African Journal of Agricultural Research

Volume 10 Number 41 8 October 2015

ISSN 1991-637X

Academic Journals

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African Journal of Agricultural Research

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Vol. 10(41), pp. 3860-3869, 8 October, 2015
DOI: 10.5897/AJAR2015.9956
Article Number: 997CE0155802
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Issues and constraints for emerging farmers in the Eastern Cape Province, South Africa

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Received 1 June, 2015; Accepted 12 August, 2015

The aim of the study was to investigate the main limiting factors that prevent emerging farmers from progressing from subsistence to commercial agricultural farming in the Eastern Cape Province. The study was conducted in the King William's Town area by means of a structured questionnaire survey. A sample of 50 households was drawn from the research area which was chosen owing to its uniqueness with regard to agricultural potential. A descriptive analysis technique was employed to investigate the main limiting factors faced by farming households in migrating towards commercial agricultural markets. The findings demonstrated that the specific limiting factors emerging farmers face are poor physical infrastructure such as poor roads, lack of transportation to the markets from the farms, lack of marketing skills and information, poor market infrastructure, and high transaction costs, insufficient land availability to expand production, lack of agricultural implements to better production, poor production and farm management skills, as well as low education levels which results in an inability to interpret market information to be used in production planning and marketing. The results from the study highlighted that the government has a crucial role to play in increasing market participation of emerging farmers through encouraging group marketing, upgrading of roads to enable smooth accessibility of farmers to output markets and the establishment of local point sales in farming rural areas. Finally the study recommended that government provides planned workshops to all farmers in order to equip them with marketing knowledge.

Key words: Emerging farmer, constraints, Eastern Cape Province.

INTRODUCTION

A number of studies have been conducted on the topic of commercialisation of emerging farmers with the aim of broadening the knowledge on the challenges that limit such migration from subsistence to commercial farming. Despite the valuable knowledge generated by these studies, there is still a remarkable scarcity of scientific information describing a more detailed picture of major challenges that affect emerging farmers. The South African government has in the past 18 years implemented several policies and programmes as well as increased the budget spent on the agricultural sector supporting emerging farmers (Department of Agriculture Forestry and Fisheries, 2010; Frequin et al., 2012; National Treasury, 2003, 2006, 2008, 2009, 2010; Aliber

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and Hall, 2012). However to-date there is inadequate evidence that these attempts had been successful (Frequin et al., 2012; National Treasury, 2003, 2006, 2008, 2009, 2010; Aliber and Hall, 2012). Indeed programmes and other attempts by government and development agencies have exacerbated rather than alleviated the difficulties emerging farmers face (Frequin et al., 2012). To-date emerging farmers still living below the poverty line are faced with difficulties in migrating into the commercial agricultural sector (Frequin et al., 2012; Aliber and Hall, 2012). The failure of several attempts by government to integrate emerging farmers into the commercial agricultural economy has increased the need for a well-grounded scientific knowledge and a thorough understanding of these challenges that emerging farmers' face.

A better understanding of specific factors that limit the development of emerging farmers is crucial in order to effectively prepare policies, development strategies, programmes and models aimed at supporting and enhancing the transition of emerging farmers into commercial agricultural farming. South Africa can no longer afford to run the risk of development programmes and policy intervention aimed at emerging farmers that do not work seemingly not because they are not working but because the challenges emerging farmers face were not correctly identified. Given the prominence of the topic the main objective of this article is to provide a scientific understanding of the challenges, issues and barriers emerging farmers face at transitioning into commercial farming as well as provide the relationship limiting factors have with the commercialisation of emerging farmers. Commercialisation of emerging farmers is a crucial topic for the current times in South Africa. The present study is primarily focused on the factors that limit the transition of emerging farmers in the King William's Town area from successfully participating in commercially viable markets. In the study it is assumed that commercialisation of subsistence agriculture implies increased participation in remunerative agricultural commercial output markets.

Issues and challenges for emerging farmers in South Africa

The South African agricultural economy has little room for emerging farmers. There is no strong support system available to support previously disadvantaged farmers (Chikazunga and Paradza, 2012), causing such farmers to be unable to take advantage of the various opportunities that the South African government has been instituting (Moloi, 2008; Anyike, 2011). According to a study by Chikazunga et al. (2012), South African agricultural economy grew rapidly under the previous South African government owing to strong state subsidies and support programmes aimed at supporting commercial farmers. Similar support programmes and state subsidies were seen as encouraging the agricultural economy of the United States of America and Europe (Chikazunga et al., 2012). Currently South African agriculture depends heavily on world markets for marketing agricultural products (Chikazunga and Paradza, 2012). The removal of marketing boards' state subsidies along with the de-regulation of the agricultural sector subsequent to the democratic transition in 1994 caused serious problems for commercial farmers in particular to previously disadvantaged farmers. By 1997 interest rate subsidies and export subsidies had ended completely and by late 1998 all marketing control boards were privatised with only the sugar industry continuing to have price support from the government (Chikazunga and Paradza, 2012).

Many emerging farmers face difficulties in accessing formal agricultural markets. As a result formal markets do not interest emerging farmers. Lack of market participation is a common feature of emerging farmers world-wide and is identified by Bie'nabe and Vermuelen (2011) as a constraint to emergent farmer development. In South African under-developed rural areas emerging farmers find it difficult to participate in commercial markets because of a range of constraints (Makura, 2001; Wynne, 2003). Attempts by farmers to market their commodity are mostly affected by poor infrastructure, inadequate property rights (Lyne, 2006), low education levels amongst the farmers, lack of credit access, absence of innovative production implements needed in-order to increase yield of commodity produced and poor entrepreneurial skills needed to make the efforts of the farmers a success (Bie'nabe and Vermuelen, 2011). Research conducted by the National Emergent Red Meat Producer's Organisation (2006) identified a number of skills shortages among emerging farmers such as a major constraint of growth. NERPO (2006) suggested that the new South African government must improve its efforts in attracting young people into the agricultural industry. Poor financial and social capital and limited access to legal resources make it difficult for emerging farmers to change negative market factors individually. As a result emerging farmers continue to be trapped in a cycle of operating within the given market from which their agricultural activities do not receive rewards (Makhura, 2003).

METHODOLOGY

It has been noted in the literature that some researchers use the term methods and methodology interchangeable (Hussey and Hussey, 1997:35). Many researchers consider methodology as an overall study approach that is undertaken and methods as various means by which data is collected and analysed. According to Mason (2002:30) the concept of methodology is separated from method. A method is part of the methodological strategy (Mason, 2003:30). Saunders et al. (2009:3) and Collins and Hussey (2009:11) explain methodology as the theory of how a research study should be undertaken and that it entails the study design and

the methods that are used for data collection and analysis.

The approach taken in this study is to include all aspects of the research process under the heading of methodology. Therefore the research design, the approach taken in this study, the type of data collection methods selected and the means of data analysis are all considered to be part of the study's methodology.

To ensure that the survey will get to the heart of the research problem and enable the researcher to answer the research questions a pilot study was conducted in Port Elizabeth, Rocklands and Uitenhange. The pilot study was also conducted to pre-test the questionnaires used for the present study data collection. The main survey to obtain the primary data for this study was conducted in King William's Town an area situated in the central region of the Eastern Cape Province. Agriculture in this area is the most used enterprise for household survival followed by operating shebbeens and taverns and the State grant. Most inhabitants in the identified area relied on farming for household survival. Data was collected using structured questionnaires. A survey of households provided much needed information on the demographics of the group of farmers such as their socio-economic characteristics. The survey involved 50 households selected in the research area.

In selecting a suitable representative sample the researcher followed a two-stage sampling process, in which the first stage involved selecting the survey area. This was followed by selecting the total number of emerging farmers engaged in agriculture in the area. It was found that the total number of agricultural households in the area is 43624 (Statistics South Africa, 2011). This consisted of mainly commercial farmers (Statistics South Africa, 2011). According to Statistics South Africa (2009), the country lacks information on smallholder and subsistence agriculture. The current list of farmers being used to conduct surveys is mainly confined to commercial agriculture. Until now agricultural censuses and surveys have largely concentrated on commercial agriculture and ignored small-scale and subsistence agriculture (Statistics South Africa, 2009, 2011). Thus a total number of subsistence farmers in the survey area could not be realized from Statistics South Africa or the Department of Agriculture.

The researcher established that emerging farmers in the area had formed associations as farmers. A list of emerging farmers' associations and the members of the associations was obtained from a non-government organization (NGO) aimed at assisting emerging farmers in the area. The researcher approached the chairman of the NGO and explained the objectives and the purpose of the study. No objections were made by the chairman. The researcher attempted to interview all the farmers on the list, but many farmers declined to participate in the study for a variety reason; some farmers were farming for household consumption where only surplus was marketed; some farmers were involved in other businesses, thus farming part-time; and some farmers were inactive owing to various constraints. These farmers could not participate in the study. The target group of the study was on emerging farmers that depend on the farm sources for household survival, farming full-time and producing mainly for the market. The researcher applied criteria of availability, willingness to cooperate and a sampling size of 50 was realised amongst those farmers available and willing to participate.

In general researchers prefer probabilistic or random sampling methods over non-probabilistic ones. They consider the former to be more accurate and rigorous. However, in applied social research there are circumstances where it is not feasible, practical or theoretically sensible to apply a random sampling method (Trochim, 2006a:361). A non-probabilistic method was used in the present study. According to Trochim (2006b:10) purposive sampling is one of the methods of non-probability sampling. It is approached with a specific plan in mind and targets a specific sample. The sampling procedure is further subcategorised as snowballing. In snowball sampling the researcher identifies the participants that meet the criteria for inclusion, and the participants are then asked to recommend others who they may know, who also meet the criteria for inclusion in the study. Although this method would hardly lead to representative samples, there are times when it may be the best method available (Trochim, 2006:10). In the case of the present study, farmers had formed associations as mentioned earlier, which made it easy for participants to recommend others as they knew each other well.

A questionnaire was used to collect data. According to Truckman (2000) and Van Niekerk (2002:35), questionnaire survey methods make it possible to measure what a person knows and the type of information he/she has, the values and beliefs of the person and the attitudes towards what the questionnaire is about. When conducting a questionnaire survey it is better to use an administered questionnaire for better results (Van Niekerk, 2002:36). The questionnaire survey can be used in three different ways namely: personal interviews, telephonic interviews and mail interviews (Randela, 2005:9). The present study made use of personal interviews because they enable the interviewer to observe behaviour that the questionnaire is not designed to detect. The questionnaire was relevant to the objectives of the study and the respondents involved in the study.

The questionnaire consisted of section A and section B. Section A contained questions on demographic characteristics and socioeconomic factors. While section B contained questions covering farming skills of the respondents and production and marketing challenges the respondents face. The questions in the questionnaire were designed in English but during the interviews they were translated in isiXhosa by the researcher, the language of the survey area and understood by the participants. The researcher understands that most of emerging farmers have low education levels and people express their views better when they express them in their own language. The respondents were asked to select the challenges that affect them the most from all the common challenges that were listed in the questionnaire. All data collected were based on the main factors affecting the migration of emerging farmers into commercial markets in the survey area.

Data analysis

The data collected from the questionnaire in this study was coded by the researcher by assigning a numerical value in order to facilitate easier workability on the SPSSx program version 21 and Statistica version 11. The Microsoft Office Excel 2010 software package was used to capture the coded data. This made it easier for the researcher to check for mistakes before analysing the data in SPSSx and Statistica. The Institute for Statistical Consultation at the Nelson Mandela Metropolitan University carried out the processing of the data, using the statistical Package for Social Science and Statistica, software used for statistical analyses. The percentages calculated were based on the total number of farmers who responded to that particular question. The farmers that did not respond to a particular question were excluded from the calculation of percentage values for that question. When a farmer selected more than one answer or gave more than one method to a question, percentages were calculated for each group of similar answers.

RESULTS

The study used data collected from a sample of 50 emerging farmers using a questionnaire survey. All 50 farmers were engaged in livestock rearing; about 30 of whom were also engaged in crop production. The information that follows result from a descriptive analysis

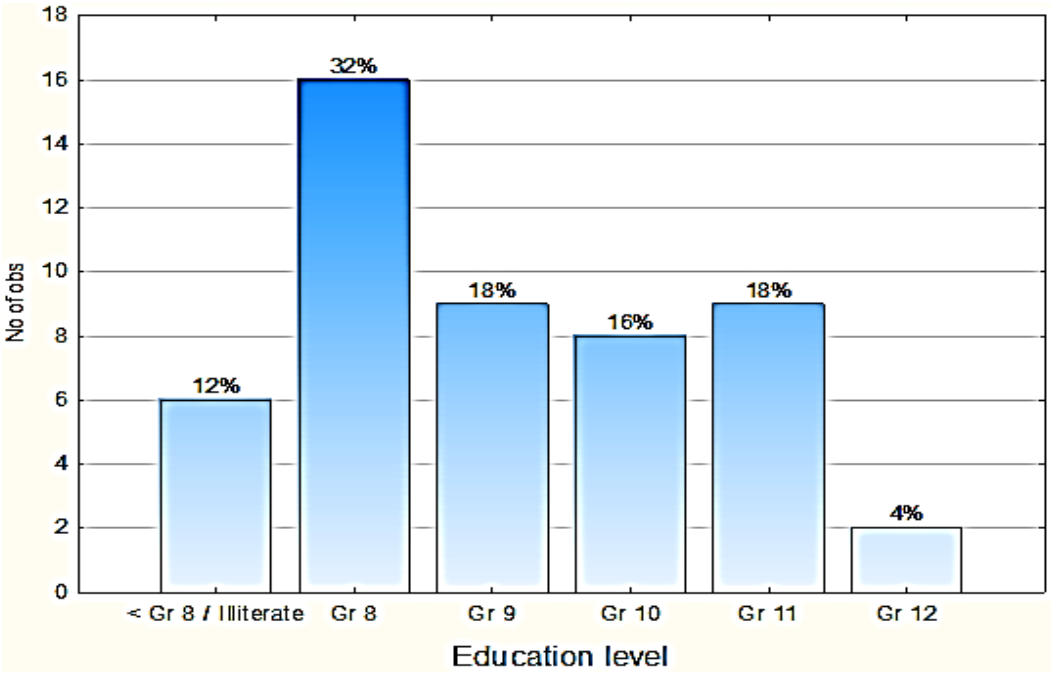

Figure 1. Distribution of farmers by education level. Source: Field survey 2013

of the data collected. The results are presented using descriptive statistics frequencies, counts, charts, percentages and standard deviations. All respondents were interviewed on their farms by appointment. Interviewing the respondents in their farms permitted the researcher to observe their farming areas. The respondents were asked to motivate their answers to verify whether they understood what they were being asked. In this regard all the results in the present study are assumed to be correct and valid.

Figure 1 shows the distribution of the farmers by their level of education. The education levels of the farmers were low. Figure 1 shows that 12% of the farmers had less than grade 8 school level; 32% had grade 8; 18% had grade 9; 16% had grade 10; 18% had grade 11; and 4% had completed grade 12. This means that 62% of the farmers had less than a grade 10 school level. None of the heads of households had tertiary education.

Farming skills

Twenty-six items were used to measure the level of adequacy of the farmers on farming and management skills necessary to operate crop and livestock production farms. The farmers were measured according to their line of production. Farmers solely engaged in livestock production were asked to respond to livestock production questions and management questions in the questionnaire only. The farmers that were also engaged in crop production were asked to respond to the livestock and crop production questions since they were also engaged in livestock production as well as the management questions. The questions were designed using four point Likert scale. Likert scale is a psychometric scale commonly used in researches which employs questionnaire as a survey instrument. After the questionnaires were completed each item was analysed and presented separately. In this study experience is equal to knowledge. It is assumed that if a farmer has knowledge of a certain aspect it will be easier to find experience along the way. Similarly if a farmer has experience about certain aspects then the farmer will have knowledge on that experience.

Crop production skills

Twelve items were used to measure the level of adequacy of the respondents on crop production skills necessary to operate a crop production enterprise. The results are shown on Table 1. For most of the items the farmers perceived their level of adequacy to be inadequate or they did not know. The farmers said that all the skills listed were important for them to know as farmers and knowing them would contribute towards their development to commercial farming. The farmers further claimed that given an opportunity to learn the skills they would. Limited knowledge as well as lack of skills in crop production among farmers constrains crop production, particularly in small scale irrigation systems. Table 1 shows the distribution of the sampled farmers experience

Table 1. Distribution of farmers experience on crop production skills.

Farming skills (N=30)	Level of adequacy %			
	Do not know	Inadequate	Adequate	Outstanding
Mulching skill	57% (17)	33% (10)	7% (2)	3% (1)
Seed bed and care skill	-	47% (14)	50% (15)	3% (1)
Soil preparation skill	3% (10)	13% (4)	73% (22)	3% (1)
Tillage skill	83% (25)	17% (5)	-	-
Transplant skill	30% (9)	36% (11)	27% (8)	7% (2)
Irrigation skill	57% (17)	43% (13)	-	-
Pest control skill	90% (27)	10% (3)	-	-
Weed control skill	20% (6)	57% (17)	23% (7)	-
Knowledge of fertilizers	17% (5)	37% (11)	47% (14)	-
Harvesting planning skill	37% (11)	37% (11)	27% (8)	-
Packing skill	63% (19)	30% (9)	7% (2)	-
Grading skill	83% (25)	13% (4)	3% (1)	-

Source: Field survey, 2013.

Table 2. Distribution of farmers experience on livestock farming skills.

Management skills (n)=50	Level of adequacy, %			
	Do not know	Adequate	Inadequate	Outstanding
Labour management skill	8% (4)	66%(33)	20% (10)	6% (3)
Equipment maintenance skill	12% (6)	38%(19)	38% (19)	12% (6)
Banking skill	10% (5)	66%(33)	18% (9)	6% (3)
Entrepreneurial skill	20% (10)	68%(34)	8% (4)	4% (2)
Bookkeeping or recordkeeping (financial management)	10% (5)	56%(28)	22% (11)	12% (6)
Marketing skill	10% (5)	74%(37)	14% (7)	2% (1)
Farm planning skill	52% (26)	40%(20)	6% (3)	2% (1)

Source: Field survey (2013).

on skills need for successful crop production.

Livestock rearing skills

Seven items were used to measure the farmers' level of experience in livestock skills necessary to operate a livestock enterprise. The results are shown in Table 2. For most of the items farmers perceived their level of experience to be either no experience or inadequate experience. Calf rearing is an important activity on a livestock farm because young calves are the future of the herd. Well reared healthy calves produce high yielding healthy adults. The majority of the sampled respondents namely 44% had no experience in calf rearing and 52% had inadequate experience in calf rearing while 2% had adequate experience in calf rearing. The majority of the farmers 48% claimed not to plan for the weaning of their calves, 46% had adequate experience in calf weaning and 6% had outstanding experience in calf weaning and claimed to plan for weaning of their calves as well as to keep records of everything such as the mother of the calf and the father.

The majority of sampled households were not able to identify sick animals, diagnose them or solve the health problem prior to it becoming a severe condition. The respondents relied mostly on other neighbouring farmers and animal technicians that rarely visit the farms. However they were able to recognise animals with foot-rot and mastitis. The hygiene of livestock and the farm helps to prevent disease build up and outbreaks among livestock. Calf pneumonia can be minimised by cleaning the houses where calves are kept. Hygienic feeding equipment helps to prevent the transmission of disease. Trimming and cleaning hooves of livestock help to prevent foot rot disease among livestock. The majority of the farmers (98%) claimed not to be informed about animal and farm hygiene and as a result it was not a concern of theirs. The majority of farmers had no systematic breeding programme or approach they were

Table 3. Distribution of farmers by experience on management skills.

Farming skills (n=50)	Level of adequacy %			
	Do not know	Inadequate	Adequate	Outstanding
Calf rearing skill	44% (22)	52% (26)	2% (1)	2% (1)
Weaning skill	48% (24)	-	46% (23)	6% (3)
Animal health skill	2% (1)	46% (23)	46% (23)	6% (3)
Animal hygiene	42% (21)	52% (26)	6% (3)	-
Livestock breeding skill	14% (7)	60% (30)	24% (12)	2% (1)
Artificial insemination skill	88% (44)	8% (4)	4% (2)	-
Animal nutrition skill	10% (5)	20% (10)	62% (31)	8% (4)

Source: Field Survey (2013).

Table 4. Marketing challenges faced by farmers.

Description (n=50)	Challenging factor	Not a challenging factor
Insufficient marketing facilities, such as sale pens and loading ramps	100% (50)	-
Long distance from farms to markets	100% (50)	-
Insufficient market information	100% (50)	-
Physical infrastructure such as poor roads to markets and lack of storage facilities	100% (50)	-
Changes in the markets such as price changes in relation to supply and demand	92% (46)	8% (2)
Transaction costs	94% (47)	6% (3)
Cheap food imports from other countries	98% (49)	2% (1)

Source: Field Survey (2013).

following for breeding their livestock. The farmers had no experience or inadequate experience of breeding skill. With artificial insemination skill farmers can breed their own desired breeds. This is a cheaper method than buying breeds from certified breeders. A total of 88% of the farmers claimed not to have experience on artificial insemination skill; 8% perceived their level of experience on artificial insemination skill to be inadequate and 4% of the farmers perceive their level of experience in artificial insemination skills to be adequate. The following table shows the distribution of the farmer's experience on skills necessary for livestock production.

Management skill

Seven items were used to measure the level of adequacy of the sampled farmers in different farming management skills necessary to operate a livestock or crop production farm. The frequencies for these items are shown in Table 3. For most of the items the farmers perceived their experience to be inadequate or adequate. However, there were some farmers with no knowledge in certain management skills although some farmers had outstanding experience in certain management skills. The farmers agreed that all the management skills listed were very important to them and knowing them could contribute towards the success of their farms.

Marketing challenges

Seven items were used to identify the marketing challenges faced by the farmers. The frequencies for the items are shown in Table 4. The findings showed that the sampled farmers are faced with marketing challenges such as insufficient market facilities, scale pens and loading ramps for livestock farmers, insufficient market information, low prices, cheap food imports coming from other countries and high transaction costs. Even farmers who managed to produce products of good quality were not realising good profits from their produce owing to insufficient markets being available. The harvests of the farmers were lost after harvesting because of spoilage. Many of the farmers tried to sell their produce to big supermarkets but were told that their produce does not meet the requirements specified by the supermarkets and lack certificate for Good Agricultural Practises. The following table shows the distribution of marketing challenges that mostly affect the sampled farmers.

Transportation costs

Amongst the marketing challenges the farmers face were high transportation costs. Marketing transport is important as it links the farmers to the markets or consumers on time. The availability of one's own market transport

Table 5. Costs of transportation per annum.

Varibles	Count	Percentage
R0 - R1000	1	2.00
R1001 - R2000	1	2.00
R2001 - R3000	5	10.00
R3001 - R4000	13	26.00
R4001 - R5000	8	16.00
Above R5000	22	44.00

Source: Field Survey (2013).

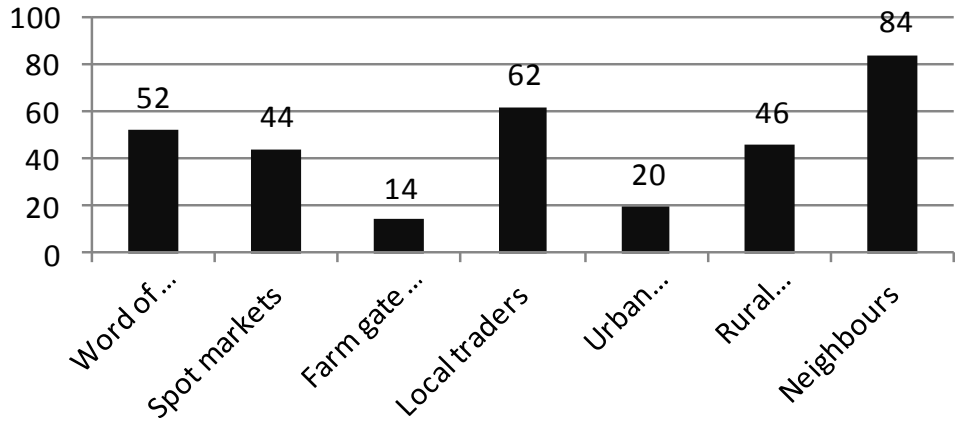

Figure 2. Distribution of marketing channels used by farmers. Source: Field Survey (2013).

influences the delivery time of produce to the markets, unlike the case of farmers who depend on hired transport or public transport to transport their produce. Transport availability determines the quality of the delivered produce. Unreliable transport can lead to the late delivery of produce. In the case of emerging farmers who lack storage facilities late delivery of produce can result in loss of produce quality and rendering the producer unreliable to the buyer. Table 5 shows the transportation cost of emerging farmers per year. The majority of the farmers were spending between R 3000 to above R 5000 on transportation per annum.

Marketing channels

Emerging farmers use different types of marketing channels to market their produce. Each marketing channel has associated costs such as transportation costs, profits and prices of produce. Before choosing a marketing channel a farmer has to consider these costs. The farmers' choice of marketing channel can pose problems and result in lower earnings. In general, the income of the farmer can be determined by the choice of marketing channel used. The farmers claimed to be using more than one marketing channel. In that case they were allowed to choose more than one answer from the questionnaire. The results were treated separately and are presented separately in Figure 2. Therefore the percentages in Figure 2 are expected not to add up to 100% as one answer was chosen more than once from the questionnaire. The majority of the sampled farmers appeared to be using informal markets to market their commodity, such as neighbours, rural consumers, farm gates and urban spot markets.

Poor access to market information

As shown in Figure 3 the majority of the sampled farmers (55%) did not have access to market information. Such farmers are unlikely to participate in marketing because they are not well informed of what is happening in the markets. The farmers were not well informed of market prices, products in supply or the products in demand. Only 45% of the farmers had access to market information. The majority of the farmers with access to market information relied on family members, self-research and other farmers for market information. The farmers claimed that the information was not timely, was

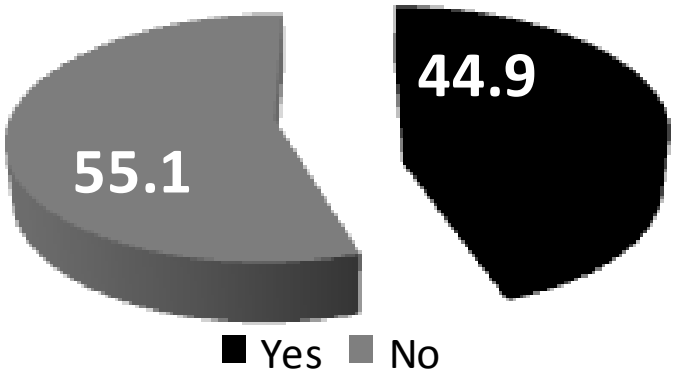

Figure 3. Distribution of farmers with access to market information. Source: Field survey (2013).

Table 6. Support services received by farmers.

Support received		
	Count	**Percentage**
Yes	32	64
No	18	36

Source: Field Survey (2013).

sometimes biased and was unreliable making the usefulness of the information doubtful.

Support services

The provision of support services remain one of the major important interventions in the agricultural sector for rural development, commercialisation, food security, poverty alleviation and income generation of emerging farmers. The commercialisation of emerging farmers cannot be achieved without appropriate farmer support services. With adequate access to farmer support services, emerging agriculture can contribute to an increased agricultural growth, rural development and have a positive impact on the farm income. When the respondents were asked about support services various answers were given. The majority of the sampled farmers namely 64% claimed to be receiving support services for their farming enterprises (Table 6) while 36% claimed not to be receiving any support service but had to rely on their own resources. When the farmers' were asked about extension services stated that the extension officer visited them once in a while. None of them could recall the visit routine.

The availability of land by the farmers seems to be also a concern in market participation (Figure 4). It is important that farmers have enough land to produce if they are to participate in commercial agricultural markets. The study found that 72% of the farmers are producing on land less than 10 ha. Only 28% of the farmers are producing on land more than 10 ha. This demonstrates that insufficient land availability in South Africa is still a challenge that many emerging farmers face. This has negative implications for sustainability and farm income, especially for livestock emerging farmers who depend on the availability of land for grazing and expansion of livestock production.

DISCUSSION

The emergent agricultural sector in South Africa has the potential to contribute to the growth of rural areas, and the reduction of unemployment, poverty and inequalities. The potential of emerging farmers to participate in this sector is untapped. Emerging farmers do not participate in markets that yield high returns. In order for emerging farmers to contribute to rural development and transit into the commercial farming sector the above mentioned aspects need to be addressed effectively. The main objective of the present study was to identify the challenges that prevent the commercialisation of emerging farmers into commercial agricultural markets. The study identified seven specific challenges to emerging farmers in the area. These were low education levels, lack of farming skills on crop and livestock production, poor management skills, high transportation

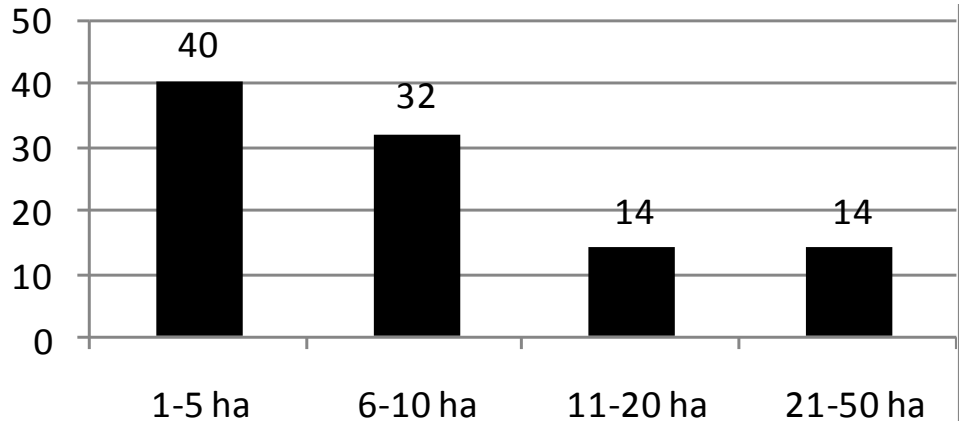

Figure 4. Land availability. Source: Field Survey (2013).

cost, lack of market information, poor support services from the government, and participating in low remunerative marketing channels.

The majority of the produce produced by the farmers is sold to informal markets with low market value. Some of the farmers used more than one marketing channel. The distance to output markets is an important factor. Long distances to the market can be discouraging to farmers who want to commercialise. All the remunerative markets were located far away from the localities of the farmers. This implied that farmers had to travel long distances to formal markets on gravel road with their commodity loaded on poor transportation. Poor infrastructures and a lack of transportation infrastructure affect the quality of produce thus causing farmers' produce to be uneconomical or to lose quality.

The finding also showed that most respondents suffer from a lack of market information owing to a lack of communication, tools and support services from the government and extension officers. The majority of the farmers relied on word-of-mouth, family and self-research for information regarding market prices which in most cases was biased not accurate or up-to-date. Marketing information is very important for the market participation of emerging farmers. The availability of market information with regard to prices can boost the confidence of a farmer in marketing his or her produce. It can also help a farmer to choose marketing channels which ensure a better profit. The availability of market information helps farmers to make informed decisions about the marketing channels in which to participate. Farmers with no access to market information often make poor decisions. Market information helps the farmer by enhancing his or her bargaining power. The availability of market information about market variables such as prices and products in high supply and high demand in the market is important to the market performance of emerging farmers.

A business in general requires someone who is open-minded and has a quick understanding mind, skills such as record keeping and banking skills, labour management and the ability to choose a profitable enterprise and production method for that enterprise. Agricultural production methods in particular are dynamic and require someone who is current with developments and changes. All these requirements are achievable through education. When people are uneducated they become victims of being cheated and once people are cheated they refuse to adopt further innovation or change even if it is beneficial to them. Chemicals such as pesticides and herbicides and integrated pest management methods need someone who will understand them and their instructions because they can be dangerous to humans and produce resulting in loss of produce. However this does not mean that uneducated farmers or farmers with low education levels cannot be successful commercial farmers. The majority of the farmers in the present study were uneducated or had low education levels. This contributes to their lack of participation in formal markets.

