Antonio Carlos Lofego, Universidade Estadual Paulista (UNESP) and the oribatid mites by Dra. Lucille Kriger Antony, Pedobiology Laboratory of the National Institute of Amazonian Research (INPA).

Representative specimens of each species were deposited on the mites reference collection of the Museum of Natural Science at UNIVATES University Center (ZAUMCN), Lajeado, Rio Grande do Sul.

The species Constance was classified as Constant (C > 50%), Accessory (25% < C < 50%) and Accidental (C < 25%) [27]. The Dominance (D) was defined by the formula: $D\% = (i/t) \times 100$, where, i = total number of individuals of a species and t = total of the individuals collected and clustered according to categories: eudominant (≥ 10%), dominant (5 ≤ 10%), subdominant (2 ≤ 5%), eventual (1 ≤ 2%) and rare (D < 1%).

The Pearson correlation index [28] has been applied to verify the correlation between *S. oryzae* and *N. paraibensis* and also correlation between biweekly abundance of *S. oryzae* and precipitation, temperature and relative humidity. Climate parameters were provided by UNIVATES University Center Meteorological Station, Lajeado, Rio Grande do Sul.

3. Results

A total of 1,626 mites belonging to 14 species from 12 families were collected (Table 1). Specimens of the families Ascidae, Cheyletidae, Cunaxidae, Diptilomiopidae, Histiostomidae, Iolinidae, Phytoseiidae, Scheloribatidae, Tarsonemidae, Tetranychidae and Tydeidae were collected. Also immature species of super family *Brachystonoidea* were found. Of the total specimens collected, 34.56% was on IRGA 424, in Taquari, 32.47% on IRGA 424-120 and 28.35% on IRGA 424-60, in Cachoeirinha and 4.61% on INTA PUITÁ CL, in Taquari.

Family Ascidae showed great richness, with four species followed by Tetranychidae, with two species. *Schizotetranychus oryzae* (86.65%) was the phytophagous mite more abundant, while among the predators *Neoseiulus paraibensis* (6.88%) stood out and *Tarsonemus bilobatus* Suski (3.25%) was the generalist species more abundant. The sampling has been enough, because the collector curve show most areas reached stability in fifth collect, except in IRGA 424-120, whose stability did not observe until the sixth sampling (Fig. 1).

In Taquari region, *N. paraibensis* was constant and subdominant, *S. oryzae*, constant and eudominant and *Tarsonemus bilobatus* accidental and subdominant on IRGA 424 (Table 1). The other species were accessory, accidental or rare. *Schyzotetranychus oryzae* and *N. paraibensis* have had the population peak on march, with 20 mites/plant and 0.65 mites/plant, respectively (Fig. 2). On INTA PUITÁ CL cultivar, only *N. paraibensis* has been constant and eudominant; *S. oryzae* accidental and dominant. The population peak of *S. oryzae* and *N. paraibensis* also happened on march with 0.25 and 1.40 mites/plant, respectively (Fig. 2). In both areas the evaluated ecologic indices were low, but on INTA PUITÁ CL superiors (Table 2).

In Cachoeirinha, *N. paraibensis* was accessory and subdominant on IRGA 424-60; *S. oryzae*, was constant and eudominant; *Tarsonemus bilobatus* accessory and subdominant. *N. paraibensis* showed population peak on February, with 0.25 mites/plant and *S. oryzae* on march, with 10.60 mites/plant (Fig. 3). In IRGA 424-120 cultivar, *N. paraibensis* was constant and subdominant; *S. oryzae*, constant and eudominant; *Tarsonemus bilobatus*, constant and dominant. *Schyzotetranychus oryzae* and *N. paraibensis* population peak happened on March, with 10.25 mites/plant and 0.25 mites/plant, respectively. In both areas evaluated ecologic indices were low, but higher on IRGA 424-120 (Table 2).

In all studied areas the correlation between the population of *S. oryzae* and *N. paraibensis* was

e 1 Mite fauna associated to rice plants of IRGA 424 and INTA PUITÁ CL cultivars, harvest 2010-2011, in the municipalities of Taquari and Cachoei nde do Sul, Brazil.

y	GENRE-SPECIES	Taquari														Cachoeirinha														TO
		IRGA 424							INTA PUITÁ CL							IRGA 424-60							IRGA 424-120							
		2010	2011						2010	2011						2010	2011						2010	2011						
		XII	I		II	III		C* D**	XII	I		II	III		C D	XII	I		II		III	C D	XII	I		II		III	C D	
		A 1	A 1	A 2	A 1	A 1	A 2		A 1	A 1	A 2	A 1	A 1	A 2		A 1	A 1	A 2	A 1	A 2	A 1		A 1	A 1	A 2	A 1	A 2	A 1		
ostomidae	*Histiostoma* sp.	-	-	-	-	1	-	A R	-	-	-	-	1	-	A Ev	-	-	-	1	-	-	A R	-	-	-	-	3	-	A R	6 (
	Lasioseius sp.1	-	-	-	-	-	1	A R	-	-	-	-	1	3	AcD	-	-	-	-	1	2	Ac R	-	-	-	1	1	4	Ac Ev	14
ae	*Lasioseius* sp.2	-	-	1	-	-	-	A R								-	-	-	-	-	1	A R								2 (
	Proctolaelaps sp.																						-	-	-	-	-	3	A R	3 (
	Cheiroseius sp.								-	-	-	-	1	-	A Ev															1 (
seiidae	*Neoseiulus paraibensis*	-	-	-	2	13	11	C S	-	1	5	-	28	22	C E	-	-	-	5	3	3	Ac S	-	-	-	4	4	11	C S	11
etidae	Immature																						-	-	-	-	-	2	A R	2 (
xidae	*Neocunaxoides rykei*	-	-	-	1	-	-	A R																						1 (
omiopidae	-	-	-	-	1	-	-	A R																						1 (
nychidae	*Schizotetranychus oryzae*	-	-	3	21	77	416	C E	-	-	-	-	5	-	A D	-	-	5	13	159	249	C E	-	-	1	-	115	345	C E	14
	Oligonychus sp.																						-	-	1	-	-	-	A R	1 (
nemidae	*Tarsonemus bilobatus*	-	-	1	13	-	-	Ac S	-	-	-	-	-	1	A Ev	-	-	-	6	2	3	Ac S	-	-	15	7	1	4	C D	53
dae	*Pronematus anconai*															-	-	-	2	2	1	Ac Ev	-	-	-	1	2	1	Ac R	9 (
dae	Immature															-	-	-	-	1	-	A R								1 (
oribatidae	Species 1								-	-	2	-	2	2	C D	-	-	-	-	-	2	A R	-	-	-	-	1	-	A R	9 (
ystonoidea	Species 1								-	-	-	-	-	1	A Ev								-	-	-	-	-	1	A R	2 (
dance		-	-	5	37	91	428		-	1	7	-	38	29		-	-	5	27	168	261		-	-	17	13	127	216		
ness		-	-	3	5	3	3		-	1	2	-	6	5		-	-	1	5	6	6		-	-	3	3	7	4		1,6
		562 (34.56%)							75 (4.61%)							461 (28.35%)							528 (32.47%)							

notes: A = Sampling. *C = Constance; C= Constant, Ac= Accessory, A = Accidental, ** D = Dominance; E= Eudominant, D= Dominant, S= Subdominant, Ev = Ev