Infrastructure such as roads, communication lines and farming facilities needed to be upgraded as it was restricting emerging farmers from commercialising to commercial farming. Fencing was required in the localities of the farmers. Irrigation equipment for crop farmers is important and needed for vegetable farming as water is a fundamental necessity. The unavailability of cultivation infrastructure such as cultivation tractors and ploughing implements was found to limit the farmers' productivity. The majority of the respondents claimed to be using the little money they have to hire these implements when needed. The implements of those who had them were old and poor in quality.

The farmers needed training in various production skills of which could boost their productivity and farm income. Skills such as training in the development of marketing strategies could help them access and secure marketing channels. Overcoming the challenges emerging farmers

face can induce the farmers to move towards commercial agricultural systems. In order for emerging farmers to withstand both local and international competition, the South African government needs to consider support policies and regulation that are necessary to stimulate growth among emerging farmers. The State has a crucial role to play in increasing market participation of emerging farmers through encouraging group marketing, the upgrading of roads to enable smooth accessibility of farmers to output markets and the establishment of local point sales in farming rural areas. The government should enact laws and implement policies that are favourable to emerging farmers. Finally, the present study recommends that government provides planned workshops to all farmers in order to equip them with marketing knowledge.

Conclusion

Many limiting factors that affect the migration of emerging farmers into commercial agricultural farming throughout the Eastern Cape Province have been discussed in this paper. The main factors are low education levels among the farmers in order to understand the dynamics of agriculture, poor management, lack of farming skills, poor access to formal remunerative markets, high transportation costs to formal markets, poor market information and insufficient support services from the government. It was found that the government needs to take a leading role in investing in these support services. Access to productive land, production inputs, infrastructure, extension services, and value adding facilities in the location of the farmers, market information and transport logistics has been found to be the key factors influencing emerging farmers' participation in remunerative agricultural markets.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

Financial assistance from the National Research Foundation and The Nelson Mandela Metropolitan University for this study is heartily acknowledged. The technical assistance of Distinguished Professor Mark Watson is highly appreciated.

REFERENCES

Aliber M, Hall R (2012). Support for smallholder farmers in South Africa: Challenges of scale and strategy. Dev. South. Afr. 29(4):548-562.
Ayinke OO (2011). Contract farming in developing emerging farmers in South Africa: Exploring the Gledhow Mansomini sugarcane Scheme. Master's thesis. University of Stellenbosch.
Biénabe E, Vermeulen H (2011). Improving smallholders' market participation: Insights from a business scheme for maize in Limpopo Province, South Africa. Dev. South. Afr. *28*(4):493-507.
Chikazunga D, Paradza G (2012). Can smallholder farmers find a home in South Africa's food-system? Lessons from Limpopo Province. The Institute for Poverty, Land and Agriculture Studies, pp. 3-4 (PLAAS) Blog. http://www.plaas.org.za/blog/can-smallholderfarmers- find-home-South-Africa's-food-system-lessons-Limpopo province. Accessed 9 November 2012.
Department of Agriculture Forestry and Fisheries (2010). The Strategic Plan for South African Agriculture. Department of Agriculture, Directorate of Agricultural Information Services, Pretoria.
Department of Agriculture, Forestry and Fisheries (DAFF) (2010). Integrated Growth and Development Plan 2011-2031. [Online] Available.
Freguin S, Anseeuw W, D'haese M (2012). Demystifying contract farming: evidence from rural South Africa. Department of Agricultural Economics. Ghent University. Belgium.
Hart T, Aliber M (2010). The need for an engendered approach to agricultural technology. Agenda 24(84):75-90.
Heinemann E (2002). The role and limitations of producer associations. European Forum for Rural Development Cooperation. 4 Sept., Montpellier.
Hussey J, Hussey R (1997). Business research. A practical guide for undergraduate and postgraduate students. Hounds mills: Macmillan.
Makhura M, Mokoena M (2003). Market access for small-scale farmers in South Africa. Pietermaritzburg: University of Natal Press.
Mason J (2002). Qualitative researching. Sage. https://uk.sagepub.com/en-gb/eur/qualitative-researching/book224801
NERPO (National Emergent Red Meat Producers Organisation) (2004). Marketing infrastructure development. [Online] Available at: [Accessed on 27 March 2005] http://www.nerpo.org.za.
National Treasury (2004). Land Affairs Vote 30; Estimates of National Expenditure 2003. Government Printers: Pretoria.
National Treasury (2005). Inter-governmental fiscal Review. National Treasury, Pretoria.
Randela R (2005). Integration of emerging cotton farmers into the commercial agricultural economy (Doctoral dissertation, University of the Free State Bloemfontein South Africa).
Trochim WM (2006). Descriptive statistics. Research Measures Knowledge Base, pp. 10-11.
Trochim WM (2006). Qualitative measures. Research Measures Knowledge Base, pp. 361-9433.
Van Niekerk PP (2002). Product development as part of a positioning strategy for the hunting industry in the Eastern Cape, D-Tech. Thesis. Port Elizabeth: Port Elizabeth Technikon.

academicJournals

Vol. 10(41), pp. 3870-3877, 8 October, 2015
DOI: 10.5897/AJAR2015.10377
Article Number: CCE8D8F55814
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Postharvest fruit quality of new papaya tree hybrids produced in semiarid region

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Received 1 September, 2015; Accepted 25 September, 2015

This work aimed to evaluate the new postharvest quality of papaya tree hybrids grown in a region of semi-arid climate, aiming to find the most suitable for marketing as new cultivars. It was conducted in a commercial farm in the municipality of Mossoró-RN, an experiment in a randomized block design with 14 treatments (12 hybrids in testing, plus two more cultivars already marketed, Tainung No. 1 and Sunrise Solo), with 4 replications. The fruits were harvested during the second maturation stage (fruit with up to 25% yellow skin) and taken to the Postharvest Laboratory of UFERSA, where they were accommodated at room temperature (22 ± 1°C and 60 ± 5% RH) until they reaches the fifth maturation stage (fully ripe fruit, with 76-100% of yellow skin), and analyzed for their physical, physical-chemical and biochemical traits. There was great variability among hybrids for the evaluated quality characteristics. However, the hybrids showed characteristics such as mass and dimensions of fruits, vitamin C, soluble solids, pulp thickness that fall under the classification for commercialization both in the domestic and international markets, with the exception of the hybrids H36.45, H36.56, and H45.56, which have yellow pulp, which is not favored in the consumer market.

Key words: *Carica papaya* L., vitamin C, soluble solid, firmness of fruit.

INTRODUCTION

The papaya tree *(Carica papaya* L.) is a fruit tree grown in almost all the national territory, with emphasis in the states Bahia with 718.000 tons in 2013, followed by Espírito Santo, Minas Gerais, Ceará and Rio Grande do Norte. Brazil is the second largest producer in the world with a total production of 1.6 million tonnes in a cultivated area of 31.989 ha and with the yield 51.02 t/ha in 2013 (Reetz et al., 2015).

Among the difficulties faced by papaya tree crop, there is the limitation of alternatives, the choice of cultivars and/or commercial hybrids for planting that meet both the requirements of the domestic and international markets. Additionally, there is also the high price of hybrid seeds of papaya tree of the Formosa group, which has led many growers to perform successive plantings with the F2, F3 and F4 generations of hybrids, which causes several

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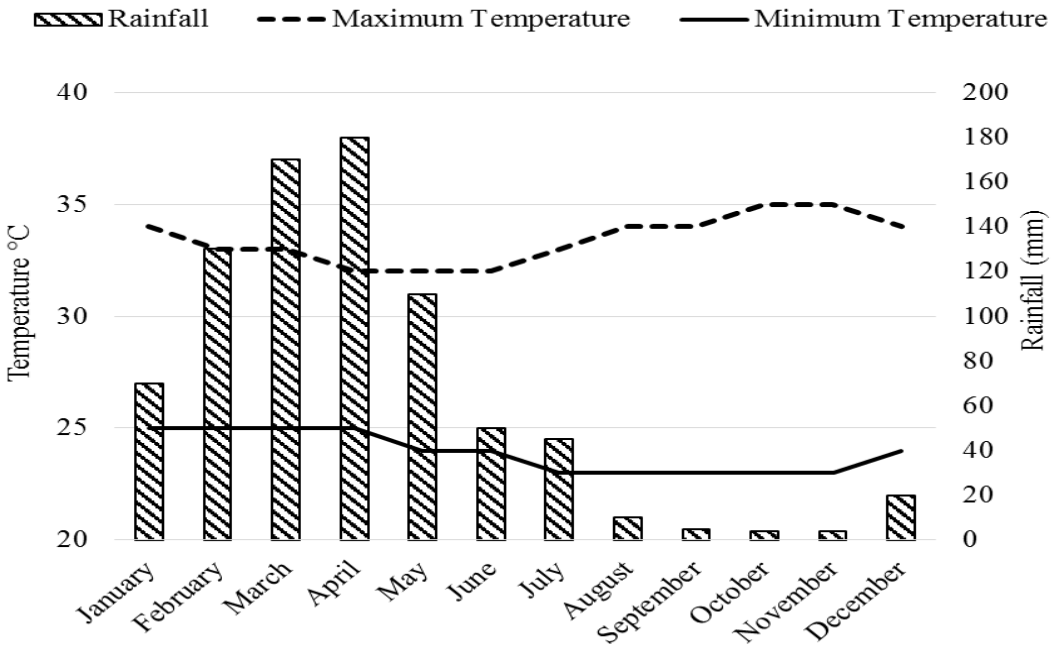

Figure 1. Temperature Analysis, maximum and minimum, and monthly rainfall in the municipality of Mossoro-RN in 2012.

problems, among them the loss of vigor and segregation to fruit shape (Marin et al., 2001; Serrano and Cattaneo, 2010).

Commercially the most cultivated varieties belong to the Solo and Formosa groups. Preferred for producing fruit in the export process, with smaller sizes, varieties of the Solo group are explored in various regions of the world. On the other hand, the fruits of the Formosa group are larger and usually commercial hybrids that are gaining space both in the domestic and foreign markets, where it has been seeing strong growth in sales mainly to Europe, Canada and the United States (Dantas and Oliveira, 2009).

From these two groups, only three cultivars occupy most of the commercial plantations. The most exploited cultivars in Brazil are 'Sunrise Solo' and 'Improved Sunrise Solo cv. 72/12' belonging to the Solo group, better known as Havaí Papaya or Amazônia and 'Tainung 01' and Tainung 02' of the Formosa group (Embrapa, 2013).

There is a low availability of cultivars for use in papaya crop. This fact makes her more vulnerable to crop diseases, pests and soil and edaphoclimatic variations, which can affect the sustainability of this agribusiness. Thus, the development work with new materials can help to increase the genetic variability of papaya tree crops through the selection of new genotypes showing resistance to major pests and diseases, good productivity and fruit quality that meets consumer demands (Oliveira et al., 2010), as consumers are increasingly demanding about the quality of the fruits that they are consuming, this being a prime factor to overcome competitive markets.

Studies on indicators of irrigated agriculture in different continents has shown great variations to this type of crop, drawing attention to the best investments and risk reduction through the programming of the use of water resources and standardization of suitable crops to the farmer (Valipour, 2015a; Valipour, 2015b). However, the development of new genotypes become extremely important, due obtain new cultivars that are productive, with good quality fruit and that adapt to different regions with low rainfall and limited availability of water for irrigation.

Fruit quality is influenced by edaphoclimatic conditions, variety, plant nutrition, timing and production site (Aular and Natale, 2013). This work aimed to evaluate the postharvest quality of new papaya tree hybrid fruits resulting from research on genetic improvement for this culture, and cultivated in the municipality of Mossoró-RN a semiarid climate region, aiming to find the most useful to marketing as new cultivars.

MATERIALS AND METHODS

The experiment was conducted in a commercial area of the company Agrícola Famosa SA in the rural municipality of Mossoró-RN. According to Köppen climate classification, the climate in the region is the BSwh' type, that is, hot and dry steppe with rainy season lingering from summer to autumn (Carmo Filho et al., 1987). The annual rainfall is around 450 to 600 mm, with the months from February to May being the wettest four months and from August to November the driest four months (Figure 1), soil

type Quartzarenic Neosoil.

The experiment was designed in randomized blocks, with four repetitions, with 14 treatments evaluated, with plots constituted of six plants, spaced 4.0 × 2.0 m. The treatments or genotypes were: two cultivars already commercialized, the Tainung No. 01 and Sunrise, plus twelve more hybrids in testing (H10.26, H10.60, H10.72, H26.60, H26.72, H33.36, H33.45, H33.56, H36.45, H36.56, H45.56, H60.72) provided by Embrapa Mandioca e Fruticultura, Cruz das Almas - BA.

In the months of October to December of 2012 the harvest took place, when fruits were harvested and selected in maturation stage 2 (fruit up to 25% of the surface with yellow skin) and then transported to the postharvest laboratory of the Federal Rural University of the Semi-Arid, where they were accommodated at room temperature (22 ± 1°C and 60 ± 5% RH) until they reached the maturity stage 5 (fully ripe fruit, with 76-100% of yellow skin), when the same were fully mature (Sanches, 2003).

The following characteristics were evaluated: fruit mass, determined by the average value of individual weighting with the results expressed in grams (g); length and diameter with the aid of a caliper rule and results expressed in centimeters (cm), relation length/diameter (fruit shape) obtained by calculating the ratio between the two, and the classification made according to scale adapted from Lopes (1982), in which fruit have compressed (RF <0.9), spherical (RF ≤ 0.9 ≤ 1.1), caplet (1.1 <RF ≤ 1 , 7) and cylindrical (RF> 1.7) format; internal cavity and pulp thickness using a digital caliper in millimeters (mm); pulp coloration using a 1-5 color scale (1 - yellow, 2 - intense yellow, 3 - salmon, 4 - orange, 5 - intense orange); fruit firmness, determined with the fruit pressure tester penetrometer, obtaining readings in lbf, lately converted into (N) vitamin C determined by titration with Tilman solution (DFI - 2,6-dichloro-phenol-indophenol 0.02%) according to the methodology proposed by Strohecker and Henning (1967) and the results expressed in ascorbic acid 100 g^{-1} mg; soluble solids (SS) directly in the homogenized juice of the edible fraction through reading in digital refractometer (PR model -. 100, Palette, Atago Co, LTD, Japan), results expressed in percentage (%) (AOAC, 2005); titratable acidity (TA) according to the method of the Association of Official Analytical Chemistry (2005), the results expressed as citric acid percentage, and the ratio soluble solids/titratable acidity was obtained by the ratio between soluble solids and titratable acidity of the fruit pulp; pH, with the aid of a potentiometer (AOAC, 2005), the soluble sugar by Antrona method according to Yemn and Willis (1954); total and soluble pectin were extracted by the methodology described by McCready and MacComb (1952) and determined according to Blumenkrantz and Asboe-Hansen (1973), the results expressed in mg of galacturonic acid per 100 g fresh pulp mass, using a standard curve for galacturonic acid; pectinmethylesterases enzyme activity (PMEs), determined by the method of Jen and Robinson (1984), result expressed in EU/min/g of tissue; and the activity of the polygalacturonase enzyme (PG), for extracting the enzyme source (Pressey and Avants, 1973), the determination was performed by the DNS method (Miller, 1959) and the results expressed as UAE/g fresh weight.

The results were submitted to analysis of variance and the average of qualitative data compared by the Scott-Knott test at 5% with the aid of the SISVAR (System for Analysis of Variance) statistical software.

RESULTS AND DISCUSSION

The fruits of the hybrids showed average values for mass ranging from 461.1 g (hybrid H10.72) to 946.2 g (hybrid H10.60). These values were lower than those obtained for Tainung No. 1 and higher than those found for the Sunrise Solo, which was statistically similar to the hybrid H10.72 (Table 1). Dias et al. (2011) evaluating the papaya genotypes, revealed a large variation for the fruit mass, with values ranging from 260 to 1890 g. Given the results for this variable, it can be seen that the hybrids have potential for selecting plants for the production of fruits with standards that meet the international market, since it requires mass around 500 g, as well as the domestic market requiring fruit with mass between 800 and 1500 g (Dantas and Lima, 2001; Ocampo et al., 2006; Dias et al., 2011).

The dimensions of the fruit also showed a large variation. For the length of the fruit, the hybrids that got the highest values were H10.60, H26.72, H33.36, H33.45, H33.56, H36.45, H36.56 and H45.56, which are lower than the Tainung no. 1 and higher than the Sunrise Solo, which presented lower undifferentiated length from the hybrid H60.72. As for the diameter of the fruit, the hybrids that had higher values were H10.26, H10.60, H33.45, H33.56, H36.45 and H45.56, not differentiating from the Tainung No. 1, and the other hybrids showed lower values, but higher than the Sunrise Solo who got smaller diameter (Table 1). For these characteristics, in the types of papaya tree of the Solo and Formosa groups, classification is very subjective when it comes to genotypes with high variability in the size and shape of fruit (Dias et al., 2011) as evaluated in this work.

For the relationship between length and diameter of the fruits it can be observed a separation between two groups, one in which the hybrids that resembled Tainung No. 1 of the Formosa group, with values above 2, with fruits having a cylindrical shape, and another group in which the hybrids resembled the Sunrise Soil Soil group, with values around 1.7 featuring an oblong shape fruit (Table 1). However, the present hybrids present format suitable for marketing, since they have shapes similar to those genotypes that are currently available on the market.

Regarding the color of the pulp, most hybrids have orange coloring similar to Tainung No. 1 and Sunrise Solo. With the exception of H10.60 and H26.72 hybrids, which have intense orange pulp color, and hybrids H36.45, H36.56 H45.56 which have yellow color (Table 2), a characteristic that is not the preferred by most consumers due to the habit of consumption of fruits with red pulp and associating this coloration that the fruits are not fully mature.

The thickness of the pulp showed a small variation between the values, with observed formation of two groups, one with the hybrids H10.60, H33.45, H33.56, H36.45 and H45.56, which did not differentiate from the Tainung No. 1, obtaining the highest values, and another group with the hybrids H10.26, H10.72, H26.60, H26.72, H33.36, H36.56 and H60.72 that were similar to Sunrise Solo with lower values (Table 2). All genotypes showed pulp thickness higher than 20 mm, considered ideal for papaya marketing (Martins et al., 2006). This variable is

Table 1. Means of mass (MASS), length (LENG), diameter (DIAM) and length and diameter ratio (COMP/DIAM) of fruits papaya tree hybrids.

Genotypes	MASS(g)	LENG(cm)	DIAM(cm)	LENG/DIAM
Tainungnº1	1337.9[a]	24.7[a]	11.2[a]	2.2[a]
Sunrisesolo	262.2d	11.3[d]	6.5[c]	1.7[b]
H10.26	762.9[b]	16.9[c]	9.7[a]	1.7[b]
H10.60	946.2[b]	19.2[b]	10.6[a]	1.8[b]
H10.72	461.1d	16.3[c]	8.1[b]	2.0[a]
H26.60	576.7[c]	16.5[c]	8.7[b]	1.8[b]
H26.72	711.7[c]	19.5[b]	9.2[b]	2.1[a]
H33.36	629.1[c]	19.1[b]	8.6[b]	2.2[a]
H33.45	886.6[b]	20.5[b]	9.8[a]	2.1[a]
H33.56	936.9[b]	20.5[b]	10.2[a]	2.0[a]
H36.45	853.2[b]	20.7[b]	9.4[a]	2.2[a]
H36.56	717.1[c]	19.9[b]	9.0[b]	2.2[a]
H45.56	812.1[b]	19.9[b]	10.4[a]	1.9[b]
H60.72	531.6[c]	14.2[d]	8.7[b]	1.7[b]
General mean	744.7	18.5	9.3	1.9
CV(%)	25.87	11.93	10.30	10.63

Means followed by the same letter do not differ by the Scott-Knott test at 5% probability.

Table 2. Means of color pulp, pulp thickness (PT), internal cavity (IC) of fruits papaya tree hybrids.

Genotypes	Pulp color	PT (mm)	IC (mm)
Tainung nº1	Orange	28.8[a]	54.2[a]
Sunrise solo	Orange	21.1[b]	22.7[c]
H10.26	Orange	24.5[b]	48.1[a]
H10.60	Intense orange	27.9[a]	49.8[a]
H10.72	Orange	22.8[b]	35.3[b]
H26.60	Orange	23.9[b]	39.4[b]
H26.72	Intense orange	23.9[b]	44.0[b]
H33.36	Orange	22.4[b]	40.7[b]
H33.45	Orange	28.6[a]	40.6[b]
H33.56	Orange	27.7[a]	46.6[a]
H36.45	Yellow	27.0[a]	39.3[b]
H36.56	Yellow	24.3[b]	41.0[b]
H45.56	Yellow	28.3[a]	46.9[a]
H60.72	Orange	22.1[b]	43.2[b]
General mean	-	25.2	42.3
CV (%)	-	12.56	12.49

Means followed by the same letter do not differ by the Scott-Knott test at 5% probability.

closely related to the quality of the fruits, because fruits with thicker pulp tend to have higher firmness (Oliveira et al., 2010), confirming what was also observed in this study.

The values for internal cavity of the fruit ranged from 22.7 to 54.2 mm. With the hybrids H33.56, H10.26, H10.60 and H45.56 presenting larger cavity not differentiating from the Tainung No. 1. The hybrids H10.72, H26.60, H26.72, H33.36, H33.45, H36.45, H36.56 and H60.72 had lower diameter of the inner cavity, but superior to the Sunrise Solo, that presented the smaller values for this variable (Table 2). Fruits with lower internal cavity, greater firmness and thickness pulp, have a higher pulp yield and are more resistant to transport to distant markets (Fioravanço et al., 1992).

The firmness of the fruit is a characteristic of

Table 3. Means of fruit firmness (FIRM), total pectin (TP), soluble pectin (SP) polygalacturonase (PG) and pectinmethylesterase (PME) of fruits papaya tree hybrids.

Genotypes	FIRM (N)	TP[1]	SP[1]	PG[2]	PME[3]
Tainung nº1	23.6[b]	444.9[a]	344.4[b]	239.8[a]	1081.4[d]
Sunrise solo	14.4[d]	462.3[a]	373.7[a]	301.9[a]	4127.3[a]
H10.26	21.2[c]	391.2[b]	356.4[a]	271.7[a]	1471.8d
H10.60	23.2[b]	366.3[b]	366.1[a]	137.9[b]	2672.8[b]
H10.72	15.1[d]	415.2[b]	373.4[a]	243.6[a]	2702.0[b]
H26.60	16.6[d]	466.9[a]	370.2[a]	243.2[a]	2887.6[b]
H26.72	15.2[d]	405.9[b]	370.9[a]	237.7[a]	2690.7[b]
H33.36	12.9[d]	398.0[b]	321.1[b]	269.2[a]	2771.4[b]
H33.45	19.8[c]	447.5[a]	331.5[b]	180.9[b]	2119.1[c]
H33.56	17.2[c]	444.3[a]	396.3[a]	231.1[a]	2292.6[c]
H36.45	19.5[c]	436.8[a]	296.1[b]	140.8[b]	1350.9[d]
H36.56	18.2[c]	480.1[a]	317.2[b]	149.6[b]	2855.6[b]
H45.56	32.6[a]	450.8[a]	343.9[b]	178.8[b]	1751.6[c]
H60.72	17.4[c]	451.4[a]	385.0[a]	307.8[a]	2465.5[b]
General mean	19.1	432.9	355.8	223.8	2374.3
CV (%)	11.69	10.62	5.97	26.44	11.82

Means followed by the same letter do not differ by the Scott-Knott test at 5% probability. [1]mg Á. Gal. 100 g^{-1}; [2]UAE g^{-1} of fresh weight; [3]U.E min^{-1} g^{-1} of tissue.

fundamental importance in the assessment of fruits, both for management and for the acceptance by the consumer market (Cuquel et al., 2012). Directly influencing the strength of the fruit from mechanical shocks during transportation and marketing, providing a longer shelf life. The hybrid H45.56 showed the highest firmness. It can be observed in a cluster with the lowest values for the hybrids H10.72, H26.60, H26.72 and H33.36 (range 12.9 to 15.1 N) that were similar to the Sunrise Solo; this lower firmness is directly related to the high activity of pectinmethylesterase and polygalacturonase, and high content of soluble pectin in these hybrids (Table 3).

For total pectin, hybrids who obtained the highest values were H26.60, H33.45, H33.56, H36.45, H36.56, H45.56 and H60.72 that did not differentiate from the Tainung 1 and Sunrise Solo (Table 3). Higher total pectin rates are important in the postharvest fruit conservation because pectins influence the texture of the fruit, as well as reduce costs in the industrial processing area due to less need for the addition of commercial pectin and reduction of sweet mass manufacturing time (Chitarra and Chitarra, 2005).

As for the soluble pectin the highest values were found for the hybrids H10.26, H10.60, H10.72, H26.60, H26.72, H33.56 and H60.72 that did not differentiate from Sunrise Solo (Table 3) . The high percentage of soluble pectin indicates fruits with less firmness, which makes the fruit more susceptible to mechanical damage, reducing its post-harvest life and renders the transport of fruits to great distances impractical.

Regarding the enzymes that degrade the cell wall, the polygalacturonase showed lower enzymatic activity in the hybrids H10.60, H33.45, H36.45, H36.56 and H45.56, as for pectinmethylesterase the least activity was in the hybrids H10.26 and H36.45 that not differed from Tainung No. 1 (Table 3). These genotypes showed greater fruit firmness, as the lower activity of these enzymes along with other pectinases, the greater the firmness of the fruit. Ethylene production during papaya ripening stage has strong participation in modulating the activity of these enzymes (Krongyut et al., 2011). According to Antunes et al. (2006), the activity of PMEs must precede PG activity, since the former has a demethylating function and prepares the polygalacturonic chain for th PG action, depolymerizing it. After the PG action, undemethylated pectic chains are exposed and may suffer, although to a lesser extent, the action of PMEs.

The fruits presented high levels of vitamin C, which ranged from 96.5 to 122.3 mg/100 g. The hybrids H10.26, H10.72, H26.72, H33.36, H33.56, H36.45 and H36.56 showed vitamin C content higher than the other hybrids and Tainung No. 1 and Sunrise Solo (Table 4). Wide variation in vitamin C values can be found among the fruits of different genotypes, probably due to the effect of weather conditions and soil nutrition, besides the characteristics of the genotype itself.

The titratable acidity showed little variation despite significant differences, with all hybrids presented lower acidity than the Sunrise Solo, and the hybrids H33.36, H33.45, H33.56, H36.45, H45.56 and H60.72, similar acidity to Tainung No. 1 (Table 4). These values were

Table 4. Means of vitamin C (VIT C, mg 100 g^{-1}), titratable acidity (TA) and pH of fruits papaya tree hybrids.

Genotypes	VIT C (mg 100 g^{-1})	TA (% Á. cítrico)	pH
Tainung nº1	97.6[c]	0.125[d]	4.55[a]
Sunrise solo	97.7[c]	0.180[a]	4.80[a]
H10.26	113.2[a]	0.166[b]	4.78[a]
H10.60	105.5[b]	0.156[c]	4.87[a]
H10.72	122.3[a]	0.156[c]	4.95[a]
H26.60	94.8[c]	0.147[c]	4.75[a]
H26.72	114.2[a]	0.167[b]	4.89[a]
H33.36	119.5[a]	0.132[d]	4.98[a]
H33.45	105.6[b]	0.126[d]	4.98[a]
H33.56	117.1[a]	0.136[d]	4.91[a]
H36.45	118.7[a]	0.123[d]	4.79[a]
H36.56	112.5[a]	0.109[e]	4.92[a]
H45.56	105.6[b]	0.129[d]	4.83[a]
H60.72	105.7[b]	0.126[d]	4.89[a]
General mean	109.3	0.141	4.85
CV (%)	4.67	6.45	3.54

Means followed by the same letter do not differ by the Scott-Knott test at 5% probability.

Table 5. Means of soluble solids (SS), relation SS/TA and soluble sugars (SA) of fruits papaya tree hybrids.

Genotypes	SS (%)	SS/TA	SA (%)
Tainung nº1	13.1[b]	104.8[b]	9.0[c]
Sunrise solo	14.3[a]	79.4[d]	10.3[b]
H10.26	15.3[a]	92.2[c]	11.2[a]
H10.60	13.0[b]	83.3[d]	8.8[c]
H10.72	15.7[a]	100.6[b]	10.9[a]
H26.60	13.7[b]	93.2[c]	9.9[b]
H26.72	14.5[a]	86.8[c]	10.5[b]
H33.36	13.9[b]	105.3[b]	10.8[a]
H33.45	13.1[b]	104.4[b]	10.3[b]
H33.56	13.5[b]	99.3[b]	10.8[a]
H36.45	12.6[b]	102.4[b]	10.1[b]
H36.56	13.3[b]	122.0[a]	10.3[b]
H45.56	12.7[b]	98.4[b]	8.9[c]
H60.72	14.8[a]	117.5[a]	10.9[a]
General mean	13.8	97.9	10.2
CV (%)	5.94	6.37	6.03

Means followed by the same letter do not differ by the Scott-Knott test at 5% probability.

superior to the variation found by Alonso et al. (2008) from 0.012 to 0.034% by evaluating the productive behavior of different varieties of papaya tree in an experiment carried out in Cuba. Large variations in papaya in acidity values can occur due to variations of each genetic material in the use of organic acids as respiratory process substrates, the management of fertilizer and spacing, as well as harvest date (Souza et al., 2009; Fontes et al., 2012).

For the pH there was no significant difference between hybrids and cultivars already marketed (Table 4). These values were lower than those found by Dias et al. (2011) that found 5.22 and 5.64 in the evaluation of papaya tree genotypes with use of agronomic descriptors.

Regarding the soluble solids, hybrids H10.26 H10.72 H26.72 H60.72 showed higher values, not differentiating between them and the Sunrise Solo (Table 5). The hybrids evaluated in this work fall within the requirements

for marketing based on this variable, both for the domestic market, where the minimum requirement for this feature is 11%, according to rules Instruction No. 4 of January 22, 2010 for papaya; as well as for the foreign market, where the minimum required is 12% (Manica, 1996).

For the SS/TA relation there was a change from 79.4 to 117.5, and the lower values were observed for hybrid H10.60 that didn't differ from Sunrise Solo. The hybrids H36.56 and H60.72 showed higher values than the others (Table 5). The relationship between sugars and organic acids becomes more representative than the isolated measurement of these, providing good perception of the balance between these two, resulting in a pleasant feeling for the consumer's taste (Fernandes et al., 2010).

For the soluble sugars, there was a group with the highest values for the hybrids H60.72, H33.56, H33.36, H10.72 and H10.26; and one with the lowest values for the hybrids H10.60 and H45.56 that did not differ among themselves and from Tainung No. 1 (Table 5). The concentration and content of sugars play a fundamental role in flavor, also being indicators of fruit maturity stage. This composition can vary due to environmental factors, sunlight quality, temperature, maturity stage, among cultivars, planting practices, as well as the type and dosage of fertilizers (Nascimento et al., 2003).

Conclusion

There was great variability among hybrids for the evaluated quality characteristics. However, all studied hybrids have characteristics such as weight, firmness, pulp thickness, vitamin C, soluble solids, titratable acidity and total sugars, within the quality standards for commercialization both in the domestic market and internationally, except for the the H36.45, H36.56, and H45.56 hybrids, that have yellow flesh that does not meet the preference of most of the consumer markets.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Embrapa Mandioca e Fruticultura and company Agrícola Famosa AS.

REFERENCES

Alonso M, Tornet Y, Aranguren M, Ramos R, Rodríguez K, Pastor MCR (2008). Caracterización de los frutos de cuatro cultivares de papaya del grupo Solo, introducidos em Cuba. Agro. Costar. 32:169-175.

Antunes LEC, Gonçalves ED, Trevisan R (2006). Alterações da atividade da poligalacturonase e pectinametilesterase em amora-preta (*Rubus* spp.) durante o armazenamento**.** Rev. Bras. de Agro. 12(1):63-66.

Association of Official Analytical Chemists (2005). Official methods of analysis of the association of official analytical chemists. 18.ed. Maryland.

Aular J, Natale W (2013). Nutrição mineral e qualidade do fruto de algumas frutíferas tropicais: goiabeira, mangueira, bananeira e mamoeiro. Rev. Bras. de Frut. 35(4):1214-1231.

Blumenkrantz N, Asboe-Hansen G (1973).New method for quantitative determination of uronic acids. Anal Biochem.54(2):484-9.