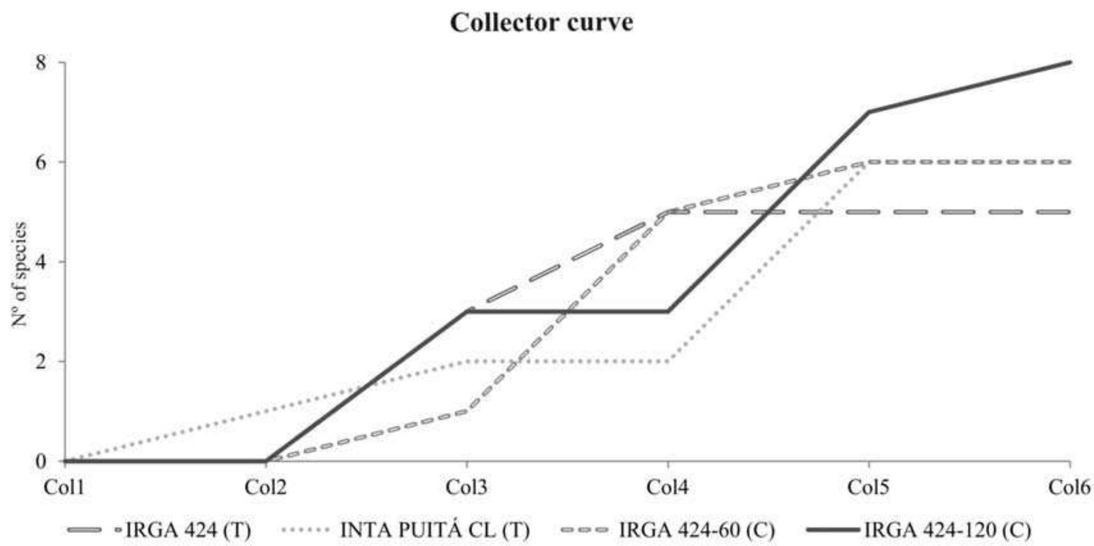


Fig. 1 Collector curve showing sampling effort carried out in rice culture of IRGA 424 and INTA PUITÁ CL in Taquari (T), IRGA 424-60 and IRGA 424-120 in Cachoeirinha (C), harvest 2010-2011.

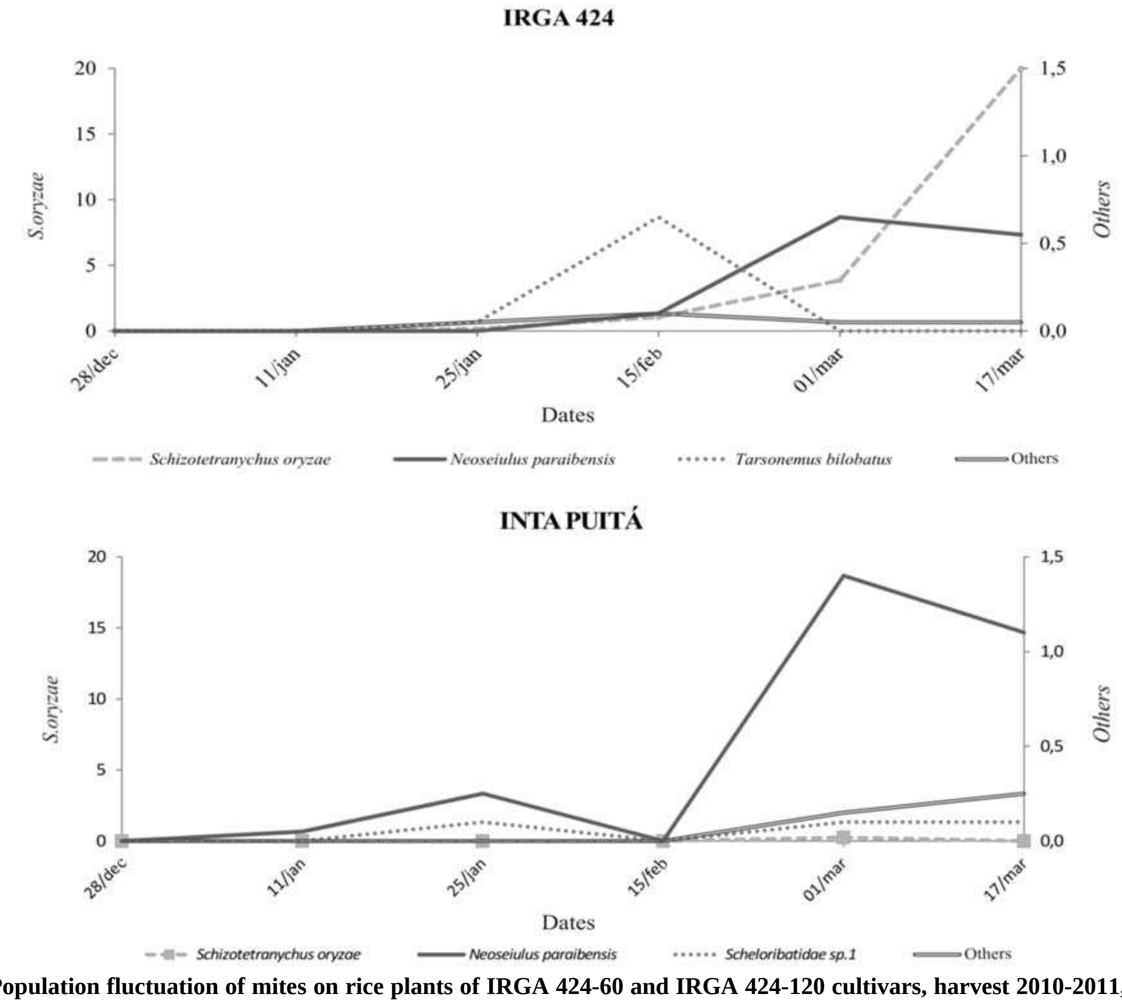
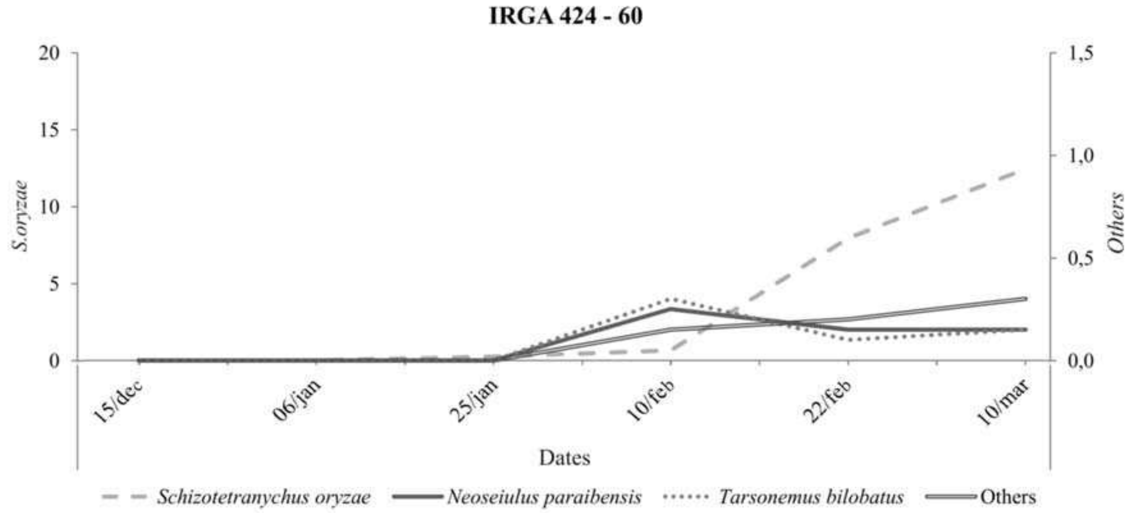