Carmo Filho F, Espínola Sobrinho J, Amorim AP (1987). Dados meteorológicos de Mossoró (janeiro de 1898 a dezembro de 1986). Mossoró: ESAM/FGD. 341:325.

Chitarra MIF, Chitarra AB (2005). Pós-colheita de frutos e hortaliças: fisiologia e manuseio. 2.ed. Lavras: UFLA. P. 785.

Cuquel FL, Oliveira CFS, Lavoranti OJ (2012). Sensory profile of eleven peach cultivars. Ciên. Tecn. Alim. 32(1):70-75.

Dantas JLL, Lima JF (2001). Seleção e recomendação de variedades de mamoeiro - avaliação de linhagens e híbridos. Rev. Bras. de Frut. 23:617-621.

Dantas JLL, Oliveira EJ (2009). O melhoramento genético do mamoeiro: avanços, desafios e perspectivas. In: I Simpósio Nordestino de Genética e Melhoramento de Plantas, 2009, Fortaleza - CE. O melhoramento genético no contexto atual. Fortaleza - CE: Embrapa Agroindústria Tropical. 1:151-180.

Dias TC, Mota WF, Oton IBS, Mizobutsi GP, Santos MGP (2011). Conservação pós-colheita de mamão formosa com filme de pvc e refrigeração. Rev. Bras. de Frut. 33(2):666-670.

EMBRAPA (2013). Mandioca e Fruticultura Tropical: Mamão. Disponível em: < http://www.cnpmf.embrapa.br/index.php?p=pesquisa-culturas_pesquisadas-mamao.php&menu=2>. Acesso em: 13 de dezembro 2013.

Fernandes AM, Soratto RP, Evangelista RM, Nardin I (2010). Qualidade físico-química e de fritura de tubérculos de cultivares de batata na safra de inverno. Hortic. Bras. 28:299-304.

Fioravanço JC, Paiva MC, Carvalho RIN, Manica I (1992). Qualidade de mamão solo que são comercializados em Porto Alegre de outubro/91 a junho/92. Rev. Ciên. Agron. 23(3):1-5.

Fontes RV, Viana AP, Pereira MG, Oliveira JG, Vieira HD (2012). Manejo da cultura do híbrido de mamoeiro (*Carica papaya* L.) do grupo 'formosa' UENF/CALIMAN - 01 para melhoria na qualidade do fruto com menor aplicação de adubação NPK. Rev. Bras. de Frut. 34(1):143-151.

Jen JJ, Robinson MLP (1984). Pectolytic enzymes in sweet bell peppers (*Capsicum annuum* L.). J. Food Sci. 49:1045-1087.

Krongyut W, Srilaong V, Uthairatanakij A, Wongs-aree C, Esguerra EB, Kanlayanarat S (2011). Physiological changes and cell wall degradation in papaya fruits cv. 'Kaek Dum' and 'Red Maradol' treated with 1- methylcyclopropene. Inter. Food Res. J. 18(4):1251-1259. http://www.ifrj.upm.edu.my/18%20(04)%202011/(6)IFRJ-2011-051.pdf

Lopes JF (1982). Melhoramento genético (chuchu, melancia, melão e pepino). In: LOPES, J. F. Cucurbitáceas: informativo agropecuário. [s.n.]61-65.

Manica I (1996). Cultivares e melhoramento de mamoeiro. In: MENDES, L. G.; DANTAS, J. L. L.; MORALES, C. F. G. Mamão no Brasil. Cruz das Almas, EMBRAPA- CNPMF. P. 179.

McCready RM, McComb EA (1952). Extraction and determination of total pectic materials in fruit. Anal. Chem. 24(12):1586-1588.

Marin SLD (2001). Melhoramento genético do mamoeiro (*Carica papaya* L.): habilidade combinatória de genótipos dos grupos "Solo" e "Formosa". 117 f. Tese (Doutorado) - Universidade Estadual do Norte Fluminense, Campos dos Goitacazes.

Martins VA, Yamanish OK, Mello RM, Lima LA, Fagundes GR (2006). Comportamento do mamoeiro Sekati nas condições o oeste da Bahia. Rev. Bras. Frut. 28(1):79-82.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of

reducing sugars. Anal. Chem. 31:426-428.
Nascimento WMO, Tomé AT, Oliveira MSP, Müller CH, Carvalho JEU (2003). Seleção de progênies de maracujazeiro-amarelo (*Passiflora edulis* f. flavicarpa) quanto à qualidade de frutos. Rev. Bras. Frut. 25(1):186-188.
Ocampo J, D'Eeckenbruggeb GC, Bruyére S, Bellaire LL, Ollitrault P (2006). Organization of morphological and genetic diversity of Caribbean and Venezuelan papaya germplasm. Fruits. 61:25-37.
Oliveira EJ, Lima DS, Lucena RS, Motta TBN, Dantas JLL (2010). Correlações genéticas e análise de trilha para número de frutos comerciais por planta em mamoeiro. Pesq. Agrop. Bras. 45:855‑862.
Pressey R, Avants JK (1973). Separation and characterization of the exopolygalacturonase and endopolygalacturonase from peaches. Plant Phys. 52(3):252-256.
Reetz ER, Kist BB, Santos CE, Carvalho C, Drum M (2015). Anuário brasileiro da Fruticultura 2014. Santa Cruz do Sul: Ed. Gazeta Santa Cruz. P. 104.
Sanches J (2003) Pós-colheita de mamão, In: Informe-on-line Toda Fruta. Edição de 24/09/2003, disponível em: <http://www.todafruta.com.br>. Acesso em: 17 de dezembro de 2013.
Serrano LAL, Cattaneo LF (2010). O cultivo do mamoeiro no Brasil. Rev. Bras. Frut. 32(3):76-82.
Souza TV, Coelho EF, Paz VPS, Ledo CAS (2009). Avaliação física e química de frutos de mamoeiro 'Tainung n°1', fertirrigado com diferentes combinações de fontes nitrogenadas. Agrária. 4(2):179-184.
Strohecker R, Henining HM (1967). Análises de vitaminas: métodos comprobrados. Madrid: Paz Montalvo. P. 42.
Valipour M (2015a). Variations of irrigated agriculture indicators in different continents from 1962 to 2011. Adv. Water Sci. Technol. 1(1):1-14.
Valipour M (2015b). What is the tendency to cultivate plants for designing cropping intensity in irrigated area? Adv. Water Sci. Technol. 2(1):1-12.
Yemn EW, Willis AJ (1954). The estimation of carbohydrate in plant extracts by anthrone. Biochem. J. 57:508-514.

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Vol. 10(41), pp. 3878-3884, 8 October, 2015
DOI: 10.5897/AJAR2015.9882
Article Number: CF8E0B755816
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Enhancing dissemination of *Beauveria bassiana* with host plant base incision trapfor the management of the banana weevil *Cosmopolites sordidus*

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Received 5 May, 2015; Accepted 12 August, 2015

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an important pest of highland banana in East and central Africa. It causes yield loss of up to 100% in heavily infested fields. Studies were carried out in Uganda to evaluate the efficacy of the the plant base incision trap in attracting C. *sordidus* and to determine the potential of using the trap in enhancing dissemination of the entomopathogenic fungi, *Beauveria bassiana* for the pest control. Field experiments were carried out in an established banana field of C. *sordidus* susceptible East African Highland Banana cultivar, Nabusa (AAA-EA). There were significantly more weevils recaptured in the incision trap (14.4%) than the conventional pseudostem trap (4.7%). After laboratory incubation, more weevils died due to pathogen infection from plots where the incision trap was used in combination with *B. bassiana* (25.9%) compared to where the pathogen was applied around the plant base without the incision (15.9%). Weevils showing signs of mycosis were recovered at 6 and 9 m from the pathogen release point, suggesting that the weevils can pick the pathogen from the aggregation point and disperse it. This data demonstrates that the incision trap is more attractive to *C. sordidus* than the conventional pseudostem trap and might be used to enhance dissemination of *B. bassiana* among adults.

Key words: Entomopathogenic fungi, horizontal transmission, pseudostem trap.

INTRODUCTION

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an important pest of highland banana in Uganda. The biology and pest status of *C. sordidus* have been reviewed by Gold et al. (2001)

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and Masanza (2003). The adults are most commonly associated with banana mats (a banana mat consists of plants coming from the common corm) and crop residues (Gold et al., 2001). They crawl short distances and may be sedentary for extended periods (Gold et al. 1999). Few disperse more than 50 m in three months (Gold et al., 2001, 2004). Eggs are deposited in the leaf sheaths and corm at the base of the banana mat (Abera et al., 2000). The larvae tunnel in the corm, damaging the vascular system and compromise the stability of the plant. Yield losses of 100% have been observed in farmers' fields (Sengooba, 1986), while losses of more than 40% have been reported in on-station trials (Rukazambuga et al., 1998; Gold et al., 2004).

Control of *Cosmopolites sordidus* is difficult because the immature stages (including the most destructive stage) are within the plant, often below the soil level, making them largely inaccessible to natural enemies (Gold et al., 2001). Therefore, control strategies often target adults. Currently, control options available to the farmers in Uganda include pesticides and cultural methods. Chemical control is regarded by farmers as easy to manage, fast acting and effective (Gold et al., 1993). The chemicals are however, costly and are thus not affordable by resource poor farmers in Uganda. Weevil resistance towards these chemicals has also been reported in some countries (Collins et al., 1991; Gold et al., 1999). Cultural control practices currently in use include crop sanitation and trapping using pseudostem but are of limited application due to being labour intensive and ineffective (Gold et al., 2002; Masanza, 2003). Farmer adoption of this method has also been limited by the availability of trapping material and lack of confidence of its effectiveness (Gold et al., 1993). Alternative control methods especially those that are ecologically sound, less expensive and safer to non-target species and environment than pesticides are being sought.

Bio-control using fungal microbes offers a promising means of managing this pest. For example, studies conducted in Uganda have identified eastern African strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo Vuillemin) (Ascomycota: Hypocreales) (Ugandan isolate G41) which effect > 95% weevil adult mortality in the laboratory and can significantly reduce adult population and damage under field conditions (Nankinga, 1999; Nankinga and Moore, 2000; Godonou et al., 2000). However, fungal microbes have not been used for the management of the pest by banana growers because of being expensive and not economically feasible.. This is because the weevil is a relatively sedentary insect, thereby necessitating the application of the fungus throughout the banana field. The possible use of infochemicals, especially kairomones, could provide a means of aggregating weevils at entomopathogen delivery sites in cuts made in residue corms, thereby reducing the amount of fungus and associated costs required (Tinzaara et al., 2002). The current delivery systems and their comparative advantage have not been investigated.

Cosmopolites sordidus is attracted to the kairomones from the host plant (Budenberg et al., 1993). The potential use of kairomones to aggregate banana weevils at delivery sites for entomopathogenic fungi has been reported (Tinzaara et al., 2002). The potential for kairomones to enhance transmission of *B. bassiana* needs to be demonstrated in Ugandan conditions. Treverrow and Bedding (1993) and Treverrow (1994) effectively used plant kairomones for the delivering of entomopathogenic nematodes by making conical cuts in the corms of banana stumps. Successful use of pseudostem traps or kairomones to enhance dissemination of *B. bassiana* would attract a large number of adults which have more chances to get in contact with the pathogen and infect healthy individuals outside the trap (horizontal transmission). This delivery system would have the advantage of reduced costs due to limited amount of the pathogen needed in traps and extra field application would not be necessary. Thus, the evaluation of techniques based on used of kairomones to enhance transmission of *B. bassiana* needs to be demonstrated in Ugandan conditions.

The objectives were to: (i) evaluate the efficacy of banana plant base incision trap for attracting *C. sordidus*, and (ii) determine the effectiveness of using host plant incision traps in enhancing the dissemination of *B. bassiana* in Ugandan conditions.

MATERIALS AND METHODS

Study site description

Laboratory and on-station field studies were conducted on the International Institute of Tropical Agriculture's (IITA) Sendusu Farm (0°32'N, 32°35'E, 1260 m.a.s.l) located 28 km northeast of Kampala, Uganda. The site has two rainy seasons (March-May and September-November) with mean annual rainfall of 1200 to 1300 mm and a mean daily temperature of 21°C.

Source of *B. bassiana*

Beauveria bassiana (isolate G41) (3×10^9 conidia/g) was obtained from the Pathology laboratory at Sendusu in a crushed maize formulation (Nankinga and Moore, 2000). This isolate had been previously shown to be effective against *C. sordidus* in the laboratory and field conditions (Nankinga and Moore, 2000).

Experiment 1: Efficacy of the incision traps

Experimental design

The objective of this experiment was to evaluate the effectiveness of using incision traps as compared to the conventional

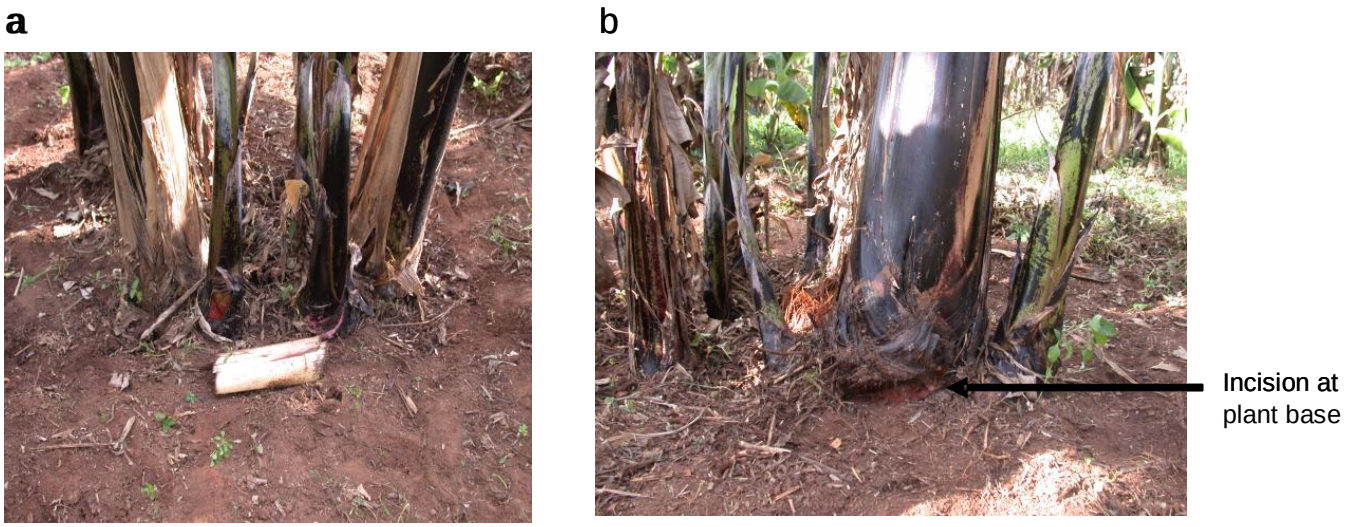

Figure 1. Trap types: (a) split pseudostem trap; (b) incision trap.

pseudostem traps in capturing *C. sordidus*. The experiment was conducted in five year old banana plots of East Africa highland banana, Nabusa cultivar (AAA-EA) mulched with dry banana leaves and well weeded. Plots of 7 rows by 7 columns of banana mats each (*Musa* spp., cv Nabusa, AAA-EA type) planted in a 3 m × 3 m arrangement were used. Plots were 10 m apart. The experiment comprised of two treatments: (a) Conventional pseudostem trap, and (b) Incision trap. Each treatment appeared in five randomly selected plots (that is, was replicated five times).

C. sordidus release and trap placement: Adult *C. sordidus* were collected from banana fields within the station at Sendusu using pseudostem traps (Mitchell, 1978). Weevil sex was determined using curvature of the last abdominal segment and punctuation on the rostrum (Longoria, 1968). Before release, weevils were scratched on the elytra with distinct marks for each banana mat per treatment using a surgical blade. A total of 72 weevils (36 female and 36 male) were released for each replicate on banana mats at varied distances (12 females: 12 males at 0, 3 and 6 m) from the traps for both treatments. Weevils were released in the evening (7.00-8.00 pm) at the base of the plants in each plot by placing them in shallow holes.

The split pseudostem traps were made from freshly harvested Nabusa cultivar plants obtained from the same field. The pseudostem was cut into pieces of about 30 cm long and split longitudinally to make uniform traps (Figure 1a). The traps were laid close to mats with their freshly cut surfaces in contact with the ground (Mitchell, 1978). The incision traps were made by making a horizontal cut/incision into the stump base at soil level using a strong sharp knife (Figure 1b). The incision was cut up to a half of the stump diameter and maintained slightly gapping by inserting small banana corm pieces (of about 2 cm^2 each).

Sampling and data collection

Weevils were picked from each trap after 3 and 6 days and identified using the marks to determine sex and the distance moved from the release point to the trap. The number of marked (males and females) and unmarked weevils recaptured in traps at 0, 3, 6 m from the point of release were recorded and the percentage of recaptured weevils calculated.

Experiment 2: Enhancing dissemination of *B. bassiana* using incision traps

Experimental design

The experiment was carried out in an established banana field at Sendusu and comprised of two treatments: (i) *B. bassiana* applied around the mat, and (ii) *B. bassiana* applied around the incision traps. Each treatment was replicated four times and each replicate was applied to separate plots of 7 by 7 plants (mats). Plots of 306.3 m^2 with 49 banana mats each (*Musa* spp., cv Nabusa, AAA-EA type) planted at spacing of 2.5 m × 2.5 m within rows were used. Plots were 5 m apart.

Application of B. bassiana

Two hundred grams (200 g) of *B. bassiana* maize substrate (5.9×10^7spores/g) was applied around one intact plant per plot for the treatment where *B. bassiana* was applied alone and around the base of incised banana stump for the incision treatment (Figure 2). The pathogen was applied on only one central mat per plot in both treatments. A treatment was applied to one plot. The plots where *B. bassiana* was applied were mulched using dry banana leaves obtained from the same field prior to treatments application (Figure 2). This was meant to prevent sunlight/heat effect on the pathogen.

Weevil release

The initial inoculum of *B. bassiana* in the banana plots was determined before release of weevils. Weevils were captured from plots using pseudostem traps and taken to the laboratory for incubation. Of the 100 weevils which were incubated for six days in the laboratory, none showed mycosis due to pathogen infection confirming no pathogen inoculum on weevils at the start of the experiment.

The weevils which were released in plots were first sexed and marked according to distances of release as described above. Twenty weevils (10 females: 10 males) were released on the mats at 0, 3, 6 and 9 m from the pathogen release point. Weevils were

Figure 2. *B. bassiana* applied around an incised plant base.

released in the evening (7.00-8.00 pm) at the base of the mats in each plot by placing them in shallow holes around the base.

Sampling and data collection

The weevils were captured weekly following their release and taken to the laboratory for incubation. The weevils were incubated (at 25 - 27°C, 80-90% r.h.) in separate glass petri dishes (90 mm diameter) in accordance to distance of capture from the *B. bassiana* release point (0, 3, 6 and 9 m). The incubated weevils were observed for any mycosis (whitish fungal mycelial growth) weekly for three weeks. The distance of infected weevil recovery from the nearest *B. bassiana* application point was noted.

Data analysis

Data of weevils re-captured from plots with pseudostem and incision traps was analysed using a chi squared test. The percentage of weevils recovered by pseudostem trapping from plots with different pathogen delivery systems that showed mycosis after incubation were analysed using contingency table test of statgraphics plus version 7 (StatPoint, Inc., USA) on numbers.

RESULTS AND DISCUSSION

Experiment 1: Efficacy of incision traps

The number of weevils recaptured from the incision trap was significantly higher (14.4%; n =360) compared to that from the conventional split pseudostem trap (4.7%) (t-test, $P<0.05$) (Figure 3). Males and females were attracted equally to either the incision trap or the pseudostem trap. There were significantly more weevils recaptured in incision traps compared to pseudostem traps from all distances from the trap (Figure 4). The incision trap attracted over 20% of the weevils from 3 m and beyond compared to 3% for pseudostem traps.

The banana weevil is attracted to the kairomones from the host plants (Budenberg et al., 1993). Treverrow (1994) identified iso-butyl-aldehyde and limonene to be the major components of the banana corm that are attractive to *C. sordidus*. In the current study, significantly more weevils were captured in the incision trap than the conventional pseudostem trap. This might be because of the higher concentration of attractive components such as limonene in the incision traps than in the conventional psuedostem traps (Treverrow, 1994). The results of this study demonstrate that incision traps are significantly more attractive to the *C. sordidus* compared to the conventional pseudostem trap.

The percentage of weevils captured from 3m and beyond was higher in incision traps than in pseudostem traps. The capacity for incision trap to attract large numbers of the weevils can be exploited for pest management where they are aggregated at delivery sites for entomopathogenic fungi for subsequent dissemination. If the incision trap attracts a higher proportion of the population to the pathogen delivery site, chances of transmission would be increased (Gold et al.,

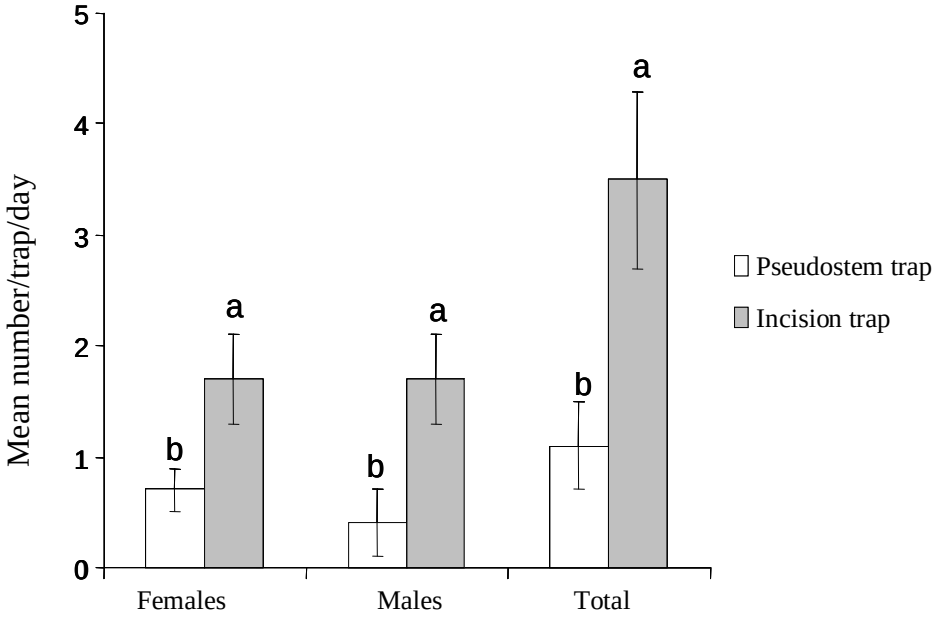

Figure 3. Mean number of *C. sordidus* captured in the incision trap compared with conventional pseudostem trap. Bars with different letters are significantly different, P < 0.05.

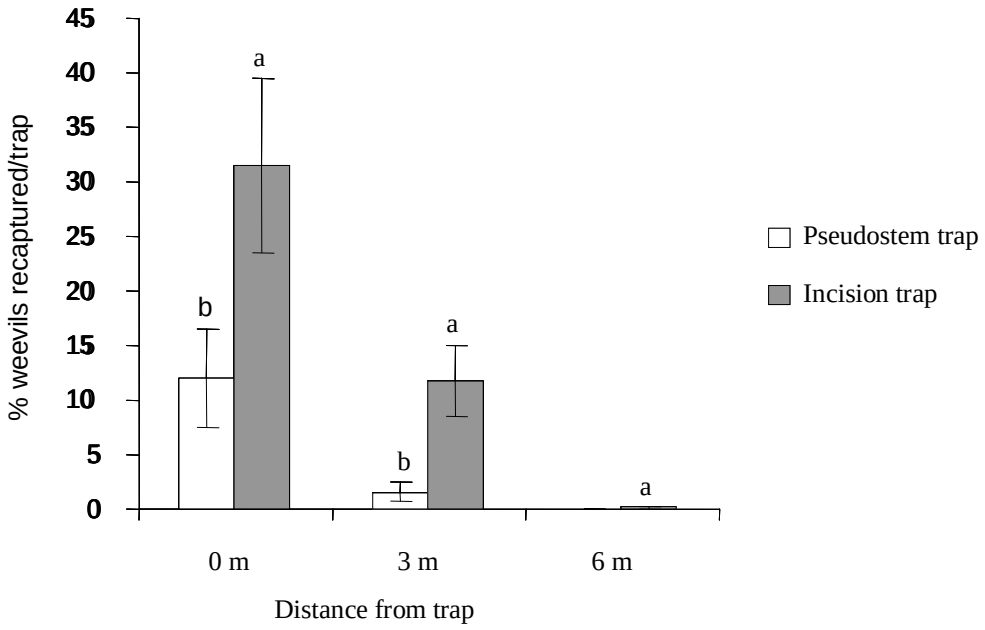

Figure 4. Percentage of *C. sordidus* recaptured in the incision trap and conventional pseudostem trap at different distance from the trap in the banana field at Sendusu, Uganda, 2006. Bars with different letters are significantly different (t-test, P < 0.05).

2001). Therefore, increasing trap efficacy will lead to higher transmission rates of *B. bassiana.* Theoretically, the strategy of using the kairomone to aggregate weevils on the pathogen delivery sites would have the advantage of reducing the amount of fungal pathogen applied per unit area compared to the technique of treating individual plants. Our results indicate that the incision trap can be used to attract *C. sordidus* to sites where the

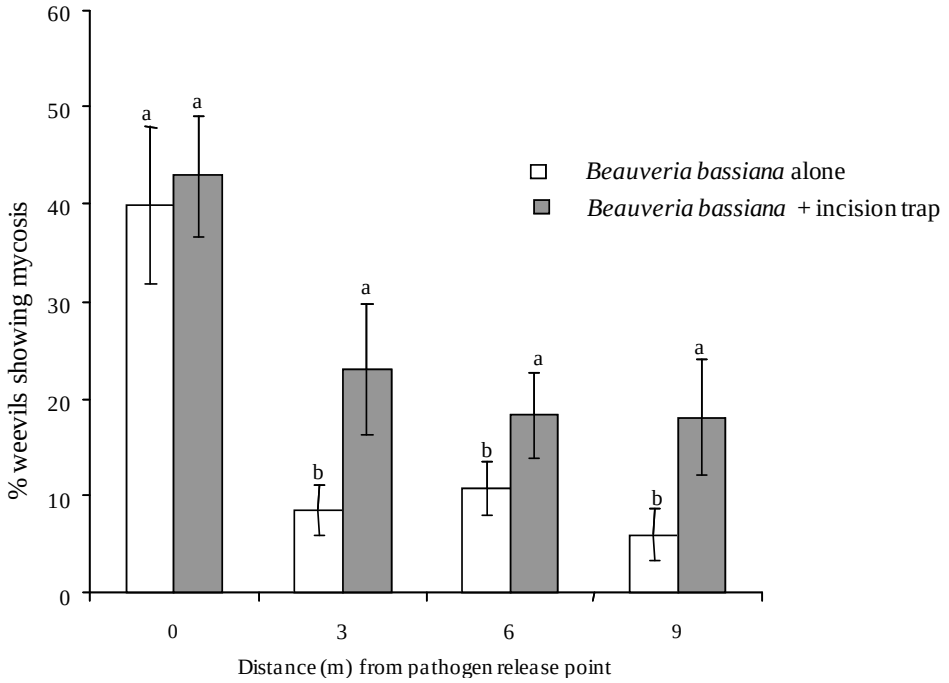

Figure 5. Percentage of weevils with mycosis that were recaptured at different distances from the pathogen release point in the banana field at Sendusu, Uganda, 2006. Bars with similar letters are not significantly different test, (χ^2; P <0.05).

entomopathogen *B. bassiana* is available for subsequent dissemination by the insects.

Experiment 2: Enhancing dissemination of *B. bassiana* using incision traps

Weevils recaptured from plots treated with *B. bassiana* around the incision trap showed significantly higher percentage mycosis (25.9±3.1) compared to those recaptured from plots where *B. bassiana* was applied around the banana mats without incision (15.9±2.7) (χ2=19.2, d.f.=1, P=0.0001). The percentage mycosis for weevils recaptured at 0 m from the pathogen release point was similar among traps (Figure 5). Weevils showing signs of mycosis recaptured at 6 and 9 m from the pathogen release point was significantly higher where *B. bassiana* was applied around the incision trap than when it was applied alone. This observation suggests that the incision trap can enhance pathogen dissemination from the release points.

An effective mechanism of transmission is a key factor in the ability of entomopathogens to develop epizootics (Roy and Pell, 2000). The use of an attractant in the system as a method of introducing a deleterious agent into a pest population requires that the lured individuals can sufficiently disperse after visiting the self-contaminating site (Vega et al., 1995; Klein and Lacey, 1999; Roy and Pell, 2000). In our study using the incision trap delivery system, it was observed that a number of weevils that died due to *B. bassiana* infection were recaptured more than 6 m from the pathogen source. This suggests that these weevils were contaminated with the pathogen from the delivery sites and dispersed after infection.

In our study, 20 to 40% of the incubated dead weevils recaptured at different distance for the pathogen delivery point showed signs of mycosis. The result could have been overestimation as the incubation method and keeping weevils for a long time ensure infection that may not occur in the field. On the other hand, the percentage transmission seems to be low but it is possible that under suitable conditions (e.g greater soil moisture, higher *C. sordidus* population levels) such a level of infection would increase the overall inoculum level. The percentage transmission is also likely to increase when more pathogen is placed around the trap.

Conclusions

The results demonstrate incision traps are significantly more attractive to *C. sordidus* compared to the conventional pseudostem trap and it shows potential for spreading *B. bassiana* in the integrated management of *C. sordidus*. Further studies should be conducted to

investigate the factors that can improve the percentage transmission of *B. bassiana* for the control of *C. sordidus.* There is also need to investigate the cost and benefit analysis of integrating the incision trap-*B. bassiana* delivery system in the management of *C. sordidus.*

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support provided by the International Foundation of Sciences, the Roots, Tubers and Banana (RTB) and Bioversity International based in Kampala, Uganda.

REFERENCES

Abera A, Gold CS, Kyamanya S, Karamura EB (2000). Banana weevil *Cosmopolites sordidus* (Germar) ovipositional preferences, timing of attack and larval survival survivorship in a mixed cultivar trial in Uganda. Act. Hortic. 540:487-496.

Budenberg WJ, Ndiege IO, Karago FW (1993). Evidence for volatile male-produced pheromone in Banana Weevil *Cosmopolites sordidus*. J. Chem. Ecol. 19:1905-1916.

Collins PJ, Treverrow NL, Lamkin TM (1991). Organophosphorus insecticide resistance and its management in the banana weevil borer, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), in Australia. Crop Prot. 10:212-221.

Godonou I, Green KR, Oduro KA, Lomer CJ, Afre-Nuamah K (2000). Field evaluation of selected formulation of Beauveria bassiana for the management of the banana weevil (*Cosmopolites sordidus*) in plantain (Musa spp., AAB group). Biol. Sci. Technol. 10:779-788.

Gold CS, Bagabe MI, Sendege R (1999). Banana weevil, Cosmopolites sordidus (Germar) (Coleoptera: Curculionidae): test for suspected resistance to Carbofuran and Dieldrin in Masaka District, Uganda. Afr. Entom. 7:189-196.

Gold CS, Kagezi GH, Night G, Ragama PE (2004). The effects of banana weevil, *Cosmopolites sordidus* (Germar) damage on highland banana growth, yield and stand duration in Uganda. Ann. Appl. Biol. 145:263-269.

Gold CS, Ogenga-Latigo M., Tushemereirwe W, Kashaija I, Nankinga CM (1993). Farmer perceptions of banana pest constraints in Uganda: Results from a rapid rural appraisal. In: Gold CS, Gemmil B, (eds). Biological and Integrated Control of Highland Banana and plantain. Proceedings of Research Coordination Meeting, Ibadan, IITA, pp. 3-25.

Gold CS, Okech SH, Nokoe S (2002). Evaluation of the pseudostem trapping as a control measure against banana weevil, Cosmopolites sordidus (Coleoptera: Curculionidae) in Uganda. Bull. Entom. Res. 92:35-44.