Fig. 2 Population fluctuation of mites on rice plants of IRGA 424-60 and IRGA 424-120 cultivars, harvest 2010-2011, in the municipality of Taquari, state of Rio Grande do Sul, Brazil.

Table 2 Shannon-Wiener (H') diversity and J-Shannon evenness in rice culture of IRGA 424 and INTA PUITÁ CL cultivars, in Taquari county, and IRGA 424-60 and IRGA 424-120, in Cachoeirinha county, harvest 2010-2011, state of Rio Grande do Sul, Brazil.

Indices	Taquari		Cachoeirinha	
	IRGA 424	INTA PUITÁ CL	IRGA 424-60	IRGA 424-120
H'	0.1295	0.4288	0.1722	0.2578
Evenness of J-Shannon	0.1434	0.4748	0.1805	0.2475



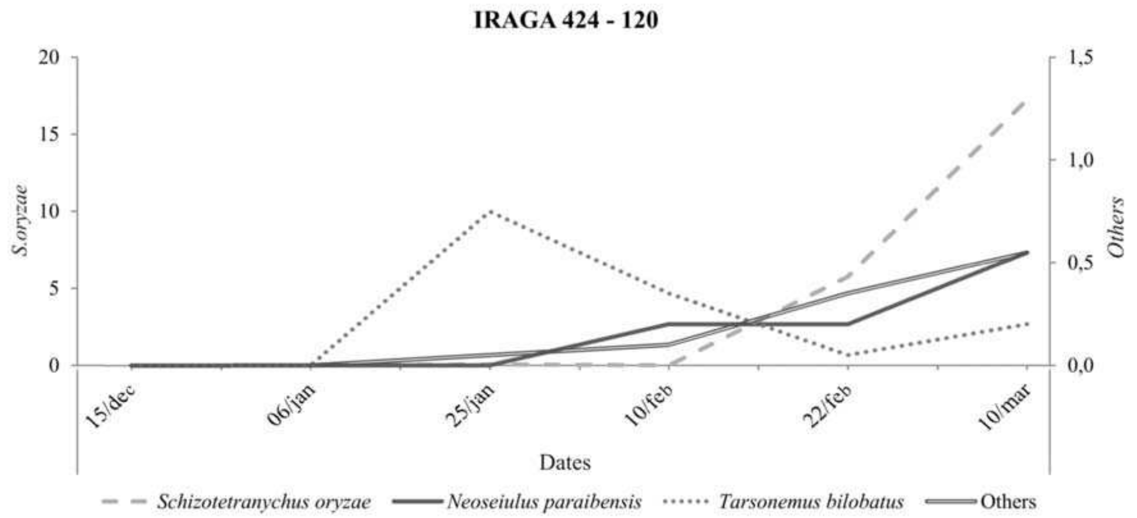


Fig. 3 Population fluctuation of mites on rice plants of IRGA 424-60 and IRGA 424-120 cultivars, harvest 2010-2011, in the municipality of Cachoeirinha, state of Rio Grande do Sul, Brazil.

Table 3 Correlation among *Schizotetranychus oryzae,* climate information and *Neoseiulus paraibensis* on rice cultivars, in the municipality of Taquari and Cachoeirinha, Rio Grande do Sul, on harvest 2010-2011.

Cultivar	Precipitation	Temperature	RH	*Neoseiulus paraibensis*
IRGA 424	r = -0.30, P = 0.55	r = -0.92, P = 0.001	r = -0.01, P = 0.97	r = 0.68, P = 0.13
INTA PUITÁ CL	r = 0.001, P = 0.99	r = -0.20, P = 0.69	r = 0.54, P = 0.26	r = 0.73, P = 0.09
IRGA 424-60	r = -0.28, P = 0.53	r = -0.26, P = 0.56	r = -0.02, P = 0.95	r = 0.44, P = 0.37
IRGA 424-120	r = -0.35, P = 0.49	r = -0.40, P = 0.43	r = -0.28, P = 0.58	r = 0.93, P = 0.006

positive, being significant only on IRGA 424-120 (r = 0.93, P = 0.006). About the climatic factors and *S. oryzae*, the correlation was negative in the majority evaluated areas, but not significant (Table 3).

4. Discussions

The mite fauna associated to rice culture was unlike in two evaluated regions, being identified only *Schizotetranychus oryzae*, *Neoseiulus paraibensis*, *Histiostoma* sp., *Lasioseius* sp. and *Tarsonemus bilobatus* as ubiquitous mites.

The present study highlights *Schizotetranychus oryzae* as the mainly phytophagous mite in rice culture in the state of Rio Grande do Sul, with populations increasing since the second half of February, reaching highest populations in the harvest, on March. This species, endemic of South America, was described from specimens collected in rice culture of Rio Grande do Sul state, Brazil and Corrientes province, Argentina [13]. It was already reported in Colombia, Cuba, Panamá and Venezuela flooded rice [18-20]. The plants attached by *Schizotetranychus oryzae* show small yellowish-white areas visible where are found the mite colonies on adaxial surface of leaves. These features are similar to those showed by Barcellos [15]. This phytophagous species would be the keystone species in this agroecosystem, being that his removal would cause population decline or change in abundance and species composition associated with this environment [29]. This phenomenon is observed on INTA PUITÁ CL where low richness and abundance occurred when a low population of *S. oryzae* has been observed. Meantime, in this survey this species did not reach to pest level.