Gold CS, Pena JE, Karamura EB (2001). Biology and integrated pest management for the banana weevil, Cosmopolites sordidus (Germar) (Coleoptera: Curculionidae). Integr. Pest Manag. Rev. 6:79-155.

Klein MG, Lacey LA (1999). An attractant trap for auto-dissemination of entomopathogenic fungi into populations of the Japanese beetle Popillia japonica (Coleoptera: Scarabaeidae). Biol. Sci. Technol. 9:151-158.

Longoria A (1968). Diferencias sexuales en la morfologia externa de *Cosmopolites sordidus* Germar (Coleoptera, Curculionidae). Ciencias Biol.l, La Habana 1:1-11.

Masanza M (2003). Effect of crop sanitation on banana weevil Cosmopolites sordidus (Germar) populations and associated damage. Ph.D Thesis, Wageningen University, P. 164.

Mitchell GA (1978). The estimation of banana borer population and resistance levels. WINBAN Research and development Technical bulletin No. 2:34.

Nankinga CM, Moore D (2000). Reduction of banana weevil populations using different formulations of the entomopathogenic fungus Beauveria bassiana. Biol. Sci. Technol. 10:645-657.

Nankinga MC (1999). Characterisation of entomopathogenic fungi and evaluation of delivery systems of Beauveria bassiana for the biological control of the banana weevil, *Cosmopolites sordidus*. Ph.D Thesis, University of Reading, UK, 276 pp.

Roy HE, Pell JK (2000). Interactions between entomopathogenic fungi and other natural enemies: implications for biological control. Biol. Sci. Technol. 10:737-752.

Rukazambuga NDTR, Gold CS, Gowen SR (1998). Yield loss in East African highland banana (Musa spp., AAA-EA group) caused by the banana weevil, *Cosmopolites sordidus* Germar. Crop Prot. 17:581-589.

Sengooba T (1986). Survey of banana pest problem complex in Rakai and Masaka districts, August 1986: Preliminary trip report Namulonge Research Station Uganda. Unpublished Ms. P. 10.

Tinzaara W, Dicke M, van Huis A, Gold CS (2002). Use of infochemicals in pest management with special reference to the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae): a review. Insect Sci. Appl. 22:241-261.

Treverrow NL (1994). Identification of components of banana corm attractive to banana weevil borer, *Cosmopolites sordidus*. Final Report HDRC Project Number Fr/227/RO, Wollongbar Agricultural Institute, New South Wales, Australia.

Treverrow NL, Bedding RA (1993). Development of a system for the control of the banana weevil borer, Cosmopolites sordidus with entompathogenic nematodes. In: Bedding R, Akhurst R, Kaya H, editors. Nematodes and the Biological Control of Insect Pests. Melbourne: CSIRO. pp. 41-47.

Vega FE, Dowd PF, Bartelt R (1995). Dissemination of microbial agents using an auto-inoculating device and several insect species as vectors. Biol. Contr. 5:545-552.

academicJournals

Vol. 10(41), pp. 3885-3892, 8 October, 2015
DOI: 10.5897/AJAR2014.8996
Article Number: C24D50155819
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Medium-term conservation and regrowth of the tropical multipurpose species *Genipa americana*

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Received 11 July, 2014; Accepted 21 August, 2015

The *Genipa americana* L., a fruit-producing species found in several countries and commonly found in the Brazilian Northeast, is very important economically, both for its wood and as a source of food and dye. This study evaluate the effects of abscisic acid (ABA) and the concentrations of salt and sugar in MS medium on reducing *Genipa americana* L. growth for *in vitro* conservation, as well as the effects of benzylaminopurine (BAP) on the regrowth phase. Seedlings from accession CZA, after 90 days of *in vitro* cultivation, were transferred to Murashige and Skoog (MS) medium with 30 g L^{-1} sucrose and various concentrations of ABA (0, 0.5, 1.0, 2.0 and 4.0 mg L^{-1}). In a second assay, various concentrations of MS medium salts and sucrose were tested. During the regrowth phase, nodal segments were placed in MS regeneration medium with 30 g L^{-1} sucrose and BAP (0 or 1 mg L^{-1}). All the ABA concentrations affected the number of leaves, but significant effects on the length of aerial portion were not observed. The MS and ½ MS media with 30 g L^{-1} sucrose slowed plant growth and are recommended for conservation protocols for slow growth. Adding 1 mg L^{-1} BAP to the culture medium caused a greater morphogenic response, and the apical, medial and basal nodes retained morphogenic potential during regrowth.

Key words: *Genipa americana* L., germplasm, abscisic acid, sucrose.

INTRODUCTION

Genipa americana (Rubiaceae) is the well-known native fruit tree in South America and West Indies. Fruits and tree bark are used medicinally as tonic, febrifuge, for venereal diseases, pharyngitis, cough, cold and others. Fruits are used to prepare beverages (Yee et al., 2010). Recently studies reported the utilization of *G. americana* as an anthelminthic in sheep (Nogueira et al., 2014).

The Brazilian Agricultural Research Agency has been increasing *G. americana* L. germplasm collection, characterization and conservation efforts through the genetic resource platform, which was started in 2009 with the creation of an active Germplasm Bank containing 195 genotypes. Each conservation method has its advantages and disadvantages, and complimentary strategies are required to maximize the conservation of genetic diversity, which varies between species (Martin and Pradeep, 2003).

G. americana L. is a perennial species, and the conservation of the genetic resources of this tree is mainly based on field collection due to its physiology. The

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species does not tolerate extreme desiccation; therefore, the behavior of *G. americana* L. during storage is less than optimal (Salomao, 2004; Magistrali et al., 2013).

In the last few years, *in vitro* culture techniques for Germplasm Bank conservation have been widely developed and applied to more than 1,000 species, many of which come from tropical regions. *In vitro* culture is thought to be a very promising method for endangered species for which the seeds cannot be conserved and are valuable for conserving plant genetic material (Harding et al., 1997).

Growth reduction is generally achieved by modifying the environmental conditions and/or the culture medium. The most widely applied technique is temperature reduction, which can be combined with a decrease in light intensity or culture in the dark. Tropical species are often cold-sensitive and have to be stored at higher temperatures, which depend on the cold sensitivity of the species. Modifications of the culture medium can include dilution of mineral elements, reduction of sugar concentration, changes in the nature and/or concentration of growth regulators and addition of osmotically active compounds (Engelmann, 2011).

Slow-growth *in vitro* conservation protocols have been created for several species, including *Plumbago indica* (Charoensub and Phansiri, 2004), *Guarianthe skinneri* (Coello et al., 2009), *Piper* sp. (Silva and Scherwinski-Pereira, 2011), *Gladiolus imbricatus* (RAKOSI-TIAN et al., 2012), *Musa balbisiana* (Kanchanapoom and Promsorn, 2012), *Lilium* sp. (Yun-Peng et al., 2012), *Vitis* sp. (Silva et al., 2012), *Glycyrrhiza glabra* (Srivastava et al., 2013), *Epidendrum chlorocorymbus* (Lopez-Puc, 2013) and *Cocos nucifera* (Lédo et al., 2014).

Numerous parameters influence the efficiency of *in vitro* slow growth storage protocols including the type of explants, their physiological state when entering storage, the type of culture vessel, its volume and the volume as well as the type of closure of the culture vessel (Engelmann, 1991). Although there is no standard procedure for all the genotypes of all the species, it will be possible to develop adequate slow-growth methods that require little manipulation for other species. Recent studies have demonstrated the organogenic capacity of *G. americana* L. plants for *in vitro* propagation (Yee et al., 2010), but strategies for the *in vitro* conservation by minimal growth were not found in the literature. The aim of this study was to evaluate the effects of abscisic acid (ABA) and the concentrations of salt and sugar in Murashige and Skoog (MS) medium on reducing *G. americana* L. growth for *in vitro* conservation, as well as the effects of benzylaminopurine (BAP) on the regrowth phase.

MATERIALS AND METHODS

Plant material and incubation conditions

Seeds taken from the mature fruit of *G. americana* L., occurring in a natural population in Cruz das Almas, BA, Brazil (12°39'10.11''S; 39°07'19.02''W)- CZA accession, were washed with running tap water for 30 min and maintained for 24 h at room temperature. The seeds were then disinfected in a laminar flow cabinet by immersion in 70% (v/v) ethanol for 60 s, followed by 20 min in 2.5% sodium hypochlorite (NaOCl) containing two drops of Tween 20. After that, the seeds were rinsed three times with sterile distilled water to remove minor amounts of disinfection liquid.

Five seeds were inoculated into 250 ml flasks containing 30 ml of MS culture medium (Murashige and Skoog, 1962) with 30 g L^{-1} of sucrose and 4.5 g L^{-1} of Phytagel™ (Sigma-Aldrich Co, Saint Louis, MO, USA) to grow healthy plants for a period of 90 days, which were then used in all the experiments.

The experiments were conducted in the Plant Tissue Culture Laboratory at Embrapa Tabuleiros Costeiros, Aracaju, Brazil. The pH of the culture media was adjusted to 5.8 ± 0.1, and the media were converted to gels with 4.5 g L^{-1} of Phytagel™, followed by autoclaving for 20 min at a temperature of 121 ± 1°C and a pressure of 1.05 atm. The cultures were maintained in a temperature-controlled growth room at 25 ± 2°C, with an average relative humidity of approximately 70% and a light/dark cycle with 12 h of light and a luminosity of 60 µmol $m^{-2}s^{-1}$.

In vitro conservation of *G. americana* L.

To study the effects of ABA and the salt and sugar concentrations of the MS culture on the *in vitro* growth, the *in vitro*-germinated plants were transferred to conservation media (CM) containing, for the assay 1, MS medium salts (Murashige and Skoog, 1962) supplemented with 30 g L^{-1} sucrose and one of five concentrations of abscisic acid (ABA) (0, 0.5, 1.0, 2.0 or 4.0 mg L^{-1}). For the assay 2, different MS salt and sucrose concentrations were used as follows: T1- MS medium + 30 g L^{-1} sucrose, T2- ½ MS + 15 g L^{-1} sucrose, T3- ½ MS + 30 g L^{-1} sucrose, T4- ¼ MS + 15 g L^{-1} sucrose, T5- ¼ MS + 30 g L^{-1} sucrose. All of the media were gelled with 4.5 g L^{-1} Phytagel™.

In vitro regrowth of *G. americana* L.

Nodal segments (apical, medial and basal) from adventitious shoots taken from the *G. americana* L. plants conserved *in vitro* for 150 days under the conditions described above were transferred to growth media. The explants were inoculated into 250 ml flasks, sealed with plastic lids, containing 30 ml of MS culture medium (MS) with 4.5 g L^{-1} Phytagel™ and supplemented with 0 to 1 mg L^{-1} BAP and 30 g L^{-1} sucrose.

Data collection and statistical analysis

The length of the aerial portion (LAP), number of leaves (NL), number of leaves with abscissions (NLA) and culture viability (CV) were evaluated in the *in vitro*-conserved plants at 60, 90, 120 and 150 days. The plant viability (plant response under conditions of limited growth) was quantified on a scale with the following categories: 5- the leaves and buds are completely green, 4- the leaves are starting to dry and die, 3- 30-50% of the leaves and buds are dry and dead, 2- more than 50% of the leaves and buds are dry and dead and 1- the leaves and buds are completely dead. The percentage of the explants with morphogenic responses was record after 50 days in the regrowth medium. The plants were considered to have a morphogenic response if they showed signs of direct or indirect organogenesis and/or callogenesis.

The *in vitro* conservation assays had a completely randomized experimental design with five treatments and four replicates, and each treatment consisted of 20 flasks with one plant/flask. The

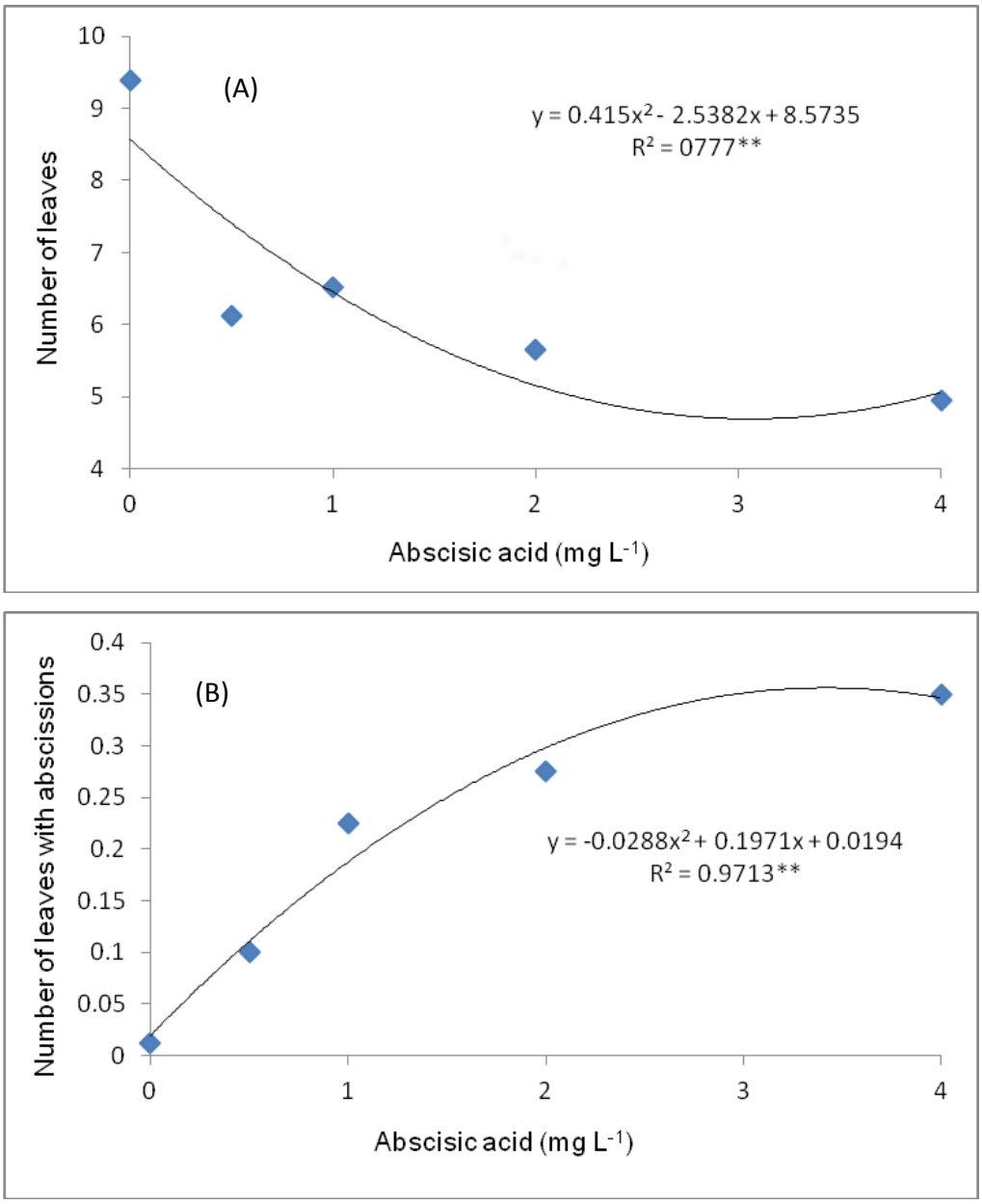

Figure 1. (A) Number of leaves (NL); (B) Number of leaves with abscissions (NLA) on *G. americana* L. plants as a function of the ABA concentration after 150 days of *in vitro* culture.

regrowth assays were completely randomized, while accounting for the factors in the *in vitro* conservation step (five treatments) combined with three types of explants and two concentrations of BAP (factorial 5 x 3 x 2) for a total of 30 treatments with three replicates each. An experimental parcel contained three flasks (one explant/flask). The data of the *in vitro* conservation assays containing ABA were used for the analysis of variance with the F-test and to calculate the fitted equations using polynomial regression. The data of the *in vitro* conservation assay containing various amounts of MS salts and sucrose were submitted to an analysis of variance and compared with the Tukey test at 1 and 5% probability. The data from the regrowth assays were compared using the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Effects of ABA, MS salts and sucrose concentrations on the *in vitro* slow-growth of *G. americana* L.

ABA significantly affected the NL and the NLA on the *G. americana* L. plantlets. The NL exhibited a quadratic behavior with a gradual reduction as a function of increasing ABA concentration (Figure 1A), as seen, increasing concentrations of ABA led to increased NLAs (Figure 1B). Although ABA affected the NL and NLA, a

Table 1. Number of leaves (NL), length of the aerial portion (LAP), number of leaves with abscissions (NLA) and culture viability (CV) in different concentrations of MS salts and sucrose.

Treatments	NL	LAP (cm)	NLA	CV
MS + 30 g L^{-1} sacarose	8.79±1.82^{c}	4.69±0.62^{b}	0.08±0.14^{a}	4.10±0.63^{c}
½ MS + 15 g L^{-1} sacarose	9.46±1.57^{bc}	5.37±0.40^{a}	0.08±0.18^{a}	4.73±0.35^{ab}
½ MS + 30 g L^{-1} sacarose	10.13±2.08^{ab}	4.73±0.77^{b}	0.05±0.10^{a}	4.44±.52^{bc}
¼ MS + 15 g L^{-1} sacarose	11.00±1.76^{a}	5.47±0.22^{a}	0.15±0.56^{a}	4.63±0.28^{ab}
¼ MS + 30 g L^{-1} sacarose	11.20±1.61^{a}	5.12±0.26^{ab}	0.00^{a}	4.94±0.11^{a}
Probability (P > F)	0.00001	0.00001	0.5655	0.00001
VC (%)	13.56	10.26	17.16	8.97

Averages in the same column followed by the same letter are not significantly different at a 5% probability level according to the Tukey test. VC= Variation Coefficient.

Table 2. Number of leaves (NL), length of the aerial portion (LAP), number of leaves with abscissions (NLA) and culture viability (CV) as a function of time cultured in different concentrations of MS salts and sucrose.

Time (days)	NL	LAP (cm)	NLA	CV
60	8.72±0.82^{b}	4.916±0.45^{a}	0.04±0.09^{a}	4.78±0.35^{a}
90	9.32±1.51^{b}	4.984±0.59^{a}	0.03±0.08^{a}	4.64±0.47^{a}
120	10.78±1.84^{a}	5.127±0.60^{a}	0.04±0.11^{a}	4.51±0.51^{ab}
150	11.64±2.01^{a}	5.273±0.64^{a}	0.17±0.05^{a}	4.33±0.55^{b}
Probability (P > F)	0.00001	0.0816	0.2453	0.0018
VC (%)	13.56	10.26	17.16	8.97

Averages in the same column followed by the same letter are not significantly different at a 5% probability level according to the Tukey test. VC= Variation Coefficient.

significant effect on the LAP (cm) and plant viability were not observed.

Although ABA participates in the regulation of many physiological processes of whole plants (Tuteja 2007), it is regarded as an inhibitor of plant growth and therefore usually used as a growth retardant in plant tissue culture (Engelmann, 1991; Rai et al., 2011). Various authors have pointed out that while exogenous ABA alone suppresses shoot regeneration, however the absence of effect on the length of the aerial portion it is possible is linked to genetic characteristics of this species.

There was no significant increase in the variables analyzed as a function of *in vitro* culture time. ABA slowed *G. americana* L. plant growth (number of leaves) at the studied concentrations and may be a promising compound for *in vitro* conservation. Studies with higher concentrations of ABA should be conducted to evaluate the effect on deceleration of growth in length of the aerial part. Regarding the MS salts and sucrose concentrations, the analysis of variance revealed significant effects on the following variables: the NL, LAP and plant viability. The *in vitro* culture time did not significantly affect the LAP or NLA.

We observed that treatments with ¼ of the MS medium salts, independent of the sucrose concentration, led to the formation of larger leaves in the plants and had greater results than the other treatments. The MS and ½ MS media with 30 g L^{-1} sucrose reduced the growth of the aerial portion (Table 1) while maintaining good plant viability.

Decreasing the salt concentrations in the culture media led to an increase in plant development, as indicated by the LAP, contrary to what was expected and what has been observed in several species when used as an *in vitro* conservation strategy (Table 2). According to Malaurie and Borges (2001), reducing the concentration of mineral salts and sucrose in the culture medium allows for the maintenance of plants at a minimal growth rate. The opposite effects observed in this study can probably be explained by endogenous factors.

Similarly, the culture media with ¼ MS salts and 15 or 30 g L^{-1} sucrose show promise for creating *in vitro* germination protocols for *G. americana* L. that decrease production costs by requiring lower concentrations of MS salts than what is required for rapid plant growth (Table 2). In addition, decreasing the salts improved plant viability, and the ¼ MS culture medium with 30 g L^{-1} sucrose had higher values than the other treatments.

The *in vitro* culture time significantly affected the NL and viability. At 150 days, there were significantly more leaves than at the other time points (Table 2). However, the *G. americana* L. plants had better viability at 60 and

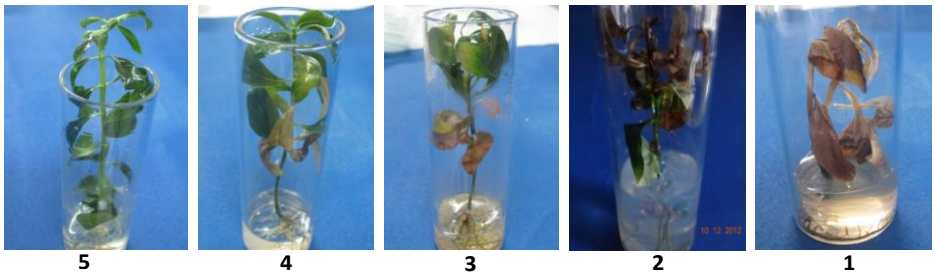

Figure 2. Rating scale for analyzing *G. americana* L. plant viability under slow-growth conditions, 5- the leaves and buds are completely green, 4- the leaves are starting to dry and die, 3- 30-50% of the leaves and buds are dry and dead, 2- more than 50% of the leaves and buds are dry and dead and 1- the leaves and buds are completely dead.

Table 3. Percentage of the morphogenic response of various types of explants (regrowth medium) in the presence of ABA (conservation medium) after 50 days of culture *in vitro*.

Types of explants	Morphogenic response (%)
Basal	78.34±24.84[b]
Medial	90.00±19.03[ab]
Apical	95.00±12.20[a]
Probability (P > F)	0.0200
VC(%)	19.11

Averages in the same column followed by the same letter are not significantly different at a 5% probability level according to the Tukey test. VC= Variation Coefficient.

90 days than at 120 and 150 days. Although the plant viability did decrease, the minimum value for this variable was 4.33 (Figure 2), indicating that the overall plants maintained excellent vigor over the culture period. The MS medium and the medium with ½ the concentration of MS salts with 30 g L^{-1} sucrose decreased the growth rate of the *G. americana* L. plants.

Effects of type of explant and BAP on the *in vitro* regrowth of *G. americana* L. from CM containing ABA

The type of explant and the interaction between the CM and BAP had significant effects during the *in vitro* regrowth of the plants. The presence of ABA in the CM or BAP and the other interactions did not significantly affect the morphogenic response. According to Table 3, the apical segment had the highest morphogenic response (93.33%), and the basal segment had the lowest response (78.34%). Overall, all of the explants had good morphogenic responses. Although ABA decreased the growth rate of the plants, the explants maintained their morphogenic potential during recovery. These results are different from those obtained by Silva and Scherwinski-Pereira (2011), who showed that ABA negatively impacted regenerations from *Piper* sp. explants.

The analysis of the morphogenic response as a function of ABA (in the CM) and BAP revealed that there was not a statistical difference between the explants grown in the presence or absence of BAP (Table 4). However, the explants with 0.5 or 2.0 mg L^{-1} of ABA during the conservation phase that were subcultured in the presence of 1.0 mg L^{-1} BAP had a greater morphogenic response than those maintained in the absence of this regulator. The explants maintained in the absence of BAP during the *in vitro* regeneration step had a larger organogenic response (84.44%). However, the explants in the presence of BAP had an 81.12% regeneration rate *in vitro* with an indirect organogenic response (Table 5). For the factors of the type of explant and the CM (ABA), we observed a morphogenic response with direct and indirect organogenesis, but a higher percentage of the explants had direct organogenesis.

Effects of type of explant and BAP on the *in vitro* regrowth of *G. americana* L. from CM with different sucrose and MS salts concentrations

The CM, BAP and the CM x BAP interaction had a significant effect on *in vitro* regrowth *in vitro,* while the explant type and the other interactions did not. As shown in Table 6, the morphogenic response of the explants maintained in MS medium + 30 g L^{-1} sucrose or ½ MS + 15 g L^{-1} sucrose during the conservation phase was lower

Table 4. Percentage of morphogenic response as a function of ABA (conservation medium) and BAP (regrowth medium) after 50 days of culture *in vitro*.

Morphogenic response (%)		
ABA (mg L^{-1})	**BAP (mg L^{-1})**	
	0	**1**
0	94.45±13.61^{aA}	88.89^{aA}
0.5	66.68^{aB}	94.45^{aA}
1.0	94.45^{aA}	83.34^{aA}
2.0	77.78^{aB}	100.00^{aA}
4.0	88.89^{aA}	83.34^{aA}
Probability (P > F)	0.0185	
VC (%)	19.11	

Averages followed by the same lowercase letter in columns and the same uppercase letter in are not significantly different at a 5% probability level according to the Tukey test. VC= Variation Coefficient.

Table 5. Percentage of morphogenic response as a function of the type of explant, the presence or absence of BAP (regrowth medium) and ABA (conservation medium) after 50 days of culture *in vitro.*

Type of explants	**EOS (%)**	**CALLO (%)**	**DO (%)**	**IO (%)**
Basal	21.67	0	38.33	40
Medial	10	0	45	45
Apical	6.67	1.67	55	36.67
BAP (mg L^{-1})	EOS (%)	CALLO (%)	DO (%)	IO (%)
0	15.56	0	84.44	0
1	10	1.1	7.78	81.12
ABA (mg L^{-1})	EOS (%)	CALLO (%)	DO (%)	IO (%)
0	8.33	0	55.56	36.11
0.5	19.44	0	41.67	38.89
1.0	11.11	0	47.22	41.67
2.0	11.11	0	38.89	50
4.0	13.89	2.78	47.22	36.11

EOS – Oxidized, contaminated and unresponsive plants; CALLO – callogenesis; DO – direct organogenesis; IO – indirect organogenesis.

in the absence of BAP than in the other treatments. These results suggest that the plants maintained in media that slow growth during the conservation phase recover less, necessitating the addition of BAP during the regrowth phase. There was no difference between the morphogenic responses of the explants maintained in the different culture media containing BAP (1 mg L^{-1}), as the media with ½ MS and 30 g L^{-1} sucrose or ¼ MS with 15 or 30 g L^{-1} sucrose resulted in a 100% morphogenic response rate in the explants. The MS medium with 30 g L^{-1} sucrose and ½ MS with 15 or 30 g L^{-1} sucrose had lower morphogenic responses in the absence of BAP than in the presence of BAP (Table 7). The presence of 1.0 mg L^{-1} BAP positively affected regrowth *in vitro*, with a significant increase in the morphogenic response of the explants grown in culture media containing the cytokine.

The inclusion of BAP combined with sucrose and various concentrations of MS salts in the culture media also led to a greater morphogenic response in the explants with callogenesis. In the absence of BAP, more explants showed direct organogenesis (Table 7). Although, the *G. americana* presents open pollination and this aspect may have influenced the higher variation coefficient in the morphogenetic responses, due to greater variation genetics of explants, the type of explant and conservation medium considerably increased regeneration with callus formation and the number of explants with a direct organogenesis response. Further studies should be performed to determine the regrowth medium that promotes the most regeneration in explants maintained under these conditions during the conservation step.

Conclusions

This is the first report on *in vitro* conservation by slow-growth of *G. americana* L., and our study is a

Table 6. Percentage of morphogenic response of the explants from accession CZA as a function of the conservation medium and the presence or absence of BAP (regrowth medium) after 50 days of culture *in vitro*.

Morphogenic response (%)		
Conservation medium	**BAP (mg L^{-1})**	
	0	**1**
MS + 30 g L^{-1} sacarose	16.67^{cB}	75.0^{aA}
½MS + 15 g L^{-1} sacarose	61.11^{bB}	94.45^{aA}
½MS + 30 g L^{-1} sacarose	72.22^{abB}	100.0^{aA}
¼MS + 15 g L^{-1} sacarose	94.45^{aA}	100.0^{aA}
¼MS + 30 g L^{-1} sacarose	88.89^{abA}	100.0^{aA}
Probability (P > F)	0.0212	
VC (%)	24.42	

Averages followed by the same lowercase letter in columns and the same uppercase letter in are not significantly different at a 5% probability level according to the Tukey test. VC= Variation Coefficient.

Table 7. Percentage of morphogenic response as a function of explant type, the presence or absence of BAP (regrowth medium) and the conservation medium after 50 days of culture *in vitro*.

Type of explants	**EOS (%)**	**CALLO (%)**	**DO (%)**	**IO (%)**
Basal	35	41.67	23.33	0
Medial	28.34	43.33	28.33	0
Apical	26.67	16.67	38.33	18.33
BAP (mg L^{-1})	EOS (%)	CALLO (%)	DO (%)	IO (%)
0	43.33	0	56.67	0
1	16.67	67.78	3.33	12.22
Conservation medium	EOS (%)	CALLO (%)	DO (%)	IO (%)
MS + 30 g L^{-1} sacarose	58.33	27.78	8.33	5.56
½MS + 15 g L^{-1} sacarose	36.11	38.89	22.22	2.78
½MS + 30 g L^{-1} sacarose	36.11	33.33	27.78	2.78
¼MS + 15 g L^{-1} sacarose	13.89	36.11	47.22	2.78
¼MS + 30 g L^{-1} sacarose	5.56	33.33	44.44	16.67

EOS – Oxidized, contaminated and unresponsive plants; CALLO – callogenesis; DO – direct organogenesis; IO – indirect organogenesis.

contribution to the germplasm conservation of this tropical multipurpose species. *G. americana* L. *in vitro* cultures can be conserved for five months in MS and ½ MS medium plus 30 g L^{-1} sucrose without losing regeneration capacity. The inclusion of 1 mg L^{-1} BAP in the regrowth medium leads to a greater morphogenic response in explants taken from plants in slow-growth conditions. The apical, medial and basal nodal segments have morphogenic potential during the regrowth phase. Studies with higher concentrations of ABA should be conducted to evaluate the effect on deceleration of growth in length of the aerial part.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

To Brazilian Agricultural Research Corporation (EMBRAPA), National Council for Scientific Development (CNPq) and Technological Support Foundation (FAPITEC/SE) for the financial support and the Coordination for the Improvement of Higher Level -or Education- Personnel (CAPES) for providing scholarship master's degree.

REFERENCES

Charoensub R, Phansiri S (2004). In vitro conservation of rose colored leadwort: Effect of mannitol on growth of plantlets. Kasetsart J. Nat. Sci. 38:97-102.

Coello CY, Miceli CL, Orantes C, Dendoove L, Gutierrez FA (2009). Optimización de reguladores de crecimiento para el cultivo in vitro de la orquídea Guarianthe skinneri (Bateman) Dressier & W.E.Higgins. Gayana Bot. 67(1):19-26.