Among predator mites, phytoseiidae had higher abundance and the ascidae higher richness. *Neoseiulus paraibensis* was the only phytoseiid species found and always associated to *S. oryzae* populations. On IRGA 424-120, the populations were higher and the association was meaningful. Higher abundance has been noted on INTA PUITÁ CL with less *S. oryzae* population. This can be correlated with management of the area, because this cultivar is resistant to herbicide that controls red rice. Other hypothesis would be that this management would optimize the action of *N. paraibensis* to control *S. oryzae*. To date, *N. paraibensis* has been reported just associated to rice culture [18-20] or in adjacent areas [30]. Owing to this fact, it is possible that *N. paraibensis* has close association with *S. oryzae*. Similar event has been described by Saitô [31] between *Typhlodromus bambusae* Ehara and *Schizotetranychus celarius* (Banks) on bamboo, where *T. bambusae* showed to be a specific predator of *S. celarius*. However, further studies need to be developed to verify the hypothesis that *N. paraibensis* has co-evolutionary association to *S. oryzae*.

Higher abundance of *Tarsonemus bilobatus* has been noted on IRGA 424-120, in Cachoeirinha, but was not observed significant damage on the plants evaluated. This fungivorous tarsonemid is widespread and reported from various regions of the world. It is known to cause injury to several flowers and seedlings of melon, watermelon and cucumber [32]. On seedlings of cucumber the symptoms are lustrous, discolored and deformed leaves with irregular folding of the upper surface.

According to the obtained results in this survey, *S. oryzae* is the phytophagous mite more important on rice cultures of the state of Rio Grande do Sul, being *N. paraibensis* the predator mite more common, also were noted strong evidences of association between these species. Meantime, lack studies to confirm this hypothesis. In view of the high abundance of this phytophagous mite in this research and the lack of information about this occurrence in other Brazilian productive regions, highlights need to do more studies in rice irrigated culture in this country.

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References

[1] G.S. Khush, Origin, dispersal, cultivation and variation of rice, Plant. Mol. Biol. 35 (1997) 25-34.

[2] E.A. Heinrichs, Management of Rice Insect Pests. Rad. IPM World Textbook, Minnesota, 1998.

[3] C.N.B. Bambaradeniya, F.P. Amerasinghe, Biodiversity associated with the rice field agro-ecosystem in Asian countries: A brief review, Int. Water Manag. Inst. 63 (2003) 24.

[4] C.L. Silva, L.L. Foloni, L.C. Paraiba, L.P.M. Plese, Environmental forecasting the distribution of pesticides applied in rice culture, Rev. Ecotox. M. Amb. 17 (2007) 75-86. (in Portuguese)

[5] N.R.A Vieira, A.B Santos, E.P Santana, The rice culture in Brazil, Embrapa Arroz e Feijão, Santo Antônio de Goiás, 1999. (in Portuguese)

[6] R.S Mendonça, D. Navia, R.I Cabrera, *Steneotarsonemus spinki* Smiley 1967 (Acari, Prostigmata:Tarsonemidae)—A threat to rice culture in Brazil, Embrapa Rec. Gen. Biotec., 2004, p. 48. (in Portuguese)

[7] Rice Planet, by the front door, Rev. Planet. Árvore. 28 (2008) 19-20. (in Portuguese)

[8] D. Navia, M.A.S. Mendes, R. Ochoa, *Steneotarsonemus furcatus* De Leon (Prostigmata: Tarsonemidae) infesting rice crops in Brazil, Int. J. Acarol. 32 (2) (2006) 189-193.

[9] R. Ochoa, R.L. Smiley, J.L. Saunders, The family Tarsonemidae in Costa Rica (Acari: Heterostigmata), Int. J. Acarol. 17 (1) (1991) 41-86.

[10] R. Ochoa, H. Aguilar, C. Vargas, Acaros fitófagos de America Central: Guia ilustrada. CATIE Costa Rica, 1991, p. 251.

[11] K.C. Lo, C.C. Ho, Ecological observations on rice tarsonemid mite, *Steneotarsonemus spinki* (Acarina: Tarsonemidae), J. Agric. Res. China 28 (1979) 181-192.

[12] G.J. Moraes, C.H.W. Flechtmann, Manual of Acarology, Basic Acarology and Mites of Cultivated Plants in Brazil, Publisher Holos, Ribeirão Preto, 2008, p. 308. (in Portuguese)

[13] N.H. Rossi de Simons, Description of *Schizotetranychus oryzae* sp.n. (Acari, Tetranychidae), Rev. Investig. Agropec. 3 (1) (1966) 1-10. (in Spanish)

[14] C.J Rosseto, N. Silveira, D. Link, J. Grazia-Vieira, E. Amante, D.M. Souza, et al., Pests of rice in Brazil, in: Technical Contributions of the Brazilian Delegation to the 2nd Meeting of the Committee of the Rice International Rice FA.