Engelmann F (1991). *In vitro* conservation of tropical plant germplasm—a review. Euphytica 57:227-243.
Engelmann F (2011). Use of biotechnologies for the conservation of plant biodiversity. *In vitro* Cell. Dev. Biol. Plant 47:5-16.
Harding K, Benson EE, Clacher K (1997). Plant conservation biotechnology: an overview. Agro-Food-Industry Hi-Tech. May/June: 24-29.
Kanchanapoom K, Promsorn N (2012). Micropropagation and in vitro germplasm conservation of endangered Musa balbisiana 'Kluai Hin' (BBB group). Afr. J. Biotecnol. 11:6464-6469.
Lédo AS, Moura CRF, Machado CA, Ramos SRR, Silva AVC, Lédo CAS (2014). Mannitol for coconut ex situ conservation by minimum growth. Pesq. Agropec. Bras. 49:1-5.
Lopez-Puc G (2013). An effective *in vitro* slow growth protocol for conservation of the orchid Epidendrum chlorocorymbos Schltr. Trop. Subtrop Agroecosys. 16:61-68.
Magistrali PR, José AC, Faria JMR, Gasparin E (2013). Physiological behavior of *Genipa americana* L. seeds regarding the capacity for desiccation and storage tolerance. J. Seed Sci. 35:495-500.
Malaurie B, Borges M (2001). Cryopreservation of coconut (Cocos nucifera L.) plumules by encapsulation/dehydration. In: International Workshop on Plant Biotechnology-Plant Breeding and Biotechnology. Bioveg. 2001. Reports…Centro de Bioplantas. Ciego de Avila. Cuba. 2001. P. 59.
Martin KP, Pradeep AK (2003). Simple strategy for the in vitro conservation of Ipsea malabarica an endemic and endangered orchid of the Western Ghats of Kerala, India. Plant Cell Tissue Org. Cult. 74:197-200.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:437-497.
Nogueira FA, Nery PS, Morais-Costa F, Oliveira NJF, Martins ER, Duarte ER (2014). Efficacy of aqueous extracts of Genipa americana L. (Rubiaceae) in inhibiting larval development and eclosion of gastrointestinal nematodes of sheep. J. Appl. Anim. Res. 42:56-360.
Rai MK, Shekhawat NS, Gupta AK, Phulwaria M, Kheta R, Jaiswal U (2011). The role of abscisic acid in plant tissue culture: A review of recent progress. Plant Cell Tiss. Organ Cult. 106:179-190.
Salomão AN (2004). Desiccation, storage and germination of Genipa americana seeds. In: Sacandé M, Joker D, Dulloo ME, Thomsen KA (Ed.) Comparative storage: biology of tropical tree seeds. IPGRI. pp. 263-269.
Silva Tls, Scherwinski-Pereira JE (2011). *In vitro* conservation of Piper aduncum and Piper hispidinervum under slow-growth conditions. Pesq. Agropec. Bras. 46:384-389.
Silva RC, Zanderluce GL, Scherwinski-Pereira JE (2012). Conservação de curto prazo in vitro e propagação em larga escala de genótipos de videira. Pesq. Agropec. Bras. 47(3):344-350.
Srivastava M, Purshottam DK, Srivastava AK, Misra P (2013). *In vitro* conservation of Glycyrrhizaglabra by slow growth culture. Int. J. Bio-Technol. Res. 3:49-58.
Tuteja N (2007). Abscisic acid and abiotic stress signalling. Plant. Signal. Behav. 2:135-138.
Yee TF, Goh CJ, Rao AN (2010). *In vitro* studies on Genipa Americana. J. Trop. Med. Plants 11:71-88.
Yun-Peng D, Wen-Yuan L, Ming-Fang Z, Heng-Bin H, Gui-Xia J (2012). The establishment of a slow-growth conservation system *in vitro* for two wild lily species. Afr. J. Biotechnol. 11:1981-1990.

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Vol. 10(41), pp. 3893-3900, 8 October, 2015
DOI: 10.5897/AJAR2014.9148
Article Number: DD5479855822
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Residuality of exogenous salicylic acid and effect on catalase activity and total antioxidant capacity in tomato leaves

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Received 14 September, 2014; Accepted 25 September, 2015

Salicylic acid (SA) is used in agricultural practices because it induces stress tolerance. The goal of this study was to determine the SA concentration in leaves, the change in catalase (CAT) activity and the change in total antioxidant capacity (TAC) that result from the exogenous application of SA (10^{-4} M) to tomato leaves every 15 days in a greenhouse with and without NaCl application. Fruit yield was also verified. Treatments included SA + 100 mM NaCl, SA + 0 mM NaCl, water + 100 mM NaCl, and water + 0 mM NaCl. As a response to the foliar application of SA, the concentration of SA in leaf tissue exhibited a dynamic behavior that was similar to that reported when SA was induced by environmental stress. A similar phenomenon was observed for CAT and TAC activity after application of SA to leaves: CAT and TAC values increased at 24 h, reached their highest values at 48 h and began decreasing at 144 h, closely following the initial dynamics of SA concentration after each application. The spray application of SA in the absence of NaCl significantly increased the concentration of SA (up to 145 and 289% for the first and second application respectively) and CAT activity (up to 182% for the third application) in leaves, without affecting fruit yield. The application of SA in combination with 100 mM NaCl had the highest values of SA (up to 381 and 258% for the second and third application respectively) and CAT activity (up to 142 and 294% for the first and third application respectively), without changing consistently the TAC of the leaves and without any effect on fruit production. The application of 100 mM NaCl did not affects the CAT activity, TAC or SA into the leaves, or fruit production.

Key words: Plant stress tolerance, elicitors, salicylates, antioxidants.

INTRODUCTION

Salicylates are involved in the control of plant growth and development and also function as signals in environmental adjustment processes (Ashraf et al., 2010). Salicylic acid (SA) is a natural plant salicylate that

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regulates growth and tolerance to abiotic (Hussain et al., 2011) and biotic stresses. Specifically, SA aids in tolerance to biotic stress by regulating internal signals or activating genes that stimulate the production of various plant defense compounds, including those involved in responses to disease, pathogens and predators (Zhang et al., 2009). The application of exogenous salicylic acid (1) induces stress tolerance mechanisms, thereby improving the biological response of the plant to adverse growth factors (Poór et al., 2011) and (2) modifies a large number of metabolic and physiological responses, including the concentration of photosynthetic pigments, minerals, carbohydrates, proteins (Azooz and Yousef, 2010), metabolites of the phenylpropanoid pathway (Mustafa et al., 2009) and certain antioxidants (Azooz, 2009).

The utilization of SA in agricultural practices has yielded beneficial results in diverse crops (Noreen and Ashraf, 2009; El-Khallal et al., 2009; Azooz and Youssef, 2010), and it has become clear that the commercial use of these compounds decreases pesticide use (Esmailzadeth et al., 2008). Scientific literature and technical studies performed for the development and legal registration of products that contain such tolerance inductors as SA (with the objective of modifying tolerance to a certain environmental factor or increasing plant yield) describe the biochemical, physiological and morphological changes that occur in plants in response to the application or induction of SA (Azooz and Youssef, 2010). However, little is known about SA concentration dynamics when it is applied exogenously to plant tissues, and the effect of SA on certain biochemical traits, such as antioxidant capacity, is unclear. This information would aid in improving the agricultural use of this compound.

Our present study aimed to determine changes in (1) SA concentration, (2) catalase (CAT) activity and (3) total antioxidant capacity (TAC) after exogenous application of SA to tomato (*Solanum lycopersicum* L.) in a greenhouse under two salinity conditions (induced with NaCl). The effect of these factors on fruit production was also verified.

MATERIALS AND METHODS

Location of the assay

The study was performed using the facilities of the Universidad Autónoma Agraria Antonio Narro, Buenavista, Saltillo, Coahuila, México.

Plant material

Tomato (*Solanum lycopersicum* L.) cv. Rio Grande (Petoseed) plants were used for all experiments. Seeds were planted in Berger BM2® commercial mix (thin peat:perlite:vermiculite 70:15:15 v/v) in polystyrene trays with 200 cavities. When the plants had produced three true leaves, the seedlings were transplanted to 20 L pots containing the same substrate. Plants were fertilized beginning on the fifth day after transplant using a Steiner solution (Steiner, 1961) diluted to 25%; when the first fruits appeared (35 days after transplant), full-strength (100%) Steiner solution was applied.

SA and NaCl treatment and application

Four treatments were utilized to examine the effects of two experimental factors: SA application to leaves and NaCl application to soil. SA was applied to leaves at a concentration of 10^{-4} M (13.8 mg L^{-1}) using reactive grade SA ($C_7H_6O_3$, CAS 67-72-7, CTR Scientific, Mexico), and NaCl was applied to soil at a concentration of 0 or 100 mM in culture medium. The four treatments were as follows: (1) Application of SA to leaves + 0 mM NaCl to soil; (2) Application of SA to leaves + 100 mM NaCl to soil; (3) application of water to leaves + 100 mM NaCl to soil, and (4) Application of water to leaves + 0 mM NaCl to soil (control). Each treatment was repeated 50 times.

Water and SA were applied to leaves at 15, 30 and 45 days after transplant during the early morning with a manual sprinkler using volumes that were large enough to achieve total coverage of the leaf area. A plastic barrier was set in between pots to prevent contamination between treatments.

NaCl (CTR Scientific, Mexico) was added to Steiner nutritional solution (Steiner, 1961) and applied beginning at 10 days after transplant. This solution was applied every two days until day 30 after transplant and daily thereafter until day 60. For all treatments, the substrate was washed every 10 days with water (1 L per pot) to avoid excessive accumulation of salts.

Collection of plant material for the determination of salicylic acid content, catalase activity and total antioxidant capacity

A single plant was one experimental unit, and the third completely developed leaf starting from the apex was used as a sample for evaluation. Plants that had already been sampled once were not used for subsequent evaluations. For each variable, treatment and sampling time, the leaves of two different plants (biological replicates) chosen at random were collected.

Leaf material used for the determination of salicylic acid and catalase activity was collected prior to application of treatments and at 12, 24, 48, 144 and 240 h after SA application. Leaf samples used for measuring total antioxidant capacity were collected at 48 and 144 h after SA application.

Prior to SA determination, cut leaves were wrapped in aluminum foil and were immediately submerged in liquid nitrogen for subsequent storage at -80°C. Samples used for verifying catalase activity and total antioxidant capacity were placed in a cooler at 4ºC and in liquid nitrogen, respectively, and were taken to the laboratory to be processed immediately.

Determination of SA

SA determination was performed using the modified method of Forcat et al. (2008), which involves (1) preparation of the sample for extraction and (2) determination of the SA concentration in the obtained extract. For (1), frozen plant material was ground in a mortar using liquid nitrogen. Ground material (0.05 g) was subsequently placed in a 1.5 ml microcentrifuge tube and 1 ml of extraction solution (10% methanol, 1% acetic acid, 89% distilled water) was added. The sample was subsequently vortexed for 20 s in a vortex mixer (Scientific products), degassed for 5 min in a Bramson gas stripper (model 1510) and centrifuged in a microcentrifuge (Labnet International, model 24D) for 10 min at 10,000 x g (10,400 rpm). After this procedure, the translucent

supernatant was extracted with a manual pipette and placed into a fresh microcentrifuge tube. The sample was re-extracted thereafter with 0.5 ml of the extraction solution; thus, approximately 1.5 ml of solution containing the compound of interest was obtained. This solution was filtered through a 0.45-µm syringe-driven filter unit (Millipore), and the SA content was determined with an Agilent 1120 liquid chromatograph (LC) using the EZChrom Elite Compact software version 3.3.0B (Agilent 2005-2008). An aliquot (20 µl) was injected into the LC, and the separation was performed using a Phenomenex Luna C18 column (100 × 20 mm) at 35°C using two solvents: A (94.9% H_2O: 5% CH_3CN: 0.1% HCOOH) and B (5% H_2O: 94.9% CH_3CN: 0.1% CHOOH). A gradient from 0 to 100% B was run over 10 min. Each leaf sample was analyzed twice. The results obtained from the chromatograph (in mg L^{-1} of SA) were converted to µg of SA per g fresh weight.

Determination of CAT enzymatic activity

Determination of CAT activity was performed on the same day that leaf samples were collected using the technique described by Masia (1998). Using an AHUS Pioneer analytical balance, 0.5 g of leaf tissue was weighed and subsequently macerated in a mortar prior to the addition of 50 mg of polyvinylpyrrolidone (Sigma-Aldrich) and 5 ml of phosphate buffer solution (100 mM, pH 7.0). The sample was aliquoted into microcentrifuge tubes and centrifuged in a microcentrifuge at 11,000 x g (11,440 rpm) for 11 min. The supernatant was diluted 1:10 with distilled water. From the resulting solution, 1 ml was removed, mixed with 3 ml of 300 µM phosphate buffer and added to 1 ml of H_2O_2 (100 µM). The reaction between CAT and H_2O_2 was terminated after one minute by applying 10 ml of 2% sulfuric acid. This was then titrated with 0.01 N potassium permanganate. Using the same procedure, a blank was produced with distilled water. Catalase activity was determined with the following formula:

$$CAT = \frac{(TV\text{-}MV)\,(KMnO_4 \text{ concentration})}{(\text{Sample / ml of NaKPi})(\text{FD sample / final NaKPi ml})}$$

where TV = volume of $KMnO_4$ consumed in the blank (ml); MV = volume of $KMnO_4$ consumed in the sample (ml); $KMnO_4$ concentration is given in moles; sample = weight of tissue used; NaKPi = sodium phosphate buffer (5 ml); FD sample = final sample dilution; final NaKPi = final amount of sodium phosphate buffer used (8 ml). One unit of catalase was defined as the amount of enzyme required to decompose 1 µM of H_2O_2 per min per g of tissue (Masia, 1998).

Determination of TAC

Determination of TAC was performed on the same day that leaf samples were collected using the technique described by Ramírez et al. (2006). The leaves were frozen in liquid nitrogen and ground in a mortar, and 4 g of the sample was added to 8 ml of 100 mM phosphate buffer (pH 7.0). Next, the sample was vortexed for 2 min using a vortex mixer (Scientific products) and centrifuged at 2880 x g (3000 rpm) for 10 min. The Calbiochem® Total Antioxidant Status Assay Kit (Miller et al., 1993) was used for determination of total antioxidant capacity. Briefly, an aliquot (20 µl) of the supernatant (extract) was combined with 1 ml of the chromogen (metmyoglobin + $ABTS^{TM}$), and the absorbance of the resulting solution was determined at a wavelength of 600 nm using a spectrophotometer (Jenway, model 6320D). Absorbance values were also determined for a blank (distilled water) and a standard (Trolox: 6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid, 2.03 mM). After 3 min, 200 µl of the substrate (stabilized hydrogen peroxide) was added to the experimental, blank and standard samples, and the absorbance at 600 nm was read again to obtain differential (Δ) measurements (Δ = initial absorbance – absorbance after 3 min). The total antioxidant capacity equivalent to Trolox (mM) was determined using the following formula:

$$TAC = \frac{(\text{Concentration of the standard})(\Delta \text{ Blank} - \Delta \text{ Sample})}{(\Delta \text{ Blank} - \Delta \text{ Standard})}$$

Determination of fruit production

Fruit from ten plants exposed to each treatment were collected for five harvests, which determined the total amount of fruit produced between 73 and 105 days after transplant (in g $plant^{-1}$). The fruit were weighed with a digital analytical balance (Explorer Pro).

Experimental design and statistical analysis

The experiment was performed using a randomized complete block design with two replicates. A single plant was the experimental unit, and two plants per assessment per treatment were considered. A total of 50 plants were included in each treatment.

A Wilcoxon matched-pairs test (Statistica for Windows, version 6.0) was used to ascertain differences between the concentrations over time of different compounds into the leaves. Similarly, a cross-correlation function test (Statistica for Windows, version 6.0) was applied to the concentration data to determine possible correlations between the levels of SA and CAT. The results from each sampling period were compared using an analysis of variance and a multiple comparison Tukey test to compare means ($p \leq 0.05$) (Statistical Analysis System for Windows, version 9.0).

RESULTS AND DISCUSSION

Change in SA concentration in leaves

The Wilcoxon test indicated a statistically significant difference ($p \leq 0.05$) between the treatments. The exogenous application of SA to leaves resulted in the highest concentrations of this compound into the leaves (Figure 1) and the salinity condition (100 mM NaCl) did not alter the SA levels into the leaves. Treatments without SA resulted in the lowest SA concentrations into the leaves, and again the salinity condition (100 mM NaCl) did not have any effect. Similar results were reported by Szalai and Janda (2009) who applied 50 and 100 mM NaCl to corn seedlings (*Zea mays* L.) and observed no increase in the concentration of leaf SA. Additionally, Hussain et al. (2011) applied 50 mM NaCl to violets (*Viola odorata* L.) without observing an increase in SA concentration in leaves, and Iqbal and Ahsraf (2006) did not observe a significant change in SA concentration in wheat seeds (*Triticum aestivum* L.) that were germinated in a culture medium containing 150 mM NaCl (electrical conductivity of 15 dS m^{-1}). It is possible that the detection of significant changes in SA concentration depends on (1) the timing of sampling and the NaCl concentration applied and (2) the specific stage of plant development and the species under study.

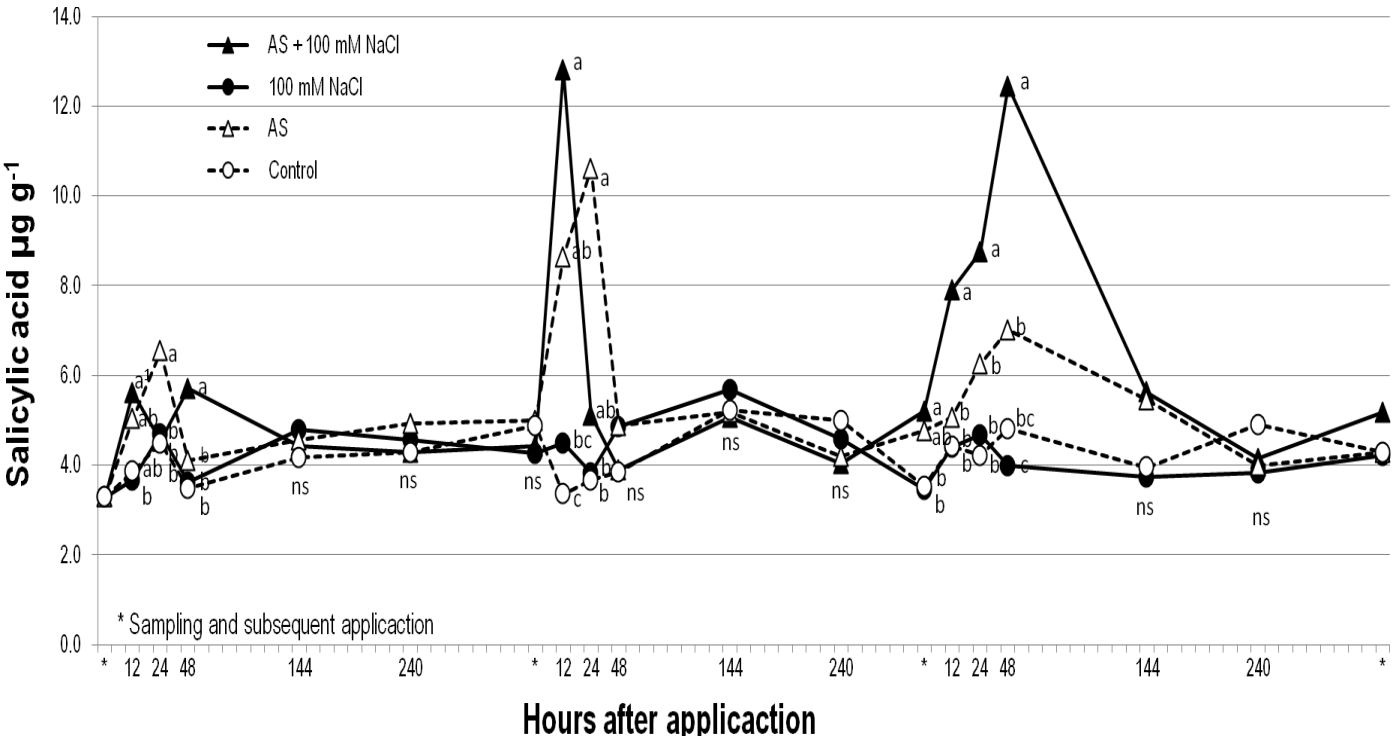

Figure 1. Dynamics of salicylic acid concentration in tomato (*Solanum lycopersicum* L.) leaves as a response to exogenous SA application at 10^{-4} M (13.8 mg L^{-1}) every 15 days under two conditions of salinity (0 and 100 mM NaCl in the culture medium). [1]The averages marked with the same letter are statistically similar (Tukey test, $p < 0.05$).

When comparing different sampling times (Figure 1), where was sprayed SA to plants without salinity conditions the SA concentration in leaf tissue was highest at 24 h after the first SA application, at 12 and 24 h after the second application and at 12, 24, and 48 h after the third application (Figure 1). The SA concentration subsequently decreased and was approximately equal to the control plants at 48 h after the first and second applications and 144 h after the third application. Although SA treatments resulted in similar increases in leaf SA levels in all sampling events regardless of the concentration of NaCl applied, we observed significant differences ($p \leq 0.05$) between the treatments at 24 h after the first application and at 12, 24, and 48 h after the third application, with the highest SA values being observed in cases where NaCl was added (Figure 1). A similar response was described by Esmailzadeth et al. (2008), who applied SA (400 µM) to tomato leaves infected by *Alternaria alternata* and observed the lowest SA values when SA and the pathogen were applied separately. The significant increase in SA concentration in saline-stressed leaves upon foliar SA application (SA on the leaves + 100 mM NaCl in the substrate) could be attributed to the known function of SA as a stress signaling compound and to the induction of a resistance response (Iwai et al., 2007). Plants exposed to the control treatment did not show significant variation in leaf SA concentration throughout the study.

Similar to what was observed in our experiments, an increase and subsequent decrease in SA levels was reported by He et al. (2007) in bean leaves (*Phaseolus vulgaris* L.) when adding the mite *Tetranichus urticae* and by Ogawa et al. (2010) in *Arabidopsis thaliana* leaves when applying mechanical stimuli. These authors also observed peak SA concentrations 24 h after the stimulus was applied with a subsequent decrease in concentration. This decrease in detectable SA (after a concentration peak) after all applications may be due to the transformation of SA into conjugated forms (Klämbt, 1962), which would mainly include salicylic and benzoic glucosides (Mustafa et al., 2009; Owaga et al., 2010), methylated forms or forms activated with coenzyme A.

The concentrations of foliar SA determined in the present study are similar to those reported by He et al. (2007) in bean leaves 24 h after infestation with *T. urticae* mites, with a maximum SA value of 12 µg g^{-1} of fresh weight. Shulaev et al. (1995) observed maximum SA concentrations equivalent to 10 µg g^{-1} fresh weight in tobacco plants (*Nicotiana tabacum* L.) infected with tobacco mosaic virus (TMV) with an increase in SA concentration in tissue closest to the infection site 144 h after the infection began. Moreover, Iwai et al. (2007) determined an SA concentration of 7.3 µg g^{-1} fresh weight when applying probenazole to rice plants (*Oryza sativa*

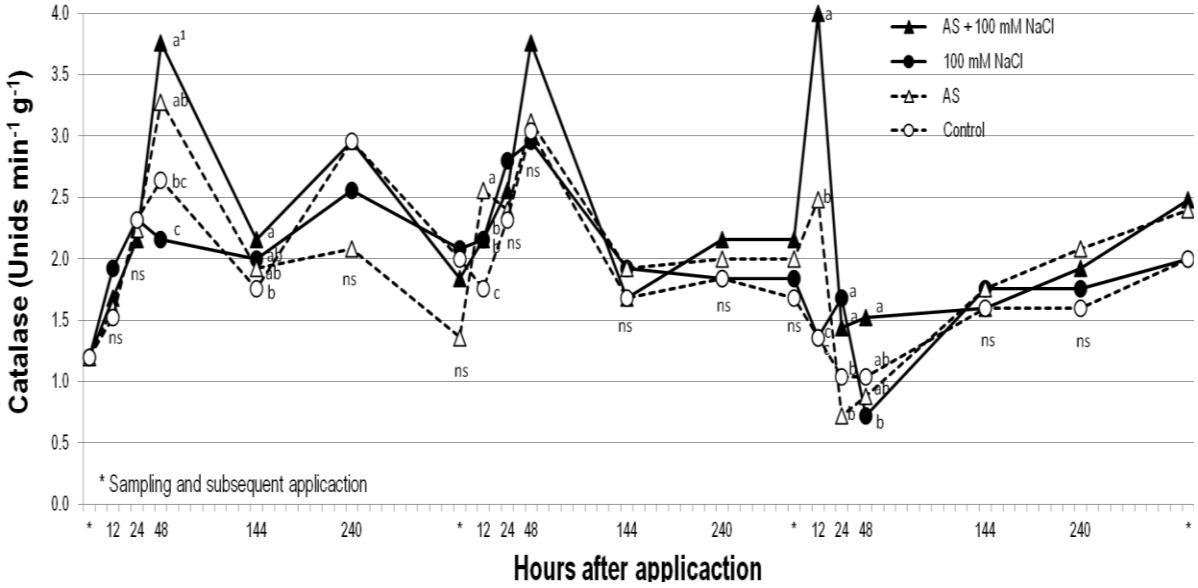

Figure 2. Dynamics of the enzymatic activity of catalase (CAT) in tomato (*S. lycopersicum* L.) leaves in response to exogenous application of salicylic acid (SA) at 10^{-4} M (13.8 mg L^{-1}) every 15 days under two conditions of salinity (0 and 100 mM NaCl in the culture medium). A CAT unit is defined as the amount of enzyme that decomposes 1 µM of H_2O_2 per minute at 25°C. [1]The averages marked with the same letter are statistically similar (Tukey test, $p \leq 0.05$).

L.). Endogenous concentration of SA may depend on the plant species in question, the concentration of applied SA, and the conditions under which the trials are performed, although the apparent transformation of a fraction of the free SA into conjugated forms is consistent even if a high concentration of SA is applied and/or measurements are obtained as early as 30 min (Norman et al., 2004). Even at the lowest concentrations, peaks and declines are observed (Figure 1) which leads to the assumption that SA is incorporated into conjugated molecules, as observed by Yalpani et al. (1991), who reported SA concentrations of 0.35 µg g^{-1} dry weight in tobacco leaves (*Nicotiana tabacum* L.) 144 h after infecting plants with TMV.

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CAT activity

The Wilcoxon test indicated a statistically significant difference ($p \leq 0.05$) in CAT activity among the different treatments. The two treatments in which NaCl was applied showed the highest CAT activity, and this activity was not affected by SA application. The two treatments in which NaCl was omitted induced a lower level of CAT activity than the ones above and did not differ from each other, which indicates that compared with SA; NaCl has a greater effect on CAT. Furthermore, when comparing the different sampling times (Figure 1), the highest CAT activity was consistently observed following treatment with both NaCl and SA (Figure 2). This apparent synergistic effect of NaCl and SA on CAT activity agrees with studies using maize (El-Khallal et al., 2009), and *Vicia faba* (Azooz, 2009). It is possible that the effect of foliar SA on CAT activity is related to the interaction between SA and reactive oxygen species (ROS) formed during oxidative stress induced by NaCl (Miller et al., 2010). To verify this finding, a cross-correlation between two time series was performed, which yielded a significant positive correlation (R=0.571) between the dynamics of foliar SA concentration and CAT activity in the treatment with NaCl and SA. The correlation was not significant ($p > 0.05$) for any of the other treatments. This result may indicate that the interaction of SA and NaCl modifies the redox balance by stimulating the formation of ROS and H_2O_2, which, in turn, stimulates the accumulation of antioxidant enzymes, such as CAT (Miller et al., 2010).

The changes in CAT activity following foliar SA application maybe were related to increases in the concentration of free radicals or compounds derived from them, such as H_2O_2 (Srivalli et al., 2003). Furthermore, Feieraband and Dehne (1996) indicated that the decreases following peaks of maximum CAT activity may result from the presence of a greater number of antioxidant molecules, such as peroxidase ascorbate, as was observed in corn by El-Khallal et al. (2009). Alternatively, the activity of proteases that degrade CAT may be induced by high levels of (or long periods of exposure to) a particular stress factor (Noreen and Ashraf, 2009).

Total antioxidant capacity (TAC)

The Wilcoxon test indicated statistically significant

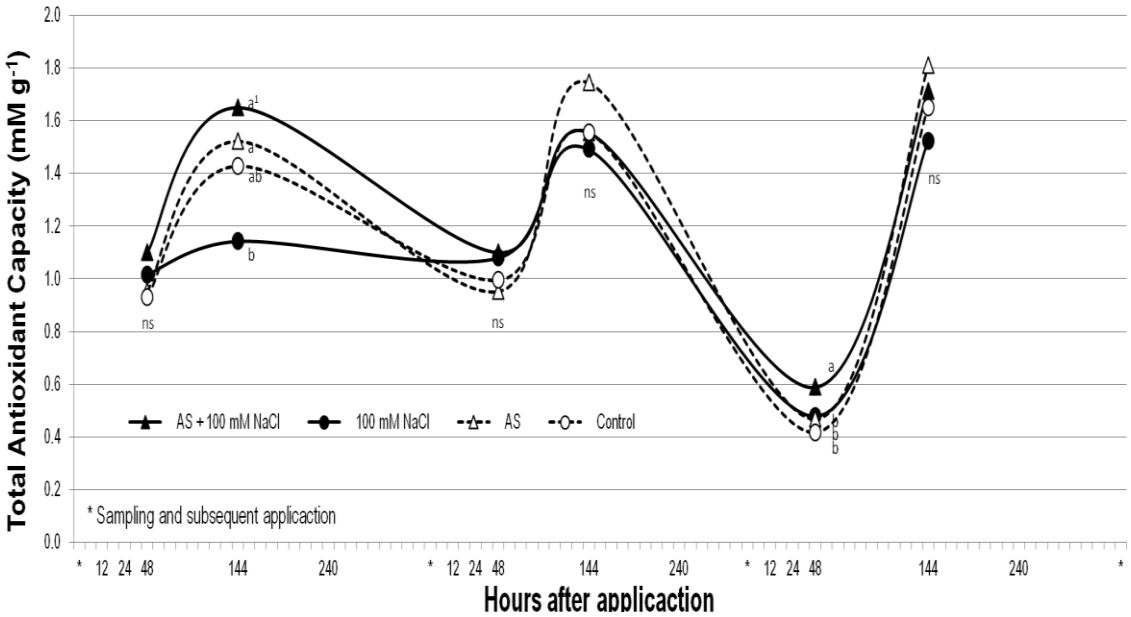

Figure 3. Dynamics of the concentration of total antioxidant capacity (TAC) in tomato (*Solanum lycopersicum* L.) leaves in response to the exogenous application of salicylic acid (SA) at 10^{-4} M (13.8 mg L^{-1}) every 15 days under two conditions of salinity (0 and 100 mM NaCl in the culture medium). [1]The averages marked with the same letter are statistically similar (Tukey test, $p \leq 0.05$).

differences ($p \leq 0.05$) between the treatments. Foliar application of SA induced the highest level of TAC in one sample at 144 h after application, while the application of NaCl did not have any effects. This response is attributed to the capacity of SA to modify the cellular redox balance by activating certain responses associated with oxidative stress, which, in turn, trigger the accumulation of antioxidant compounds (Alvarez, 2000).

The TAC of the foliar extract obtained at the different sampling times was higher at 144 h after first application in the SA application with or without salinity condition, and at 48 h after third application (Figure 3). We have no explanation about the increase of TAC in control treatment at 144 h after the second and third application, maybe has relation with an increase of midday maximum temperature into the greenhouse from 30 to 34°C, which could give rise to changes in the concentration of antioxidants (Sairam et al., 2000; Almeselmani et al., 2006).

Fruit production

Fruit production from the five cuts was not significantly different ($p > 0.05$) between plants exposed to the control treatment and those treated with foliar SA applications (Figure 4). Other authors reported increases in fruit yield (Yildirim and Dursan, 2008) after SA application. However, SA induces a wide range of metabolic responses that are generally directed toward adjusting the redox balance in the photosynthetic machinery under conditions of environmental stress. In the case of stress induced by salinity, adjustments are primarily made in the levels of antioxidant compounds that alleviate oxidative stress, and these adjustments do not always result in an increase in fruit yield (Joseph et al., 2010).

Conclusions

Foliar application of salicylic acid to tomato plants showed a residual effect in the concentration of this compound in leaves that lasted up to 48 h after application. The effect was evident in plants with and without NaCl treatment. According to the results of this assay, SA applications on tomato should be performed within a minimum interval of six to 10 days in order to maintain the SA concentration higher than the basal ones. The addition of 100 mM NaCl to the nutritive solution did not modify the catalase activity, the total antioxidant capacity, salicylic acid concentration in leaves or fruit production. This trend was modified by the application of salicylic acid, which in combination with NaCl application increased the salicylic acid concentration, catalase activity and the total antioxidant capacity on leaf tissue but did not modify fruit production. The application of salicylic acid over plants in absence of NaCl stress increased the catalase activity and the total antioxidant capacity of foliar tissue without modifying fruit production.