[15] D.F. Barcellos, A.M. Oliveira, S. Sudo, A.B. Oliveira, C.H.W. Flechtmann, Occurrence of rice mite in the states of Rio de Janeiro and Espírito Santo. Pesq. Agropec. Brasil, Brasília 14 (2) (1979) 181-184. (in Portuguese)

[16] D.A. Chant, J.A McMurtry, Illustrated keys and diagnosis for the gener and subgenera of the Phytoseiidae of the world (Acari: Mesostgmata), Indira Publishing House, Michigan, 2007, p. 220.

[17] J.A. McMurtry, B.A. Croft, Life-styles of Phytoseiid mites and their roles in biological control, Annu. Rev. Entomol. 42 (1997) 291-321.

[18] G.J. Moraes, N.C. Mesa, A. Braun, Some phytoseiid mites of Latin America (Acari: Phytoseiidae), Int. J. Acarol. 17 (1991) 117-139.

[19] M. Ramos, G.J. Moraes, Predatory mites associated with *Steneotarsonemus spinki* (Acari: Tarsonemidae) on rice in Cuba, in: J.B. Morales-Malacara, V. Behan-Pelletier, E. Ueckermann (Eds.), in: Proceedings of the International Congress, Sociedad Latinoamericana de Acarología, México, 2007.

[20] E. Quirós-Mcintire, H. Rodríguez, Predatory mites associated to *Steneotarsonemus spinki* Smiley (Acari: Tarsonemidae) in Panamá, Rev. Protec. Veg. 25 (2) (2010) 103-107. (In Spanish)

[21] E.E. Lindquist, G.O. Evans, Taxonomic concepts in the Ascidae, with a modified setal nomenclature for the idiosoma of the Gamasina (Acarina: Mesostigmata), Mem. Entomol. Soc. Can. 47 (1965) 1-64.

[22] R.B. Halliday, D.E. Walter, E.E. Linquist, Revision of the Australian Ascidae (Acarina: Mesostigmata), Invertebr. Taxon. 12 (1998) 1-54.

[23] R.L. Smiley, The Predatory Mite Family Cunaxidae (Acari) of the World with a New Classification, Indira Publishing House, 1992, p. 356.

[24] A.M. Hughes, The mites of stored food and houses, 2nd ed., Technical Bulletin No. 9, Ministry of Agriculture, London, 1976, p. 400.

[25] J.W. Amrine, Keys to the world genera of the Eriophyoidea (Acari: Prostigmata), Indira Publishin House, West Bloomfield, Michigan, 1996, p. 187.

[26] E.W. Baker, D.M. Tuttle, A Guide to the Spider Mites (*Tetranychidae*) of the United States, Indira Publishing House, Michigan, 1994, p. 347.

[27] S. Silveira-Neto, O. Nakano, D. Barbin, N.A.V. Nova, Manual of insect ecology, Agronômica Ceres, São Paulo, 1976, p. 419.

[28] J.H. Zar, Biostatistical analysis, Prentice-Hall, Inc., New Jersey, 1999, p. 663.

[29] M.E. Power, D. Tilman, J.A. Estes, B.A. Menge, W.J. Bond, L.S. Mills, et al., Challenges in the quest for keystones, BioScience 46 (1996) 609-620.

[30] G.J. Moraes, J.A. McMurtry, Phytoseiid mites (Acarina) of Northeastern Brazil with descriptions of four new species, Int. J. Acarol. 9 (3) (1983) 131-148.

[31] Y. Saito, Life-history and feeding habit of *Typhlodromus bambusae*, a specific predator of *Schizotetranychus celarius* (Acari: Phytoseiidae: Tetranychidae), Exper. Appl. Acarol. 10 (1990) 45-51.

[32] Y.H. Tseng, Mites associated with weeds, paddy rice and upland rice fields in Taiwan, Acarology VI. Ellis Horwood, Chichester 2 (1984) 770-780.