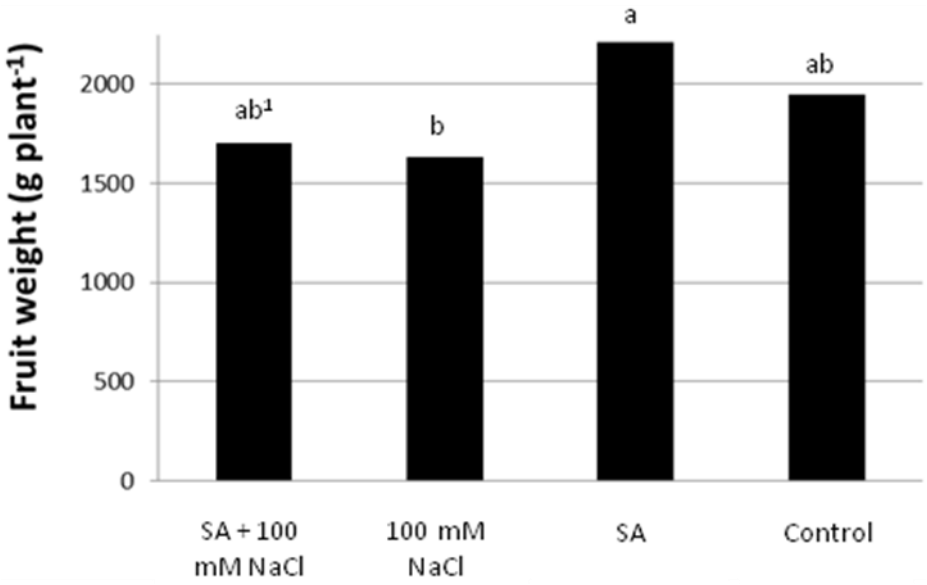

Figure 4. Tomato fruit production (g/plant) harvested between 73 and 105 days after plants were subjected to exogenous application of salicylic acid (SA) at 10^{-4} M (13.8 mg L^{-1}) and NaCl at 100 mM. [1]The averages marked with the same letter are statistically similar (Tukey test, $p > 0.05$).

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENTS

Author Guzmán-Téllez thanks CONACYT for the financial support.

REFERENCES

Almeselmani M, Deshmukh P, Sairam R, Kushwaha S, Sing T (2006). Protective roll of antioxidant enzymes under high temperature stress. Plant Sci. 171:382-388.

Alvarez M (2000). Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Plant Mol. Biol. 44:429-442.

Ashraf M, Akram NA, Arteca RN, Foolad MR (2010). The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Crit. Rev. Plant Sci. 29:162-190.

Azooz M (2009). Salt stress mitigation by seed priming with salicylic acid in two faba bean genotypes differing in salt tolerance. Int. J. Agric. Biol. 11:343-350.

Azooz M, Youseef M (2010). Evaluation of heat shock and salicylic acid treatments as inducers of drought stress tolerance in wheat. Am. J. Plant Physiol. 5:56-70.

El-Khallal S, Hathout T, Ahsour A, Kerrit A (2009). Brassinolide and salicylic acid induced antioxidant enzymes, hormonal balance and protein profile of maize plants grown under salt stress. Res. J. Agric. Biol. Sci. 5:391-402.

Esmailzadeth M, Soleimani M, Rouhani H (2008). Exogenous application of salicylic acid for induced systemic acquired resistance against tomato steam canker disease. J. Biol. Sci. 8:1039-1044.

Feieraband J, Dehne S (1996). Fate of the prophyrin cofactors during the light-dependent turnover of catalase and the photosystem it reaction-center protein DI mature rye leaves. Planta 198:413-422.

Forcat S, Bennett N, Mansfield J, Grand M (2008). A rapid and robust method for simultaneously measuring change in the phytohormones ABA, JA y SA in plants following biotic and abiotic stress. Plant Methods 4:16.

He W, Li H, Li X, Li M, Chen Y (2007). *Tetranynchus urticae* Koch induced accumulation of salicylic acid in frijole leaves. Pesticide Biochem. Physiol. 88:78-81.

Hussain M, Nawaz K, Majeed A, Ilyas U, Lin F, Ali K, Nisar MF (2011). Role of exogenous salicylic acid applications for salt tolerance in violet. Sarhad J. Agric. 27:151-175.

Iqbal M, Ahsraf M (2006). Wheat seed priming in relation to salt tolerance: growth, yield and levels of free salicylic acid and polyamides. Ann. Bot. Fennici 43:250-259.

Iwai T, Seo S, Mitsuhara I, Ohashi K (2007). Probenazole induced accumulation of salicylic acid confers resistance to *Magnaporte griseain* adults plants rice. Plant Cell Physiol. 48:915-924.

Joseph B, Jini D, Sujatha S (2010). Insight into role of exogenous salicylic acid on plants growth under salt environment. Asian J. Crop Sci. 2:226-235.

Klämbt H (1962). Conversion in plants of benzoic acid to salicylic acid and its βd-glucoside. Nature 196:491.

Masia A (1998). Superoxide dismutase and catalase activities in apple fruit during ripening and postharvest and with special reference to ethylene. Physiol. Plant 104:668-672.

Miller N, Rice-Evans C, Davies M, Gopinathan V, Milner A (1993). A novel method for measuring antioxidant capacity and its application to monitory the antioxidant status in premature neonates. Clinical Sci. 84:407-412.

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010). Reactive oxygen species homeostasis and signaling during drought and salinity stress. Plant Cell Environ. 33:453-467.

Mustafa N, Kin H, Choi Y, Vepoorte R (2009). Metabolic changes in salicylic acid-elicited *Catharanthus roseus* cell suspension cultures monitored by NMR-based metabolic. Biotechnol. Lett. 31:1967-1974.

Noreen S, Ashraf M (2009). Assessment of variation in antioxidative defense system in salt treated pea (*Pisum sativum*) and its putative

use as salinity tolerant markets. J. Plant Physiol. 166:1764-1774.
Norman C, Howell K, Millar A, Whelan J, Day D (2004). Salicylic acid is an uncoupler and inhibitor of mitochondrial electron transport. Plant Physiol. 34:492-501.
Ogawa T, Ara T, Aoki K, Suzuki H, Shibata D (2010). Transient increase in salicylic acid and its glucose conjugates after wounding in *Arabidopsis* leaves. Plant Biotechnol. 27:205-209.
Poór P, Gemes K, Horváth F, Szepesi A, Simon ML, Tari I (2011). Salicylic acid treatment via the rooting medium interferes with stomatal response, CO_2 fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. Plant Biol. 13:105-114.
Ramírez H, Rancaño-Arrioja JH, Benavides-Mendoza A, Mendoza-Villareal R, Padron-Corral E (2006). Influencia de promotores de oxidación controlada en hortalizas y su relación con antioxidantes. Revista Chapingo Serie Horticultura 12:189-195.
Sairam R, Srivastava, G, Saxena D (2000). Increased antioxidant capacity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. Biol. Plant. 43:245-251.
Shulaev V, León J, Raskin I (1995). Is salicylic acid a translocated signal of systemic acquired resistance in Tobacco? Plant Cell 7:1691-1701.
Srivalli B, Sharma G, Khanna-Chopra R (2003). Antioxidative defense systems in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. Physiol. Plant. 119:503-512.
Steiner B (1961). A universal method for preparing nutrient solution of a certain desired composition. Plant Soil 16:134-154.
Szalai G, Janda T (2009). Effect of salt stress on the salicylic acid synthesis in young maize (*Zea mays* L.) plants. J. Agron. Crop Sci. 193:165-171.
Yalpani N, Silverman P, Wilson T, Kleier D, Raskin I (1991). Salicylic acid is a systemic signal of pathogenesis related protein in virus infected tobacco. Plant Cell 3:809-818.
Yildirim E, Dursan E (2008). Effect of foliar salicylic acid applications on plant growth and yield of tomato under greenhouse condition. Acta Hortic. 807:395-400.
Zhang Z, Li Q, Li Z, Staswick P, Wang M, Zhu Y, He Z (2009). Dual regulation role of GH3.5 in salicylic acid and auxin during *Arabidopsis-Pseudomonas syringae* interaction. Plant Physiol. 145:450-464.

academicJournals

Vol. 10(41), pp. 3901-3908, 8 October, 2015
DOI: 10.5897/AJAR2015.10165
Article Number: 4E59E2D55845
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Adaptability and stability of cowpea genotypes to Brazilian Midwest

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Received 15 July, 2015; Accepted 21 August, 2015

This study aimed to evaluate cowpea genotypes for adaptability and stability of grain yield using different methods, in order to support the launch and/or cultivars recommendation for agribusiness in the Brazilian Midwest. Trials were conducted from 2010 to 2012 in four locations, totaling eight environments. We evaluated 20 cowpea genotypes arranged in a randomized block design with four replications. Studies of adaptability and stability of cowpea genotypes were estimated using different methods. The environments A1, A5, A6 and A8 are the most appropriate for selecting superior genotypes for adaptability and stability. The genotypes MNC03-737F-5-1 BRS-Tumucumaque, BRS-Guariba, MNC02-684F-5-6, MNC03-725F-3, MNC02-682F-2-6, BRS-Cauamé, BRS-Itaim and MNC03- 737F-5-11 showed adaptability and stability sufficient for recommendation for the region. Eberhart and Russell, Lin and Binns and AMMI methods can be used as a complementary way to better predict the genotype performance.

Key words: Genotype, environment interaction, selection *Vigna unguiculata.*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) has a great socioeconomic importance, both as food and as a generator of employment and income. It is rich in protein, minerals and fiber (Frota et al., 2008) and constitutes a basic food component of rural and urban populations in North and Northeast of the Brazil. Currently the use of cowpea has expanded more intensively to the Midwest region of Brazil.

Small farmers, who still practice traditional farming, cultivate cowpea, in North and Northeast of the Brazil. Large scale cultivation of cowpea in the states of Mato Grosso and Mato Grosso do Sul, in the Brazilian Midwest began from 2006, and the production comes mainly from medium and large entrepreneurs, who adopt modern technologies for crop production with an average productivity of 960 kg ha^{-1} (Freire Filho et al., 2011).

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Table 1. Characteristics of each experimental environment.

Environments	Year	Municipality	Altitude (m)	Latitude	Longitude	Biome	Climate	Soil
A1	2010	Aquidauana	430	22°01'S	54°05'W	Pantanal	AW	Oxisol red
A2	2010	Chapadão do Sul	790	18°05'S	52°04'W	Cerrado	AW	Oxisol red
A3	2010	Dourados	147	20°03'S	55°05'W	Mata Atlântica	CW	Utisol red
A4	2011	Chapadão do Sul	790	18°05'S	52°04'W	Cerrado	AW	Oxisol red
A5	2011	Dourados	147	20°03'S	55°05'W	Mata Atlântica	CW	Utisol red
A6	2011	Chapadão do Sul	790	18°05'S	52°04'W	Cerrado	AW	Oxisol red
A7	2012	Aquidauana	430	22°01'S	54°05'W	Pantanal	AW	Oxisol red
A8	2012	Dourados	147	20°03'S	55°05'W	Mata Atlântica	CW	Utisol red

An understanding of the genotype and environmental interactions in cow pea is important both for crop improvement and to ascertain the performance of genotypes to variations in the environmental factors. Despite of its importance most studies on cowpea have neglected the interactions between genotypes and environment, not providing detailed information on the performance of each genotype front to environmental variations. An analysis of adaptability and stability is essential to identify varieties with predictable performance and that are responsive to environmental variations in specific or wide conditions, making more reliable the recommendation of cultivars (Cruz et al., 2012).

Adaptability refers to the ability of genotypes to take advantage of the existing environmental conditions, whereas stability relates to the ability of a genotype response in a highly predictable manner to an environmental condition (Cruz et al., 2012). The choice of methods used for analysis depends on the experimental data, mainly related to the number of available environments, the precision required and the type of desired information. Several methods have been used to study the adaptability and stability of genotypes in cowpea (Ojo et al., 2006; Akande, 2007; Rocha et al., 2007; Barros et al., 2013).

The main purpose of a breeding program is launch superior cultivars to those existing. Thus, the lines are intensively evaluated for several agronomic characters. However, the relative ranking of the lines, in most cases, is not consistent, since variations may occur because of the interactions between genotype and environment. Therefore, this interaction takes leading role in the process of cultivar recommendation. In this way, cultivars with high yielding potential and good adaptability and stability, could be recommended with greater reliability for Brazilian regions.

Though several previous studies on cowpea have shown high genetic divergence (Santos et al., 2014a), as evidenced through correlations and path analysis (Santos et al., 2014b), there are few studies about the adaptability and stability of cowpea genotypes. Therefore, the aim of this study was to evaluate selected cowpea genotypes for the adaptability and stability of grains using different methods, in order to introduce and/or recommend cultivars for the crop agrobusiness in the Brazilian Midwest.

MATERIALS AND METHODS

Trials were conducted from February to July of 2010, 2011 and 2012 in the municipalities of Aquidauana, Chapadão do Sul and Dourados, located in the Midwest region of the Brazil. The trials in Chapadão do Sul were carried out only in 2010 and 2011, totaling eight environments (Table 1). It was used a randomized block design with four replications and 20 genotypes. Experimental plot consisted of four rows with five meters of length, spaced 0.50 m apart, considering as useful area the two central lines. The seeds of cowpea genotypes used in the trial were obtained from the Embrapa Meio-Norte, which included 16 pure lines and four commercial cultivars.

The trials were carried out in February, April and March, in Dourados, Aquidauana and Chapadão do Sul, respectively. Fertilization at the time of sowing consisted of 200 kg ha^{-1} of the commercial formulates 04-20-20 of NPK. One week after seedling emergence manual thinning was done, allowing eight plants per meter.

Data were subjected to joint analysis of variance and subsequently the adaptability and stability analysis through the methods of Eberhart and Russell (1966), Carneiro (1998) and AMMI model (Gauch and Zobel, 1988). Eberhart and Russell's (1966) regression adopted was $Y_{ij} = m_i + b_i I_j + d_{ij} + e_{ij}$, wherein Y_{ij} is the mean observed of the genotype *i* in the environment *j*; d_{ij} is the regression deviation of the genotype *i* in the environment *j*; e_{ij} is the mean error associated with the average. The environmental index was estimated according to the equation $I_j = \overline{Y}_j - \overline{Y}_m$, with $\sum_{j=1}^{n} I_j = 0$, wherein $\overline{Y}_m$ is the overall mean; $\overline{Y}_j$ is the mean in the environmental *j*; *n* is the number of environments.

According to Eberhart and Russell (1966) method, genotype adaptability was measured by the parameter β_{1i}, while the behavior stability was evaluated by the variance of the regression deviations (σ^2_{di}) and by coefficient of determination (R^2) that, according to Cruz et al. (2012), is an auxiliary measure for assessing stability. When σ^2_{di} is significant and R^2 is higher than 80%, R^2 indicates acceptable predictability.

Carneiro (1998) adopts thr P_i measure as the mean square distance between the genotype *i* and the genotype with maximum response to the environment *j*, in the following way: $P_i = \sum_{j=1}^{n} \frac{(Y_{ij} - M_j)^2}{2n}$, wherein P_i is the superiority index of genotype *i*;

Table 2. Resume from the joint analysis of variance according to Eberhart and Russell method, regarding to yield grain (kg ha^{-1}), of 20 cowpea genotypes obtained in eight environments, from 2010 to 2012.

Sources of variation	Degrees of freedom	F-value	% variation
Environment (E)	7	258.88*	78.27
Genotype (G)	19	7.34*	6.02
G x E	133	2.73*	15.70
E/G	140	15.54*	-
Linear E	1	1812.14*	83.29
Linear G x E	19	3.95*	3.44
Combined deviation (E/G)	120	2.40*	13.25
Residue	477	-	-

*Significant (P ≤ 0.01) by F-test.

Y_{ij} is the yield of the genotype *i* in the environment *j*; M_j is the yield of the genotype with maximum response among all genotypes in in the environment *j*; *n* is the number of environments. This expression can be unfolded into:

$$P_i = \frac{[n(Y_i - M_g)^2 + \sum_{j=1}^{n}(Y_{ij} - Y_g - M_j + M_g)^2]}{2n},$$

wherein Y_g is the mean of the genotype *i* and M_g is the mean of the genotypes with maximum response. Considering that M_j represents a hypothetical genotype, the first term of the equation is the sum of squares for the genetic effect and the second is the sum of squares of the genotype x environment interaction.

In AMMI-biplot analysis, we considered the genotype and environments effects as fixes and the model according to the equation:

$Y_{ij} = \mu + g_i + a_j + \sum_{k=1}^{n}\lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \bar{\varepsilon}_{ij}$ wherein: Y_{ij}: is the average response of the i-th genotype (i = 1,2, ... ,G genotypes) in the j-th environment (j = 1,2, ... , A environments); μ : the overall mean of the trials; g_i : i-th genotype effect; a_j : j-ésimo environment effect; λ_k : k-th singular value (scalar) of the original interaction matrix (named by G x E); γ_{ik} : element corresponding to i-th genotype in k-th matrix column singular vector GE; α_{jk} : element corresponding to j-th environment in k-th matrix line singular vector GE; ρ_{ij} : noise associate to term $(ga)_{ij}$ of the classic genotype *i* with environment *j* interaction; $\ddot{e}_{ij}$: average experimental error. For defining the number of axes to be retained in order to explain the pattern related to interaction, we adopted the criteria proposed by Gauch and Zobel (1988), taking into consideration the proportion of the sum of squares from the interaction ($SS_{G \times E}$) accumulated until the n-th axis. Thus, the stopping point that determines the model selection for each family member of AMMI models (AMMI0, AMMI1,..., AMMIn) was obtained based on the significance of the F tests from Gollob (1968).

The interpretation of adaptability and stability of genotypes and environments, that is, its coordinates in the main axes of interaction (IPCA) was based on biplot graphical analysis (Gabriel, 1971), graph that contains two categories of points or markers; in case, one referring to genotypes and other to environment. First, we proceeded the graphic interpretation in biplot based on the variation of the main additive effects of genotype and environment and multiplicative effect of G x E interaction, and subsequently the interpretation based only on the multiplier effect of G x E interaction. All analysis were performed with Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

In the joint analysis (Table 2), all effects were significant (P≤0.01), which indicates contrasts between the environments and the occurrence of differential response of genotypes across to environmental effects. This can be confirmed by looking up soil and climatic characteristics of each environment (Table 1), showing differences in altitude, latitude, longitude, climate and soil type, besides climatic effects such as precipitation and temperature. The existence of significant genotype x environment for grain yield that the analyzes of stability and adaptability are appropriate, the fact that the edaphoclimatic are the factors that most influence the adaptability and stability of genotypes.

We observed that the magnitude of environmental effect (78.27%) was more important than the effect of the G x E interaction (15.70%), and which was greater than the effect of genotypes (6.02%). These results indicate the existence of variability among the evaluated environments, and consequently, the performance of genotypes, justifying a more detailed study on the individual performance of cultivar in order to identify the magnitude of their interactions with the environment. These results are similar to those reported by Akande (2007), Sarvamangala et al. (2010) and Nunes et al. (2014).

Combined deviations were highly significant (P ≤ 0.01), indicating the stability of the linear and nonlinear components involved in the phenotypic performance of the genotypes in the environments under study. It is perceived that only a small part of the G x E interaction can be explained by the linear relationship among

Table 3. Overall means (β_{0i}), estimates of coefficients of regression ($\hat{\beta}_{1i}$), regression deviations ($\hat{\sigma}^2_{di}$) and coefficients of determination (R^2), according to Eberhart and Russell method, regarding to yield grain (kg ha^{-1}), of 20 cowpea genotypes obtained in eight environments, from 2010 to 2013.

Identification	Genotypes	β_{0i}	$\hat{\beta}_{1i}$ (1)	$\hat{\sigma}^2_{di}$ (2)	R² (%)
G1	MNC02-675F-4-9	802 [b]	1.00[ns]	3934.40[ns]	91.85
G2	MNC02-675F-4-2	785 [b]	0.92[ns]	11221.45[ns]	87.50
G3	MNC02-675F-9-2	698 [b]	0.94[ns]	-12555.85[ns]	98.27
G4	MNC02-675F-9-3	782 [b]	1.06[ns]	11184.20[ns]	90.33
G5	MNC02-676F-3	886 [a]	0.88[ns]	21179.47*	82.31
G6	MNC02-682F-2-6	916 [a]	0.65[ns]	36334.69**	64.51
G7	MNC02-683F-1	744 [b]	0.76[ns]	27218.04*	75.34
G8	MNC02-684F-5-6	895 [a]	0.85[ns]	-1238.08[ns]	91.74
G9	MNC03-725F-3	874 [a]	1.09[ns]	17291.85[ns]	88.94
G10	MNC03-736F-7	668 [b]	0.69[ns]	18750.77*	75.67
G11	MNC03-737F-5-1	910 [a]	1.44**	-1636.31[ns]	96.99
G12	MNC03-737F-5-4	859 [a]	1.28[ns]	-9581.43[ns]	98.26
G13	MNC03-737F-5-9	873 [a]	1.27[ns]	6974.84[ns]	94.05
G14	MNC03-737F-5-10	696 [b]	1.16[ns]	20888.70*	89.23
G15	MNC03-737F-5-11	897 [a]	1.23[ns]	62718.19**	81.14
G16	MNC03-737F-11	815 [b]	0.79[ns]	32107.30**	74.62
G17	BRS-Tumucumaque	1.173 [a]	0.92[ns]	21851.01*	83.61
G18	BRS-Cauamé	896 [a]	0.87[ns]	41770.17**	74.74
G19	BRS-Itaim	883 [a]	1.02[ns]	54517.18**	76.77
G20	BRS-Guariba	1.031 [a]	1.08[ns]	28622.44*	85.59
-	Mean			854,53	

[ns], ** and *: not significant, significant a 1 and 5% of probability respectively; [(1), (2)]: significant by t-test; significant by F-test, respectively; Means followed by the same letter in the same column do not differ by the Skott and Knott test.

genotypes and environments, indicating that the G x E interaction is not adequately explained by the regression.

The average grain yield (β_0) ranged from 668 to 1,173 kg ha^{-1} for genotypes MNC03-736F-7 and BRS-Tumucumaque, respectively, with overall mean of 854.53 kg ha^{-1}, standing out with better adaptation those genotypes with average yield grain above this mean such as BRS-Guariba, MNC02-682F-2-6, MNC03-737F-5-1 and MNC02-676F-3 (Table 3).

Considering the twelve genotypes that showed better adaptation (β_0 > overall mean), similar to the unit, indicating that the genotypes had wide adaptability to the evaluated environments. The genotype MNC03-737F-5-1 proved to be very effective in increasing average yields under unfavorable conditions ($\hat{\beta}_{1i}$ > 1), suggesting that it has a great ability to adapt to stressful environment. However, the use of this should be careful, since, to unsuitable environmental conditions, i.e., in regions involving low technological level and/or subject to soil and climatic variations, it yield can be reduced. The genotypes MNC02-676F-3, MNC02-682F-2-6, MNC02-684F-5-6, MNC03-725F-3, MNC03-737F-5-4, MNC03-737F-5-9, MNC03-737F-5-11, BRS-Tumucumaque, BRS-Cauamé, BRS-Itaim and BRS-Guariba have shown to be wide adaptation, suggesting that these genotypes tolerate different environmental conditions keeping their average yield around the overall mean.

Regarding to predictability, nine genotypes showed no significant regression deviation ($\hat{\sigma}^2_{di}$ = 0), indicating stability, it means that these genotypes not varied their average yield over the years and places, being weakly influenced by environmental conditions. We also observe that only six genotypes showed R² lower than 80%. The coefficient of determination higher than the above represents low dispersion of data, indicating high reliability in the type of environmental response determined aby regression. Thus, this value should be used as reference so that the regression satisfactorily explain the performance of a genotype depending on an environment (Cruz et al., 2012).

P_i values, according to the method proposed by Lin and Binns (1988), for all environments and their deployment to favorable and unfavorable environments according to the methodology suggested by Carneiro (1998) are shown in Table 4. We observed that among the twenty genotypes were identified five that stood out showing the lower P_i overall values: BRS-Tumucumaque, BRS-Guariba, MNC03-737F-5-1, MNC02-682F-2-6 and

Table 4. Estimates of the parameters overall, favorable and unfavorable P_i, regarding to yield grain (kg ha^{-1}), of 20 cowpea genotypes obtained in eight environments, from 2010 to 2012.

Identification	Genotypes	Overall mean	Overall P_i /1000	P_i (Favorable)	P_i (Unfavorable)
G1	MNC02-675F-4-9	802 [b]	145.50	167.27	139.04
G2	MNC02-675F-4-2	785 [b]	166.70	184.95	151.26
G3	MNC02-675F-9-2	698 [b]	198.12	205.43	153.66
G4	MNC02-675F-9-3	782 [b]	163.21	179.92	141.46
G5	MNC02-676F-3	886 [a]	112.25	109.7	91.04
G6	MNC02-682F-2-6	916 [a]	83.40	151.97	117.17
G7	MNC02-683F-1	744 [b]	203.86	310.27	157.77
G8	MNC02-684F-5-6	895 [a]	97.84	77.89	60.02
G9	MNC03-725F-3	874 [a]	113.43	123.87	96.64
G10	MNC03-736F-7	668 [b]	246.59	365.05	237.87
G11	MNC03-737F-5-1	910 [a]	83.45	65.75	51.31
G12	MNC03-737F-5-4	859 [a]	114.58	124.65	97.45
G13	MNC03-737F-5-9	873 [a]	121.17	128.42	107.3
G14	MNC03-737F-5-10	696 [b]	202.57	238.48	155.1
G15	MNC03-737F-5-11	897 [a]	130.28	161.68	128.12
G16	MNC03-737F-11	815 [b]	176.91	198.73	153.48
G17	BRS-Tumucumaque	1,173 [a]	21.75	36.91	6.59
G18	BRS-Cauamé	896 [a]	129.16	156.64	118.47
G19	BRS-Itaim	883 [a]	131.97	164.48	132.13
G20	BRS-Guariba	1,031 [a]	58.53	53.24	45.37
-	Mean		854.53		

Means followed by different letters in the same column do not differ by Skott-Knott test (P ≤ 0.05).

MNC02-684F-5-6. According to Cruz et al. (2012), a great advantage of the Lin and Binns method is the immediate identification of the most stable genotypes due to the uniqueness of the P_i parameter, but this method only shows a general parameter estimation for recommending cultivars. However, modifying the method proposed by Carneiro (1998) provides an estimate of P_i for favorable and unfavorable environments that provide greater robustness to the method.

Given the above, we verified that the genotypes BRS-Tumucumaque, BRS-Guariba, MNC03-737F-5-1 and MNC02-684F-5-6 showed the lowest P_i values both in favorable and in unfavorable environments, especially the BRS-Tumucumaque, which was the most yield, considering the overall mean, with the lowest P_i overall value and P_i in favorable and in unfavorable environments and also one of the smaller contribution to the interaction, thus can be considered of general adaptation and high predictability. In studies with cowpea, Adewale et al. (2010); Shiringani and Shimelis (2011) found results related to the P_i parameter, thus confirming that the most adapted and stable genotypes are associated with high incomes.

Another important fact was that the genotype MNC03-737F-5-1, demonstrated wide adaptability and stability by presenting lower P_i values in favorable and unfavorable environments, however, by Eberhart and Russel (1966) method this genotype has proved to be very demanding in unfavorable conditions ($\hat{\beta}_{1i} > 1$) suggesting that it has great ability to exploit advantageously improving the environment. Thus it can be inferred that the combined use of these two methods brings more detailed information about the adaptability and stability of evaluated genotypes. This results corroborate those obtained by Almeida et al. (2012), where, working with different group of genotypes and environments, the authors concluded that it is recommended the use of Lin and Binns methodology in conjunction with Eberhart and Russel (1966) method.

The genotypes MNC02-675F-4-2, MNC02-675F-9-2, MNC02-676F-3, MNC02-683F-1, MNC02-684F-5-6, MNC03-737F-11, BRS-Tumucumaque and BRS-Cauamé showed lower P_i values in the unfavorable environments when compared to favorable environments or overall P_i, indicating that these genotypes have a specific adaptation to unfavorable conditions. It should also be emphasized that smaller P_i values reduce the deviation around the maximum yield for each environment. Thus, greater stability is associated with higher yield. However, the estimation of the P_i parameter is highly correlated to yield, so genotypes with lower grain yield will have a high P_i value, without necessarily being highly unstable.

Thus, although the genotypes MNC02-675F-4-9, MNC02-675F-4-2, MNC02-675F-9-2 and MNC02-675F-

Table 5. Analysis of additive main effects and multiplicative interaction regarding to yield grain (kg ha^{-1}), of 15 cowpea genotypes obtained in eight environments, from 2010 to 2012.

S.V	Degrees of freedom	F-value	$\%SS_{G \times E}$/PCA (1)	$\%SS_{G \times E\ Acummulate}$
Genotypes (G)	19	7.34*	-	-
Environments (E)	7	258.88*	-	-
G x E	133	2.73*	-	-
PCA 1	25	6.57*	52.52	52.52
$Residue_{AMMI1}$	108	1.55*	-	
PCA 2	23	1.82*	22.15	74.67
$Residue_{AMMI2}$	85	1.21^{ns}	-	
Mean error	477	-	-	-

* and ^{ns}: not significant and significant a 1% of probability respectively; (1): Percentage of the sum of squares of the genotype x environment interaction captured by IPCA.

9-3 present high overall P_i values, it were considered by the (Eberhart and Russel, 1966) methodology as high stability (regression deviations no significant). However growing of these genotypes should be thoroughly evaluated, because it yielded below the overall average.

In AMMI analysis, the $SS_{G \times E}$ was decomposed in four IPCAs; however, only the first two axes (IPCA1 and IPCA3) were significant by the F_R test and explained 52.52 and 22.15%, respectively, covering a total of 74.67% of the $SQ_{G \times E}$. For the interpretation are shown only the first two IPCAs, since the residue of IPCA2 was not significant by the F_R test. This indicates that the most predictive model is AMMI2. So this was selected for the study of stability and adaptability of genotypes and environments (Table 5). Similar results to those reported by Freire Filho et al. (2005), where the authors observed that the first two components explained 72% of the standard variance. Already Asio et al. (2005) reported that the first principal component explained 87.9% of the entire variation.

The graphical interpretation in *biplot* was based on the variation due to the main additive effects of genotype and environment and multiplicative effect of G x E interaction (AMMI 2), as well as only based on multiplicative effect of G x E interaction (AMMI 1). In this last case, the effect of G x E interaction is analyzed, aiming to confirm the observed stability in *biplot* AMMI 2.

In the graphical representation of the AMMI2 analysis, genotypes and stable environments are those whose points are situated near to the origin, i.e., the score values are almost zero in the two axes of the interaction (IPCA1 and IPCA2). The genotypes that contribute less to the G x E interaction were MNC02-675F-9-2 (3), MNC02-675F-9-3 (4), MNC02-675F-4-2 (2), MNC02-675F-4-9 (1) and MNC02-684F-5-6 (8) and the environments A2, A4 and A8 were the most stable, whose point is located near the origin, that is, the scores are lower value for the two axes of interaction (Figure 1A and B).

The genotypes MNC02-675F-4-9 (1), MNC03-725F-3 (9) and MNC03-737F-5-11 (15) interact positively with the A2 and A5 environments because besides having the same sign scores, the points relating to these genotypes suggest a common direction and the vectors starting from the origin with a small angle between them. The genotypes MNC03-737F-11 (16), MNC03-736F-7 (10), MNC02-683F-1 (7), MNC02-684F-5-6 (8), MNC02-675F-4-2 (2) and BRS-Cauamé (18) also demonstrated positive specific interaction with the environment A3, A8 and A4 by similar interpretation. There has been a clear lack adaptation of genotypes MNC03-737F-5-11 (15) and MNC03-725F-3 (9) with the environments A7 and A6 in the two graphs (markers pointing in opposite directions).

In *biplot* AMMI1 the abscissa axis represents the main effects (mean of genotypes and environment) and the axis of ordinates represents the scores for the genotype by environment interaction of said axis. Genotypes and stable environments are those whose points are located near to zero. It are identify which genotypes that have grain yield lower or higher than the average overall production through vertical reference line.

The genotypes MNC02-675F-4-9 (1), MNC02-675F-4-2 (2), MNC02-675F-9-2 (3), MNC02-675F-9-3 (4), MNC02-683F-1 (7), MNC03-736F-7 (10), MNC03-737F-5-10 (14) and MNC03-737F-11 (16) have yield less than the overall average, which is 854.53 kg ha^{-1}, while the other genotypes have yield equal to or higher than the average. The environments with low yield are A2, A3, A4 and A7, and the environments that have the highest yield are A1, A5, A6 and A8. The points located around zero from the horizontal axis IPCA1 correspond to genotypes MNC02-675F-9-3 (4), BRS-Itaim (19), MNC03-736F-7 (10) and MNC02-684F-5-6 (8) and the environments A2, A3, A8 and A6 more stable, being the first two associated to low yield and the last two associated to high yield (Figure 1A).

The environmental stability has great importance because it informs about the reliability of ordering of genotypes in a given test environment, compared to the average rating for the tested environments. The most

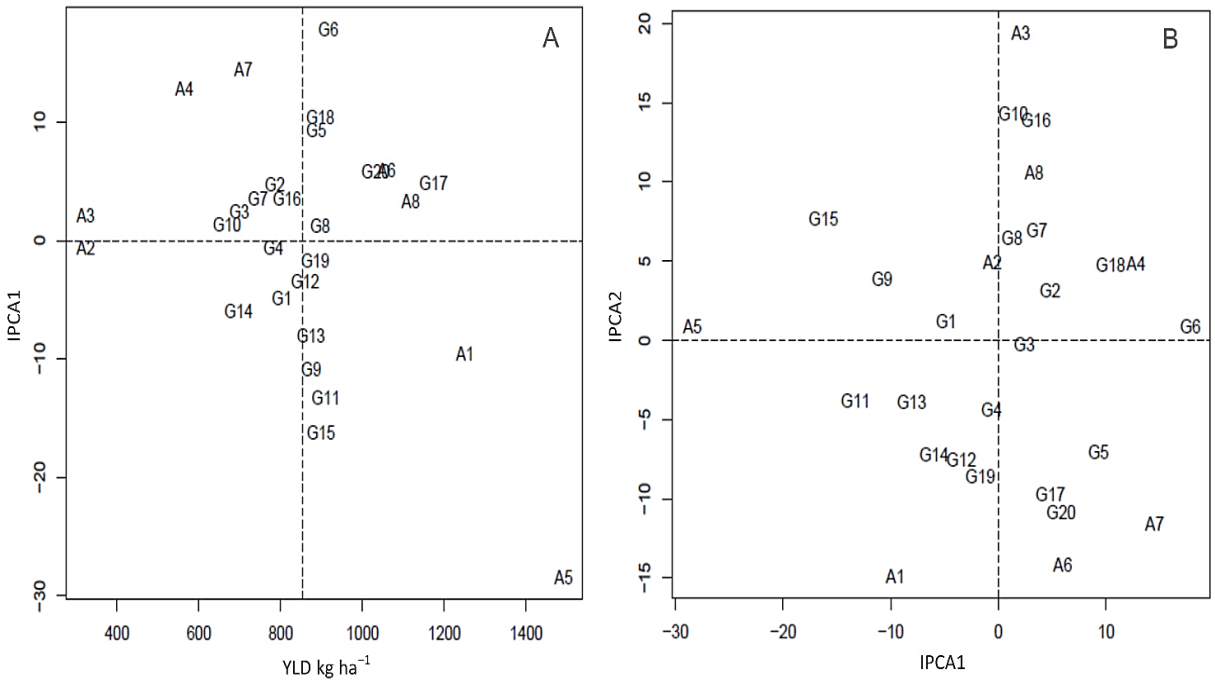

Figure 1. *Biplot* AMMI1 (A) and MMI2 (B) analysis for yield grain (kg ha^{-1}), of 20 cowpea genotypes obtained in eight environments, from 2010 to 2012. A1: Aquidauana, 2010; A2: Chapadão do Sul, 2010; A3: Dourados, 2010; A4; Aquidauana, 2011; A5: Chapadão do Sul, 2011; A6: Dourados, 2011; A7: Aquidauana, 2012 and A8: Dourados, 2012.

unstable environments were A1, A5 and A7, and the environments A5 and A5 environments showed instability associated with high grain yield, which corroborates the fact that the location Aquidauana have presented positive interaction with the agricultural year 2010 and Chapadão do Sul with 2011.

We observed that there is a disagreement among the Eberhart and Russel (1966) and AMMI methods, because the environments classified as unfavorable by environmental indices obtained by the Eberhart and Russell (1966) methodology are considered as stable in the AMMI analysis, such as CS10 and DS10. This probably is because the Eberhart and Russel (1966) methodology considers an environmental index, which refers to the average of an environment subtracted from the overall average, therefore, not take into account the present G x E interaction. The genotypes BRS-Tumucumaque (17) and BRS-Guariba (20) were the most productives, however, these genotypes presented one of the greatest deviations and contributed more to G x E interaction. In the AMMI method, the selection of genotypes with high stability usually results in low grain yields and, consequently, the selection for higher grain yield can lead to less stability (Pacheco et al., 2005). The genotypes MNC03-737F-5-1 (8) and BRS-Itaim (19) obtained grain yield above the overall average and at the same time were predictable, showing that these gather overall adaptability (Eberhart and Russel, 1966), can be grown in all studied environments.

Conclusions

The genotypes MNC03-737F-5-1, BRS-Tumucumaque, BRS-Guariba, MNC02-684F-5-6, MNC03-725F-3, MNC02-682F-2-6, BRS-Cauamé, BRS-Itaim and MNC03-737F-5-11 gather yield adaptability and stability sufficient for recommendation for Brazilian Midwest. Eberhart and Russell, Lin and Binns and AMMI methods can be used in a complementary way for the better predict the genotypes performance. The environments A1, A5, A6 and A8 are the most suitable for selecting superior genotypes for adaptability and stability.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Adewale BD, Okonji C, Oyekanmi AA, Akintobi DAC, Aremu CO (2010). Genotype variability and stability of some grain yield components of cowpea. Afr. J. Agric. Res. 5:874-880.

Akande SR (2007). Biplot analysis of genotype by environment interaction of cowpea grain yield in the forest and southern guinea savanna agro-ecologies of Nigeria. J. Food Agric. Environ 5:464-467.

Almeida WS, Fernandes FRB, Teófilo EM, Bertini CHCM (2012). Adaptability and stability of grain yield in cowpea under different biometrics. Rev. Bras. Agrocie. 18:221-228.

Asio MT, Osiru DSO, Adipala E (2005). Multilocational evaluation of

selected local and improved cowpea lines in Uganda. Afr. Crop Sci. J. 13:239-247.
Barros MA, Rocha MM, Gomes RLF, Silva KJD, Neves AC (2013). Adaptabilidade e estabilidade produtiva de feijão‑caupi de porte semiprostrado. Pesq Agropec Bras 48:403-410.
Cruz CD, Regazzi AJ, Carneiro PCS (2012). Modelos biométricos aplicados ao melhoramento genético. UFV, Viçosa.
Cruz CD (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. 35:271-276.
Eberhart SA, Russell WA (1966). Stability parameters for comparing varieties. Crop Sci. 6:36-40.
Freire Filho FR, Rocha MM, Ribeiro VQ, Lopes ACA (2005). Adaptabilidade e estabilidade produtiva de feijão‑caupi. Cien. Rur. 35:24‑30.
Freire Filho FR, Ribeiro VQ, Rocha MM, Damasceno-Silva KJ, Nogueira MR, Rodrigues EV (2011). Feijão-caupi: produção, melhoramento genético, avanços e desafios. Embrapa Meio-Norte, Teresina.
Frota KMG, Mendonça S, Saldiva PHN, Cruz RJ, Arêas JAG (2008). Cholesterol-lowering properties of whole cowpea seed and its protein isolate in hamsters. J. Food Sci. 73:235-240.
Gabriel KR (1971). The biplot-graphical display of matrices with applications to principal components analysis. Biometrika 58:453-467.
Gauch HC, Zobel RW (1988). Predictive and posdictive success of statistical analysis of yields trials. Theor. Appl. Genet. 35:905-912.
Gollob HF (1968). A statistical model which combines features of factor analytic and analysis of variance techniques. Psychometrika 33:73-115.
Lin CS, Binns MR (1988). A superiority measure of cultivar performance for cultivars x location data. Can. J. Plant Sci. 68:193-198.
Nunes, HF, Freire Filho FR, Ribeiro VQ, Gomes RLF (2014). Grain yield adaptability and stability of blackeyed cowpea genotypes under rainfed agriculture in Brazil. Afr. J. Agric. Res. 9:255-261.
Ojo DK, Oduola MS, Oduwaye OA (2006). Graphical assessment of yield stability and adaptation in cowpeas (*Vigna unguiculata* L. Walp). Niger. J. Genet. 20:17-25.
Pacheco RM, Duarte JB, Vencovsky R, Pinheiro JB, Oliveira AB (2005). Use of supplementary genotypes in AMMI analysis. Theor. Appl. Genet. 110: 812‑8185.
Rocha MM, Freire Filho FR, Ribeiro VQ, Carvalho HWL, Belarmino Filho J, Raposo JAA, Alcântara JP, Ramos SRR, Machado CF (2007). Adaptabilidade e estabilidade produtiva de genótipos de feijão-caupi de porte semi-ereto na Região Nordeste do Brasil. Pesq. Agropec. Bras. 42:1283-1289.
Santos A, Ceccon G, Davide LMC, Correa AM, Alves VB (2014b). Correlations and path analysis components in cowpea. Crop Breed. Appl. Biotechnol. 14:82-87.
Santos JAS, Teodoro PE, Correa AM, Soares CMG, Ribeiro LP, Abreu HKA (2014a). Desempenho agronômico e divergência genética entre genótipos de feijão-caupi cultivados no Ecótono Cerrado-Pantanal. Bragantia 73:1-6.
Sarvamangala C, Uma MS, Biradar S, Salimath PM (2010). Stability analysis for yield and yield components over seasons in cowpea [*Vigna unguiculata* (L.) Walp.]. Electronic. J. Plant Breed. 1:1392-1395.
Shiringani RP, Shimelis HA (2011). Yield response and stability among cowpea genotypes at three planting dates and test environment. Afr. J. Agric. Res. 6:3259-3263.

academicJournals

Vol. 10(41), pp. 3909-3912, 8 October, 2015
DOI: 10.5897/AJAR2015.10306
Article Number: 8B0A7AD55849
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effects of alternative extracts in the agronomic performance of two soybean cultivars

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Received 18 August, 2015; Accepted 18 September, 2015

The soybean cultivation along the years has required big investments and high technological level to develop new and more productive cultivars. However, the advent of biotic stress, such as diseases, can lead to losses in yield. To attenuate this interference and obtain the expected yield, chemical products have been used to control these diseases. This technique has shown good efficiency in short-term, but can damage the environment and promote the appearance of resistant biotypes in the long-term. So, alternative methods of diseases control, such as pyroligneous extract and the fungus *Pycnoporus sanguineus* have been used in soybean and other cultures to control diseases without the prior negative aspects of chemical products. The aim of this study was to evaluate possible interferences of these treatments in the agronomic performance of soybean culture. The experiment was conducted in the 2012/2013 harvest, submitting two soybean cultivars to pyroligneous aqueous extract in the 0.6 and 1.3% concentrations and the fungus *P. sanguineus* extract in the 2.5 and 5% concentrations, thus comparing their results with a positive control and water control treatment. The results showed that the 2.5% fungus concentration statistically reduced the yield of the Turbo RR® BMX cultivar and the 5% fungus concentration reduced the grain mass of Vmax RR® NK 7059. The pyroligneous extracts reduced the number of pods of the Turbo RR® BMX cultivar and the height of Vmax RR® NK 7059, but did not affect the yield. In either cultivar, the plant growth regulator obtained the best results for all characteristics.

Key words: pyroligneous, *Pycnoporus sanguineus,* plant growth regulator.

INTRODUCTION

The high technological level employed in the soybean cultivation system requires big investments, which are offset by an increasing yield. Thus, any factor that limits gains in production must be attenuated, aiming to ensure a good yield and, consequently, good profits. In this scenario, among the factors that most limit the soybean production are diseases. Most of these problems are caused by fungus, bacteria, nematodes and virus, and they have already been reported in Brazil (Yorinori, 1986).

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Commonly, the disease control in the crops is based on chemical substances. One of the main chemical products used are fungicides, which, due to its easy manipulation and good disease control, had shown an increase of use of 276.2% from 1964 to 1991 (Pansera et al., 2012). The utilization of these products has many advantages in short-term, but, in long-term, it can cause problems due to the residue accumulation in the soil and environment, being necessary the development of alternative methods of disease control (Viecelli et al., 2010; Ghini and Kimathi, 2000). So, the use of pyroligneous extract and the plant resistance induction with natural products are methods that come up with high potential (Schwan-Estrada and Stangarlin, 2005).

The pyroligneous extract, also known as pyroligneous acid, pyroligneous liquid or wood vinegar, is a natural liquid product obtained by the smoke condensation, from wood carbonization, collected under controlled temperatures (Schwengber et al., 2007; Alves et al., 2007; Silveira, 2010). This liquid is basically made of water (80 to 90%), containing around 200 different compounds, with prominence of acetic acid (8%), methanol (± 2%) and acetone (± 1%) (Zanetti et al., 2004).

This product is being used with different aims in agriculture, such as fertilizer in rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* L.) and sweet potato (*Ipomoea batatas* L.), nematicide and fungicide (Tsuzuki et al., 2000; Esechie et al., 1998; Shibayama et al., 1998; Cuadra et al., 2000). Moreover, it is being recommended to control mite and insects by foliar pulverization or straight application in soil (Schwengbers et al., 2007).

Plant resistance induction is characterized by the activation of the latent defense system in response to the interaction with an external agent (Stangarlin et al., 1999). This defense is stimulated by elicitors, which are molecules capable to protect the plant against future infections caused by pathogens (Pascholati and Leite, 1995; Smith, 1996; Stangarlin et al., 1999).

Among the elicitor agents, there are the plant extracts and essential oils, as well as mushroom extracts (Stangarlin et al., 1999; Schwan-Estrada et al., 2003; Schwan-Estrada and Stangarlin, 2005; Di Piero et al., 2005). In this last example, the basidiomycete *Pycnoporus sanguineus* (L. ex Fr.) Murr. has its aqueous extract being used in agriculture as an alternative method for diseases control. *P. sanguineus* showed an efficient control of *Colletotrichum* in bean, inhibiting the germination of conidia (Assi, 2005).

Studies that relate benefits of alternative methods of disease control do not point any possible interference to the soybean yield. So, the aim of this study was to verify any possible interference in yields of two soybean cultivars caused by the use of pyroligneous and *P. sanguineus* extracts, comparing the results with a plant growth regulator.

MATERIALS AND METHODS

The experiment was conducted during the 2012/2013 harvest, in Maripa city, located in Western Parana, in a clayey oxisol soil (Embrapa, 2006). The weather is Cfa, according to Koppen classification (Koppen and Geiger, 1928). The cultivars Turbo RR® BMX and Vmax RR® NK 7059 were chosen to be used in this study due to their large cultivation in the region. The experimental area altitude was 320 m. The plots contained six 5 m lines, spaced 45 cm between rows. The useful area was of 5.4 m^2, using the four central rows and eliminating 1 m of each extremity. The fertilization, seedling and the phytosanitary control was made according to Embrapa (2011).

The pyroligneous extract used was obtained through eucalyptus wood carbonization, using brick furnace and submitted to decantation during 6 months. After this time, the dilutions were prepared at the 0.6 and 1.3% concentrations.

Samples of *P. sanguineus* were collected from a eucalyptus cultivation area located in Maripa, PR, which was drought in forced circulation greenhouse at 45°C, until it reached constant weight. Following, they were crushed and submitted to extraction in the proportion of 1 g of the fungus and 14 mL of distillated water. The solution was kept for 24 h at 4°C and then the dilutions of 2.5 and 5% were prepared (Figueiredo, 2012).

For each soybean cultivar used, the randomized block design was considered, with six treatments in three repetitions. In each case two extract concentrations were analyzed, and also a water control (T1), and a positive control (T2) composed by the commercial product Stimulate®. The pyroligneous aqueous extract was tested in 0.6 (T3) and 1.3% (T4) concentrations, while the *P. sanguineus* extracts in the 2.5 and 5.0% (T5 and T6, respectively) concentrations. The commercial product Stimulate®, a plant growth regulator containing 0.009% of kinetin, 0.005% of gibberellic acid and 0.005% of IBA, was applied in the 0.5 L ha^{-1} dose, during the soybean fifth and sixth-node stage (Adapar, 2014).

The applications were made during the beginning bloom (R1) using a CO_2 pressurized backpack sprayer, with a 2 BAR (29 psi) constant pressure, in a flow rate of 0.65 L min^{-1} (Fehr et al., 1971). The bar contained 6 Teejet type XR 110.0 nozzles. During the application, the bar was positioned 50 cm apart the target, with a constant speed of 1 m second $^{-1}$ and 50 cm width range, providing a spray volume of 200 L ha^{-1}.

The parameters evaluated were: plant stand, plant height, number of pods, 100 grains mass and yield (kg ha^{-1}).To evaluate the plants height, 10 plants were chosen randomly in the useful area of the plot, and their heights were measured in cm by using a wood ruler. The evaluation of the number of pods was made manually, counting the number of pods of 10 random plants during the soybean full-maturity stage. To plant stand, it was counted the number of plants per meter within the floor area of the plot

The plants were harvested manually in full-maturity, when 95% of the pods had reached their mature pod color (Fehr et al., 1971). Next, the material harvested was threshed with an experimental threshing machine and stored in paper-bags, which were later weighed to obtain the yield. The determination of 100 grains mass was made separating and weighting in eight samples of each plot.

The data were analyzed according to Pimentel-Gomes and Garcia (2002), submitting the data to variance analysis. When the F value was significant, the T test (LSD) at 5% was realized.

RESULTS AND DISCUSSION

The results obtained from the Statistical evaluation of the soybean cultivar Turbo RR® BMX results are shown in Table 1. According to the results, the number of pods and

Table 1. Agronomic parameters evaluated in the soybean cultivar Turbo RR® BMX, submitted to different treatments.

Treatments	Height (cm)	Number of pods	100 grains mass (g)	Yield (kg ha^{-1})
T1	78.20^{A}	47.40^{D}	19.00^{A}	3137.50^{AB}
T2	77.10^{A}	62.55^{A}	18.25^{A}	3425.75^{A}
T3	77.90^{A}	55.05B^{C}	19.25^{A}	3194.00^{AB}
T4	78.85^{A}	57.35^{B}	18.50^{A}	3287.00^{AB}
T5	78.45^{A}	57.95^{B}	18.50^{A}	2762.50^{B}
T6	76.90^{A}	51.45^{C}	19.00^{A}	3156.75^{AB}
CV %	5.08	4.38	3.56	11.24

*Same letter on the columns are not significant different (p > 0.05) by the T test (LSD); T1 = water control; T2 = positive control; T3 = 0.6% pyroligneous extract; T4 = 1.3% pyroligneous extract; T5 = 2.5% *P. sanguineus* extract; T6 = 5% *P. sanguineus* extract.

Table 2. Results of the agronomic parameters evaluated in the soybean cultivar Vmax RR® NK 7059, submitted to different treatments.

Treatments	Height (cm)	Number of pods	100 grains mass (g)	Yield (kg ha^{-1})
T1	83.50^{AB}	50.35^{A}	16.34^{B}	3017.75^{A}
T2	87.25^{A}	45.25^{A}	17.45^{A}	3279.00^{A}
T3	86.50^{AB}	50.70^{A}	16.85^{AB}	3214.50^{A}
T4	78.85^{B}	53.20^{A}	16.73^{AB}	2660.25^{A}
T5	88.00^{A}	45.35^{A}	16.87^{AB}	2884.50^{A}
T6	82.70^{AB}	50.80^{A}	16.28^{B}	2714.75^{A}
CV %	5.08	4.38	3.56	11.24

*Same letter on the columns are not significant different (p > 0.05) by the T test (LSD); T1 = water control; T2 = positive control; T3 = 0.6% pyroligneous extract; T4 = 1.3% pyroligneous extract; T5 = 2.5% *P. sanguineus* extract; T6 = 5% *P. sanguineus* extract.

yield showed significant difference for the cultivar Turbo RR® BMX. T2 is significantly superior (p<0.05) than the other treatments for the number of pods basically because it is a commercial plant growth regulator and it contains 0.009% of kinetin, 0.005% of gibberellic acid and 0.005% of IBA, which provide a hormonal balanced dose that improves the plant growth and development (Adapar, 2014). The result in this study for Stimulate® is similar to another study, in which the application of this product also caused an increase in the soybean number of pods (Bertolin et al., 2010).

Still considering this parameter, the higher pyroligneous extract concentration (T4) presented a number of pods statistically superior (p<0.05) than the higher *P. sanguineus* extract concentration (T6). So, in this study, a difference between the two concentrations of *P. sanguineus* was noticed for the number of pods, where the lower concentration provided a bigger number of pods, indicating that the fungus can cause some injury to the plant. The two pyroligneous extract tested did not show significant difference each other, but yet provided a number of pods higher than the water control, as well as the other treatments. This parameter is inferred directly in soybean yield, but according to Braz et al. (2010) this variable is related mainly to plant genetic potential.

The yield results for the cultivar Turbo RR BMX showed that all treatments tested did not differ significantly from the water control. In a study conducted by Bertolin et al. (2010) the authors indicate possible provides increase in number of pods per plant and grain yield both application via seed as foliar, caused by the use of Stimulate®, something that seems to have occurred in present work. Viecelli et al. (2010) verified that the *P. sanguineus* had an efficient disease control in other legumes, like bean.

The results of the different treatments in the cultivar Vmax RR® NK 7059 are shown in Table 2. It is possible to see that for all agronomic parameters tested, none of the treatments differ from the water control. Beninca (2007) shows in his work the resistance induction efficiency in soybean plants by the use of *P. sanguineus* extract. This way, it can be seen that the application of these natural products did not affect the productivity of cultivars soy worked. The 100 grain mass results show that only the positive control (T2) presented a mass significantly higher (p<0.05) than the water control.

Conclusion

The pyroligneous and *P. sanguineus* extracts are

alternative methods that efficiently control plant diseases. However, the interference of these methods over the agronomic parameter evaluated in the soybean culture could not be inferred. In the conditions of this study, the interference of the extracts can be significant according to the cultivar analyzed, where the results vary from one cultivar to another.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Adapar (Agricultural Defense Agency of Paraná) (2014). Agency agricultural defense of Parana. Stimulate Agriculture Bulletin, pp. 1-11.

Alves M, Cazetta JO, Nunes MA, Oliveira A, Colombi CA (2007). Ação de diferentes preparações de extrato pirolenhoso sobre *Brevipalpus phoenicis* (GEIJSKES). Rev. Brasileira Fruticultura 29(2):382-385.

Assi L (2005). Controle de Colletotrichum lindemuthianum (Sacc. Et Magn.) Scrib na cultura do feijão (*Phaseolus vulgaris* L.) pelo extrato do cogumelo *Pycnoporus sanguineus* (L.ex Fr.). Thesis (MS in Agronomy) - State University of Western Paraná 1:51.

Bertolin DC, Sá ME, Arf O, Furlani Jr E, Colombo AS, Carvalho FLBM (2010). Aumento da produtividade de soja com a aplicação de bioestimulantes. Rev. Bragantia 69(2):339-347.

Beninca CP (2007). Indução de fitoalexinas e atividade de peroxidases em sorgo e soja tratados com extratos de basidiocarpos de *Pycnoporus sanguineus*. Thesis (MS in Agronomy) - State University of Western Paraná 1:45.

Braz GBP, Cassol GM, Ordoñez GAP, Simon GA, Procópio SO, Oliveira Neto AM, Ferreira Filho WC, Dan HA (2010). Componentes de produção e rendimento de soja em função da época de dessecação e do manejo em pós-emergência. Rev. Bras. Herbicidas 9(2):63-72.

Cuadra R, Cruz X, Pereira E, Martin E, Diaz A (2000). Algunos compuestos naturales com efecto nematicida. Rev. de Protección Vegetal 24(15):31-37.

Di Piero RM, Garcia Jr D, Tonucci NM (2005). Indutores bióticos FEALQ, pp. 29-50.

Embrapa (2006). National Center for Soil Research. Brazilian system of soil classification (2):1-306.

Embrapa Soja (2011). Soybean production technologies - central region of Brazil in 2012 and 2013, pp. 1-262.

Esechie HA, Dhaliwal GS, Arora R, Randhawa NS, Dhawan AK (1998). Assessment of pyroligneous liquid as a potential organic fertilizer. In: Ecological agriculture and sustainable development, 1997, Chandigarh, India. Proceedings. Chandigarh: Center for Research in Rural and Industrial Development 1:591-595.

Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971). Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11(6):929-931.

Figueiredo AS (2012). Avaliação "*in vitro*" dos extratos de basidiomicetos frente à fitopatógenos prejudiciais à produção de hortaliças de pequenos produtores da região do baixo Amazonas. Thesis (MS in Biotechnology and Natural Resources) - Amazon State University 1:104.

Ghini R, Kimati H (2000). Resistência de fungos a fungicidas. Embrapa Meio Ambiente, P. 78.

Koppen W, Geiger R (1928). Klimate der Erde. Gotha: Verlag Justus Perthes. Wall-map 150 cm×200 cm.

Pansera MR, Vicenço CB, Prancutti A, Sartori VC, Da Silva Ribeiro RT (2012). Controle alternativo do fungo *Sclerotinia sclerotiorum* (LIB.) De Bary causador da podridão de sclerotinia, com óleos essenciais e extratos vegetais. Rev. Brasileira de Agroecologia 7(3):126-133.

Pascholati SF, Leite B (1995). Hospedeiro: Mecanismo de resistência. Manual de fitopatologia princípios e conceitos. Ed. Agronômica Ceres 1(22):417-454.

Pimentel-Gomes F, Garcia CH (2002). Estatística aplicada a experimentos agronômicos e florestais: Exposição com exemplos e orientações pra uso de aplicativos. Fealq pp. 1-309.

Schwan-Estrada KRF, Stangarlin JR, Cruz MES (2003). Uso de plantas medicinais no controle de doenças de plantas. Fitopatologia Brasileira 28:554-556.

Schwan-Estrada KRF, Stangarlin JR (2005). Extratos de óleos essenciais de plantas medicinais na indução de resistência. In: Cavalcanti, L.S., Di Piero, R.M., Cia, P., Pascholati, S.F., Resende, M.L.V., Romeiro, R.S. (Eds.). Indução de resistência em plantas a patógenos e insetos. Piracicaba: FEALQ, pp. 125-138.

Schwengber JE, Schiedeck G, Gonçalves M (2007). Preparo e utilização de caldas nutricionais e protetoras de plantas.

Shibayama H, Mashima K, Mitsutomi M, Arima S (1998) Effects of application of pyroligneous acid solution produced in Karatsu city on growth and free sugar contents of storage roots of sweet potatoes. Marine and Highland Bioscience Center Report 7:15-23.

Silveira CM (2010). Influência do Extrato Pirolenhoso no desenvolvimento e crescimento de plantas de milho. Thesis (PhD in Agronomy). Paulista State University, pp. 1-79.

Smith CJ (1996). Accumulation of phytoalexins: defence mechanism and stimulus response system. New Phytologist 132(1):1-45.

Stangarlin JR, Scwan-Estrada KRF, Cruz MES, Nozaki MH (1999). Plantas Medicinais – Plantas medicinais e controle alternativo de fitopatógenos. Biotecnologia Ciência Desenvolvimento 2(11):16-21.

Viecelli CA, Stangarlin JR, Kuhn OJ, Schwan-Estrada KRF (2010). Indução de resistência em feijoeiro à mancha angular por extratos de micélio de *Pycnoporus sanguineus*. Summa Phytopathologica 36(1):73-80.

Tsuzuki E, Morimitsu T, Matsui T (2000). Effects of chemical compounds in pyroligneous acid on root rice plant. Japan J. Crop Sci. 66(4):15-16.

Yorinori JT (1986). Doenças da soja no Brasil. In: FUNDAÇÃO CARGILL. Soja no Brasil Central. Campinas: Fundação Cargill, pp. 301-363.

Zanetti M, Cazetta JO, Mattos Jr DD, Carvalho SAD (2004). Influência do extrato pirolenhoso na calda de pulverização sobre o teor foliar de nutrientes em Limoeiro 'Cravo'. Rev. Brasileira de Fruticultura 26(3):529-533.

academicJournals

Vol. 10(41), pp. 3913-3920, 8 October, 2015
DOI: 10.5897/AJAR2015.10335
Article Number: 8BD0E9C55851
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Sunflower behavior of on soils with water availability and addition of cattle biofertilizer

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Received 22 August, 2015; Accepted 18 September, 2015

The Sunflower plant is an oilseed of great importance on the world stage. However, their productivity may be compromised by lack of rain and availability of nutrients. Thus this study aimed to evaluate the growth of sunflower plants under different hydric regimes in two soils with the application of cattle biofertilizer. The experiment was performed in a greenhouse State University of Paraíba campus IV in Catolé do Rocha, Paraíba, Brazil. The treatments were arranged in a completely randomized design using a 2 × 2 × 5 factorial for two levels of water availability (100 and 50% WA), two soils Entisol and Alfisol and five rates of cattle biofertilizer (0.0, 2.5, 5, 7.5 and 10% of the substrate volume), totaling 20 treatments with nine replications and 180 experimental plots. At 90 days after sowing the plants were evaluated for growth and biomass accumulation. The reduction from 100 to 50% WA negatively affected the vegetative behavior of the plants in both soils. The increased growth and biomass accumulation occurred with the application of biofertilizer rates of 3.5 and 10% (v/v) in Entisol and Alfisol, and respectively.

Key words: *Helianthus annuus* L., organic fertilization, irrigation.

INTRODUCTION

The Sunflower plant (*Helianthus annuus* L.) is an oilseed of great importance on the world stage, and it is considered as the fifth largest producer of grains and the fourth in oil production (Howell et al., 2015).

In Brazil, the sunflower cultivation is concentrated in the Midwest region, which accounts for 88.8% of the national production (Conab, 2013), but there is the possibility of cultivation in several Brazilian regions as it presents a short cycle (90 to 130 days) and has desirable characteristics for biodiesel production, such as hardiness, content and quality of the oil extracted from the grains (Souza et al., 2010). There is the example of the Northeast region, which despite contributing with only 5% of the Brazilian production (Ibge, 2012), it shows a

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Table 1. Values of pH, electrical conductivity and cattle biofertilizer composition 45 days after the start of anaerobic fermentation.

pH	EC	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	Cl^{-}	CO_3^{2-}	HCO_3^{-}	SO_4^{2-}
	dS m^{-1}	$cmol_c L^{-1}$							
6.34	1.08	3.71	2.40	3.27	1.69	5.59	0.43	2.03	3.02

EC = electrical conductivity of biofertilizer.

good expansion potential, given its favorable characteristics to the sunflower cultivation, such as the great diversity of fertile soils in this region.

Sunflower cultivation in the Northeast of Brazil is mainly situated in regions of semiarid climate, which has limitations to express the potential crop productivity due to the low rainfall (< 700 mm per year) and poor distribution of rainfall, since the lack of water in the soil is a limiting factor to the growth and development of plants (Lechinoski et al., 2007). Thus, it becomes necessary to resort to the practice of irrigation, in order to reduce and / or eliminate the problems of inappropriate water supply in the agriculture of the semiarid region.

In agricultural systems where chemical fertilizers are not used, organic matter is the main source of nutrients, like in the case of subsistence agriculture of the semiarid region of the Northeast of Brazil (Tiessen et al., 2001; Jiang et al., 2014). In this context, the adoption of organic fertilization with cattle biofertilizers becomes a viable alternative because of the ease of obtainment and relatively low cost. However, there are few studies that address the organic fertilization in sunflower cultivation in semiarid, especially when related to the use of biofertilizers.

Thus, the study aimed to evaluate the growth of sunflower plants under different hydric availability. in two soils with the application of cattle biofertilizer.

MATERIALS AND METHODS

The experiment was conducted from September 2011 to March 2012, in a greenhouse, on the premises of the Centre for Human and Agricultural Sciences, of the State University of Paraíba, Campus IV, Catolé do Rocha, Paraíba, Brazil, altitude of 275 m.

The soil used in the experiment were classified as Entisol and Alfisol (Usda, 2014), from Catolé do Rocha and Brejo dos Santos municipalities in Paraíba state. Samples of these soils were collected at a depth of 0 the 20 cm of depth, air-dried, sieved through a 2.0 mm mesh sieve and characterized chemically and physically using the methods adopted by Embrapa (1997), presenting the following results: Entisol: sand = 820 g kg^{-1}; silt = 125 g kg^{-1}; clay = 55 g kg^{-1}; field capacity = 22.82% of the volume; permanent wilting point = 6.54% in volume; available water = 16.28% in volume; pH (H_2O) = 7.44; Ca = 4.44 $cmol_c$ kg^{-1}; Mg = 2.81 $cmol_c$ kg^{-1}; Na = 0.26 $cmol_c$ kg^{-1}; K = 0.57 $cmol_c$ kg^{-1}; H = 0.00 $cmol_c$ kg^{-1}; Al = 0.00 $cmol_c$ kg^{-1}; OM = 6.9 g kg^{-1}; P = 53.3 mg kg^{-1}; Alfisol: sand = 655 g kg^{-1}; silt = 228 g kg^{-1}; Clay = 117; Field capacity = 19.60% of the volume; permanent wilting point = 5.70% in volume; available water = 13.06% in volume g kg^{-1}; pH (H_2O) = 7.81; Ca = 3.97 $cmol_c$ kg^{-1}; Mg = 2.45 $cmol_c$ kg^{-1}; Na = 0.15 $cmol_c$ kg^{-1}; K = 0.18 $cmol_c$ kg^{-1}; H = 0.06 $cmol_c$ kg^{-1}; Al = 0.00 $cmol_c$ kg^{-1}; OM = 4.5 g kg^{-1}; P = 14.3 mg kg^{-1}.

The treatments were arranged in a completely randomized design with 2 × 2 × 5 factorial, referring to two levels of available water (100 and 50% WA), two soils (Entisol and Alfisol) and five rates of cattle biofertilizer (0.0, 2.5, 5, 7.5 and 10 % of the substrate volume), totaling 20 treatments with nine replications and 180 experimental plots. Each experimental unit consisted of a plastic vessel with capacity for 30 liters and a sunflower (*Helianthus annuus* L.) plant of the hybrid cultivar Helium 253.

The sowing was performed at a depth of 5 cm, using seven seeds per pot, distributed equidistantly. The seedling emergence began on the fourth day after sowing (DAS), at the ninth DAS the first thinning was made, leaving three plants per pot and the second pruning at 15 DAS, leaving only one plant, the most vigorous one. The biofertilizer rates were applied three times in 20 days, starting after the second pruning.

The cattle biofertilizer was obtained through anaerobic fermentation by mixing equal parts of fresh cattle manure and slightly saline water - EC = 0.8 dS m^{-1}, adding 2 kg of leaves and branches of the leguminous plant beans-macassar (*Vigna unguiculata* L.), in the plastic biodigester with capacity for 200 liters, kept tightly closed for 45 days. During the fermentation the methane gas produced was released through a duct connected to the upper base and its end submerged in water (Santos, 1992). As a result of being applied in liquid form, composition analysis was performed following common procedures for water sample for irrigation, according to the data in Table 1, congenerous suggestion of Cavalcante et al. (2010).

Soil water content was monitored daily by TDR probe model PR2/6 (Time Domain Reflectometry) in the treatments of 100 and 50% of the WA in four depth intervals: 0-10; 10-20; 20-30 and 30-40 cm; from the readings made with the probe model PR 2 for each depth, the values were put in a spreadsheet that accounted for the water content of each layer (four layers of 10 cm each), making a balance of water content along the existing soil profile as determined in accordance with the following equation:

$$WA = (CC–CA)/100) \times V \quad (1)$$

Where: WA = water available in cm; CC = Moisture at field capacity (dry weight basis); CA = Current capacity of soil and V = Soil volume.

Based on the probe readings a water balance was performed, which recorded the consumption of water by such treatments. Irrigation of the vessels was done daily by hand with the aid of a graduated cylinder.

At 90 DAS the irrigation was suspended, based on the criterion of the physiological maturity of the grains (Silva et al., 2007a.); on this same period, all the plants were in the phenological stage R9 (chapter inclined down, with back and bracts with a color between yellow and brown). At this time, we determined the plant height (HP), stem diameter (SD), number of leaves per plant (NLP), the dry mass of the shoot (DMS), of the root (DMR) and total (DMT) where the HP was obtained using a millimeter ruler measuring the distance between the neck of the plant and the insertion of the

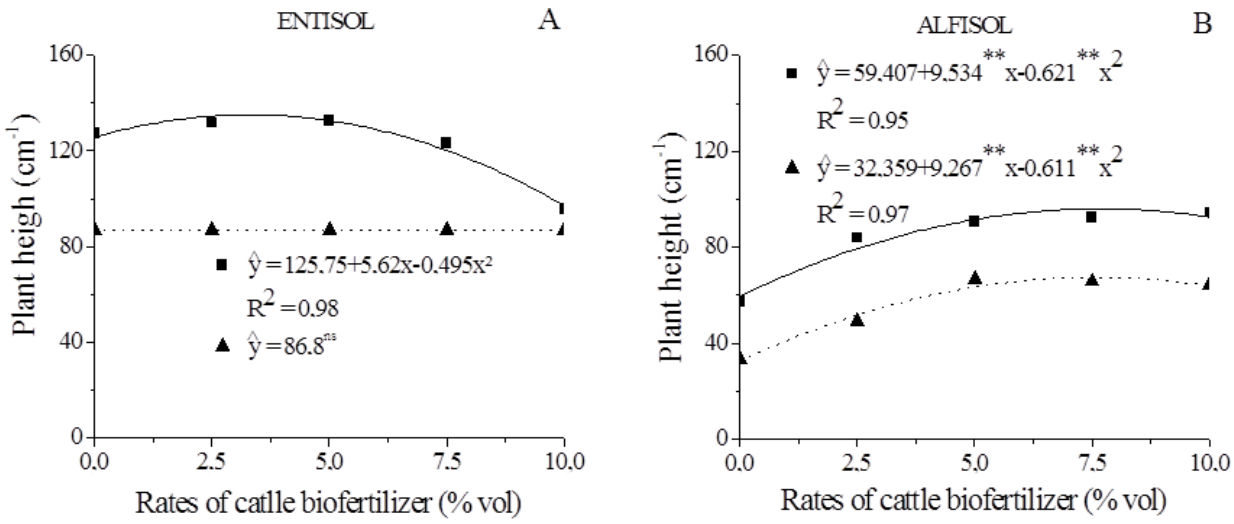

Figure 1. Sunflower plant height cv. Helium 253 under two hydric treatments 100% (—) and 50% (----) of available soil water, rates of cattle biofertilizer in two soil types.

chapter, the SD was determined using a digital caliper series 799 measuring the plant's cervical region at a height of 5 cm of soil, DMS, DMR and DMT were evaluated with a precision balance after drying in an oven with forced air ventilation at 65°C until a constant weight was obtained. It was also measured the total leaf area (TLA) obtained through Equation 2:

$$TLA = 1.7582\ L^{1.7067} \quad (2)$$

Where the "L" is the width of the leaf blade (Maldaner et al., 2009).

The results were submitted to variance analysis, F test and regression using the statistical program Sisvar© (Ferreira, 2011).

RESULTS AND DISCUSSION

A quadratic behavior of growth in height was observed on sunflower in function of the rates of biofertilizer in Entisol with 100% WA, where the maximum growth (135.07 cm) was estimated at the level of 3.3% v/v biofertilizer (Figure 1A). From the maximum point there is a tendency to reduction in plant height, a fact possibly related to the toxic effect of the gradual increase in biofertilizer rate, given that the soil is at field capacity and the cattle biofertilizer is made with nutrients readily available for the plants, thereby promoting an excessive increase in nutrients in the soil solution. Similar results were observed by Rivera-Cruz et al. (2008), who found an increased growth of the banana tree with a rate of up to 3% of biofertilizer applied in the crown projection, tending to decline since then.

However, plants grown in Entisol with 50% WA in the soil, the data did not fit any mathematical model, and an average of 86.8 cm per plant was found. It is believed that in this condition the solubility of biofertilizer in the soil did not occur, so that the nutrients were not available for the plants, such a fact being contrary to that observed in the condition of 100% WA, which favors the solubility of the biofertilizer's nutrients and their availability in the soil solution (Figure 1A).

In Alfisol, the increase in the level of biofertilizer resulted in a boost in plant height until the biofertilizer levels of 7.68 and 7.59% v/v, reaching maximum heights of 96.55 and 67.45 cm with 100 and 50% WA in the soil, respectively (Figure 1B). Regardless of the soil used, plants cultivated with 100% WA surpassed those grown with 50% WA, evidencing the effect of hydric stress on the plants. The increments found in this study were also similar to Silva et al. (2007b), who studied sunflower plants submitted to irrigation according to crop evapotranspiration and plants without hydric supplementation, finding an increase in plant height of about 20.8%. Paiva Sobrinho et al. (2011) also found that sunflower plants have a boost in height when there is an increase on the amount of available water in the soil, these responses having also being confirmed by Silva et al. (2007b) Nobre et al. (2010) and Freitas et al. (2012).

For the stem diameter variable, plants cultivated in Entisol maintained at 100% WA conformed to the polynomial model, with maximum diameter estimated at 23.07 mm, achieved with the estimated rate of 3.97% v/v of biofertilizer (Figure 2A). The stem diameter of the sunflower cultivated in Entisol submitted to 50% of WA positively conformed to the linear model, so that the unit increase of level of the soil biofertilizer provided a direct increment in diameter of 0.16 mm, with a maximum value of 17.97 mm regarding the highest rate of input.

In regard to Alfisol, it was observed that the stem diameter of sunflower plants increased linearly with higher rates of biofertilizer applied to the soil, reaching maximum diameter of 16.02 and 11.08 mm when fertilized with biofertilizer 10% in volume and kept at 100 and 50% WA, respectively (Figure 2B). The larger stem diameter in plants cultivated with 10% of biofertilizer is

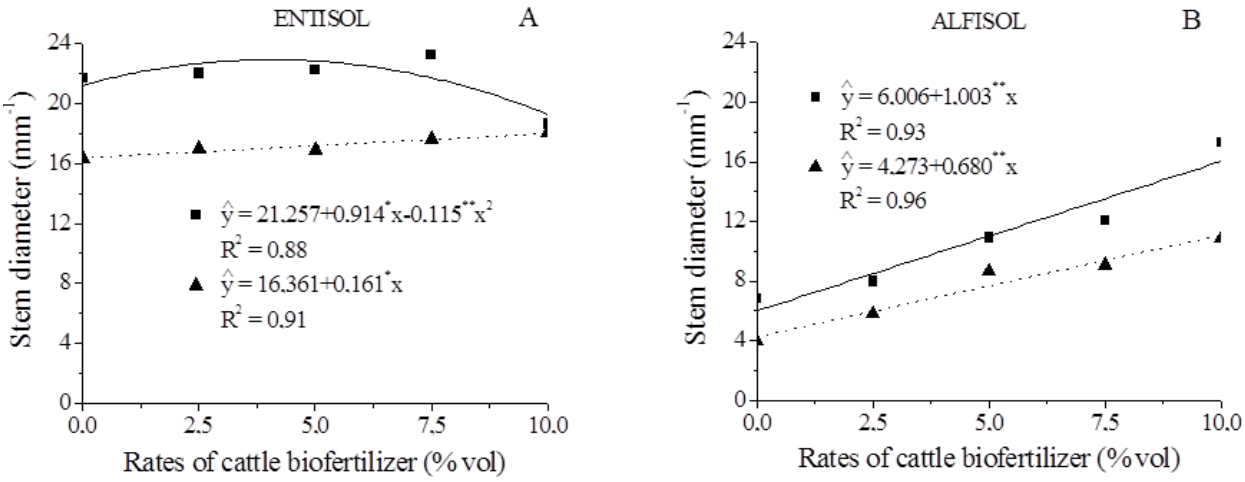

Figure 2. Sunflower plants stem diameter cv. Helium 253 under two hydric treatments 100% (—) and 50% (----) of available soil water, rates of cattle biofertilizer in two soil types.

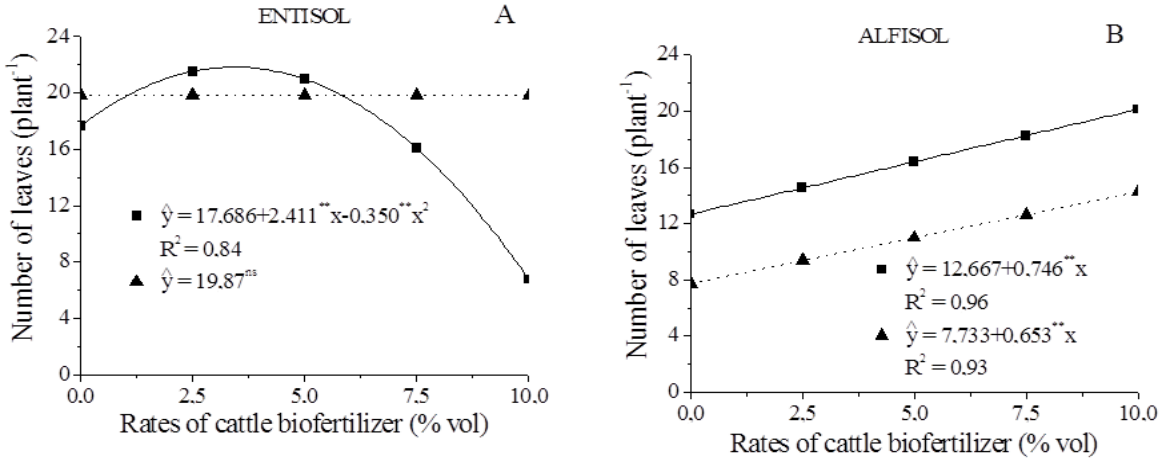

Figure 3. Number of sunflower plants leaves cv. Helium 253 under two hydric treatments 100% (—) and 50% (----) of available soil water), rates of cattle biofertilizer in two soil types.

associated with increased fertility of Alfisol, given the low levels of organic matter, phosphorus and potassium verified in this soil in comparison to the studied Entisol.

The reduction in the level of available water in the soil from 100 to 50% WA, independently of the soil studied, caused reductions in stem diameter of sunflower cultivar Helium 253, indicating that this plant does not support hydric deficit, since it provokes stem growth reduction in both height and diameter, possibly due to the fact hydric stress slows cell growth and elongation (Taiz and Zaiger, 2013). This information was also verified by Silva et al. (2007b) and Gomes et al. (2010), both studied sunflower plants submitted and not to hydric stress throughout the crop cycle. The authors ascertained increments in stem diameter of the plants, in the order of 26.48 and 22.22% for treatments without hydric stress in relation to plants under hydric stress. These results characterize sunflower as a plant highly sensitive to hydric stress.

As for the rates of biofertilizer, we verified a quadratic behaviour on Entisol maintained at 100% WA, noting that the largest number of leaves (20.76 plant^{-1}) was obtained in plants cultivated at a rate of 3.44% v/v. However, plants subjected to water deficit (50% WA in the soil) did not fit to the specific mathematical models, obtaining an average of 19.87 leaves plant^{-1} (Figure 3A). Reductions were observed in the number of leaves of sunflower plants when subjected to biofertilizer levels above 3.44% v/v on the treatments kept without hydric stress. This fact may be related to excess nutrients applied to the soil via biofertilizer, as verified by Oliveira et al. (2009), where high levels of organic matter in the form of manure can cause nutritional imbalance in the soil and, consequently,

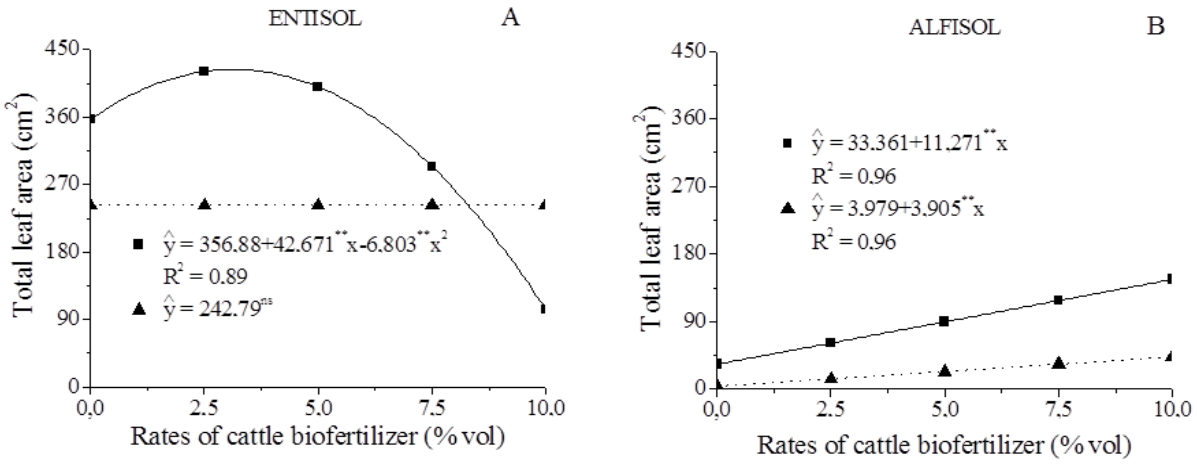

Figure 4. Leaf area of sunflower plants cv. Helium 253 under two hydric treatments (100% (—) and 50% (---) of available soil water), rates of cattle biofertilizer in two soil types.

reduced plant growth.

When studying the number of plant leaves cultivated in Alfisol, there was a linear increase with increments of 0.74 and 0.65 leaves per plant for each unit increase of biofertilizer rate for levels of 100 and 50% of WA in the soil, respectively (Figure 3B). The superiority of the number of leaves in conditions of 100% WA and the reduction in the number of leaves on plants under condition of hydric stress is explained by Taiz and Zeiger (2013), so that the lack of water in the plant tissues due to drought limits cell division and CO_2 assimilation rate, thus reducing plant growth.

However, the significant increase in the number of leaves as a result of the increase of irrigation depth is in accordance with Paiva Sobrinho et al. (2011), that evaluating sunflower under different levels of irrigation (60, 70, 80, 90 and 100% in relation to field capacity), observed a significant effect of increase in water availability on the number of leaves, being 87.34% the one that provided the highest number of leaves per plant (10.25).

An increase in leaf area of plants cultivated in Entisol at the level of 100% WA was observed when fertilized with cattle biofertilizer until the estimated rate of 3.13% v/v, with a value of 423.78 cm^2, decreasing with an increase of the rate (Figure 4A). This happens probably for nutritional imbalance, caused by an excess of nutrients, due to the natural fertility of the soil present elevated levels even before the application of biofertilizer, and also favor a possible increase of salt content in the soil solution (Epstein and Bloom, 2006).

On the plants led to 50% WA in Entisol, the data of leaf area did not adjust to the mathematical models studied, obtaining a mean of 242.79 cm^2 $plant^{-1}$ (Figure 4A), possibly under this condition of hydric availability the biofertilizer dilution did not occur, unlike it was verified in Alfisol which has higher content of silt and clay giving the this soil higher amount of micropores when compared to Entisol, which has 82% of sand in its granulometric fraction, which confers it a greater number of macropores, resulting in a greater water loss and hence in insufficient solubility of biofertilizer for the plants. This is confirmed by the positive effect of biofertilizer in Alfisol with water availability at 50% WA in the soil where your greatest clay content promoted greater water retention in the soil (Figure 4B).

For the leaf area of sunflower plants grown in Alfisol, a linear and growing behavior was observed, with an increase of 11.3 and 3.9 cm^2 in leaf area for each unit increase of biofertilizer rate, on the blades 100 and 50% WA, respectively (Figure 4B), achieving leaf area of 146.07 and 43.04 cm^2 when submitted to 10% of biofertilizer. Probably the biggest leaf area in the soil maintained at 100% of WA may be related to the higher solubility and thus availability of organic matter applied to the soil through the biofertilizer, thus improving the relationship between macro and micronutrients. In addition to the fact that the addition of biofertilizer increases the content of organic matter in the soil and consequently increases the water retention level in the soil. According to Silva Júnior and Siqueira (1997) the mineralization of organic fertilizers improves soil structure, aeration and capacity to hold water, for assisting in the formation of aggregates, and exercising with this direct effect on soil microporosity.

With respect to the water content in the soil, similar results were obtained by Silva et al. (2007b) and Paiva Sobrinho et al. (2011), who verified that 93.43% of field capacity promoted greater leaf area of sunflower cultivars Charrua and Olisun 3 evidencing that the sunflower crop requires high availability of water in the soil.

A quadratic behavior of cumulative shoot dry mass of cultivar Helium 253 sunflower's was observed depending on the biofertilizer rates applied to Entisol kept at 100% of WA, verifying the maximum accumulation of biomass of 159.25 g $plant^{-1}$, in the estimated amount of 3.40% v/v.

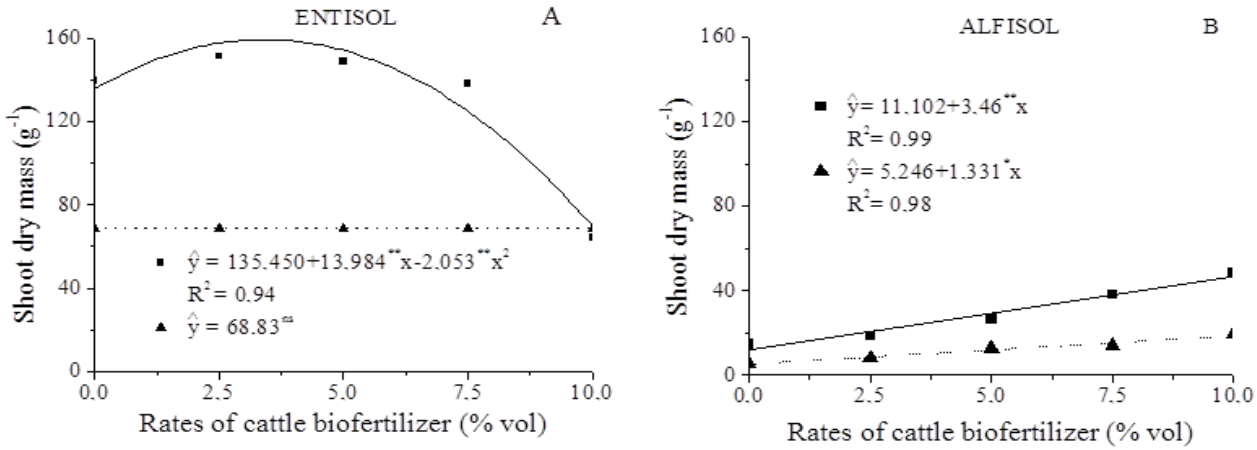

Figure 5. Shoot dry mass of sunflower plants cv. Helium 253 under two hydric treatments (100% (—) and 50% (----) of available soil water), rates of cattle biofertilizer in two soil types.

However, when the soil was maintained at 50% of WA, dry biomass data did not fit the proposed mathematical models, averaging 68.83 g plant^{-1}, these results, 43% lower than those observed in plants grown with 100% WA (Figure 5A).

However, a linear growth of the shoot dry mass in Entisol with 100 and 50% WA, with an increase of 3.46 and 1.33 g plant^{-1} for each unitary increase in biofertilizer rate, reaching maximum values of 46.51 and 18.56 g plant^{-1} at rate of 10% (v/v), respectively (Figure 5B).

The superiority of plants kept at 100% WA in the soil is observed on the order of 131.36 and 162.98% in comparison to plants submitted to 50% WA in Entisol and Alfisol, respectively. This fact shows that the Helium 253 sunflower hybrid did not support the water stress, thus evidenced the importance of adequate water supply throughout the culture cycle, in order to occur effective plant growth, resulting in great productions of biomass, in agreement with Silva et al. (2011) and Paiva Sobrinho et al. (2011), who also observed greater biomass weight of sunflower cultivated without hydric deficit in the soil.

Plants cultivated in Entisol were greater than those grown in Alfisol, with Entisol superiority of 262.65% in aerial biomass of sunflower Helium 253. This may be related to increased K, Ca and organic matter of Entisol, because according to Zobiole et al. (2010), the export order of the hybrid BRS 191 was as follows: N > P = K > Mg = S > Ca. These authors also mention the importance of adequate supply of N, K and Ca, due to high demand of the culture for these elements. These findings were also confirmed by Christin et al. (2009) by verifying the reduction in plant height and number of leaves of the sunflower grown with potassium and nitrogen deficiency in the order of (53.33 and 25%) and (77 and 66.7%), respectively, when compared to those grown without nutritional deficiency.

The root biomass per plant was influenced by the biofertilizer rates applied at Entisol and Alfisol at 100% WA, observing the maximum value of 22.30 and 5.18 g plant^{-1} with application of 3.14 and 10% of the volume, respectively. In the unfolding of the triple interaction of biofertilizer rates within the blade of 50% of WA in both soils, root dry mass per plant did not fit any proposed mathematical model, with averages of 6.85 and 1.2 g plant^{-1}, respectively (Figure 6A and B). This reduction in root dry weight of the plants submitted to hydric stress in the soil may be a reflection of lower absorption of essential elements, according to a research conducted by Paiva Sobrinho et al. (2011), who found that 60% WA in the soil caused a reduction of perspiration, leading to lower water absorption and therefore lower nutrient absorption by the plants promoting limitations to their growth. Vazin et al. (2011) found a reduction of 38% of the shoot dry mass of the sunflower in vegetative phase and only 6% in the production phase when submitted to hydric stress compared to plants grown without soil water deficit.

Plants cultivated in Entisol overlapped the root dry mass of those cultivated in Alfisol, regardless of the water level in the soil. Possibly, the greater content of phosphorus and organic matter in Entisol provided a bigger root growth. Phosphorus is a primary macronutrient, and albeit absorbed in smaller quantities than nitrogen and potassium, its presence on the soil is essential for the growth and production of plants, and organic matter can increase its availability.

Conclusion

Sunflower cultivar Helium 253 proved to be highly sensitive to hydric deficit, showing a significant reduction in growth when cultured at 50% WA. Thus, it is recommended that the cultivation of this crop should be done keeping the soil water levels at 100% WA. The cattle biofertilizer can be used as an important approach

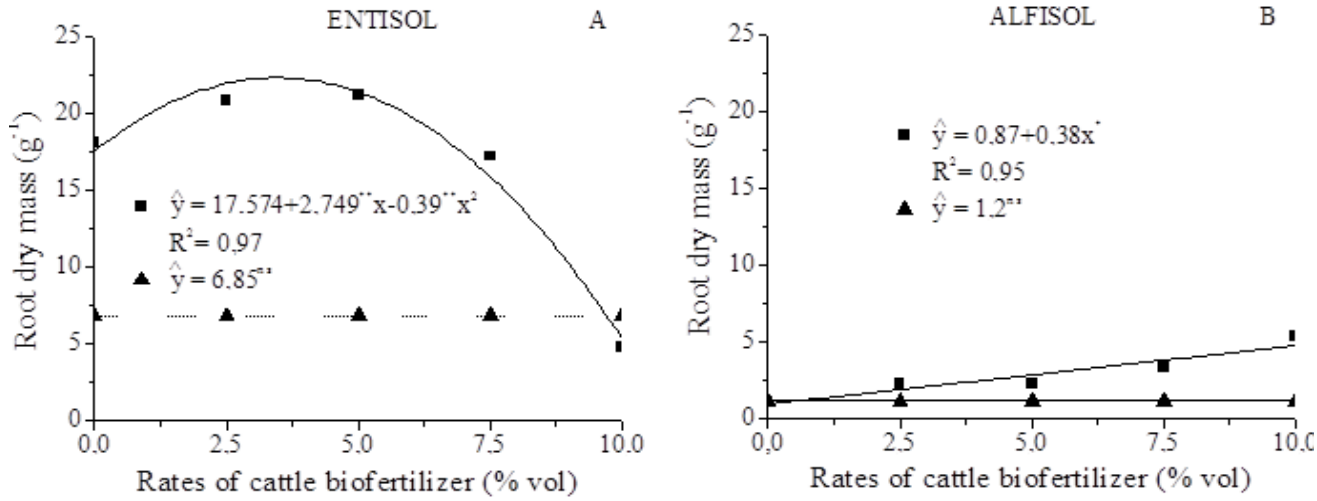

Figure 6. Root dry mass of sunflower plants root cv. Helium 253 under two hydric treatments 100% (—) and 50% (----) of available soil water, rates of cattle biofertilizer in two soil types.

to increase soil fertility. Rates of 3.5 and 10% v/v to Entisol and Alfisol, respectively, were the ones that provided the best results. However, Entisol presented the greater aptitude for the cultivation of sunflower Helium 253.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the UniversidadeEstadual da Paraíba - UEPB for providing the necessary infrastructure to carry out this experiment and also to the National Council for Scientific and Technological Development - CNPq for providing scientific initiation scholarship to the first author.

REFERENCES

Cavalcante LF, Vieira MS, Santos AF, Oliveira WM, Nascimento JAM (2010). Água salina e esterco bovino líquido na formação de mudas de goiabeira cultivar Paluma. R. Bras. de Frut. 32(1):251-261.

Christin H, Petty P, Ouertani K, Burgado S, Lawrence C, Kassem A (2009). Influence of Iron, Potassium, Magnesium, and Nitrogen Deficiencies on the Growth and Development of Sorghum (*Sorghum bicolor* L.) and Sunflower (*Helianthus annuus* L.). J. Bio. Resour. 1(1):64-71.

Conab - Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira: 4º levantamento de Grãos - Safra 2012/2013 (2013). Disponível em: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/13_01_09_17_44_20_boletim_graos_janeiro_2013.pdf. Acesso em: 15 de Abri. 2013.

Empresa Brasileira de Pesquisa Agropecuária (1997) - Embrapa Centro Nacional de Pesquisa de Solos. Ministério da Agricultura e do Abastecimento. Manual de métodos de análise de solo. 2. ed. P. 212.

Epstein E, Bloom AJ (2006). Nutrição mineral de plantas: Princípios e perspectivas. P. 403.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciên. e Agrote. 35:1039-1042.

Freitas CS, Silva ARA, Bezerra FML, Andrade RR, Mota FSB, Aquino BF (2012). Crescimento da cultura do girassol irrigado com diferentes tipos de água e adubação nitrogenada. R. Bras. Eng. Agrí. Amb. 16(10):1031-1039.

Gomes EP, Ávila MR, Rickli ME, Petri F, Fedri G (2010). Desenvolvimento e produtividade do girassol sob lâminas de irrigação em semeadura direta na região do arenito caiuá, estado do Paraná. Irrigation 15(4):373-385.

Howell TA, Evett SR, Tolk JA, Copeland KS, Marek TH (2015). Evapotranspiration, water productivity and crop coefficients for irrigated sunflower in the U.S. Southern High Plains. Agric. Water Manag. (162):33-46.

Ibge: instituto brasileiro de geografia e estatística (2012). Disponível em: http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/lspa/default.shtm. Acesso: 02 jan. 2014, 10:30:25.

Jiang X, Chen Z, Dharmasena M (2014). The role of animal manure in the contamination of fresh food. Adv. Microb. Food Saf. (2):312.

Lechinoski AL, Freitas JMN, Castro DS, Lobato AKS, Oliveira Neto CF, Cunha RLM, Costa RCL (2007). Influência do estresse hídrico nos teores de proteínas e aminoácidos solúveis totais em folhas de Teca (*Tectona grandis* L. f.). R. Bras. Bioci. 5(1):927-929.

Maldaner IC, Heldwein AB, Loose LH, Lucas DDP, Guse FI, Bortoluzzi MP (2009). Modelos de determinação não-destrutiva da área foliar em girassol. R Ciên. Ru. 39(5):1356-1361.

Nobre RG, Ghyi HR, Soares FAL, Andrade LO, Nascimento ECS (2010). Produção do girassol sob diferentes lâminas com efluentes domésticos e adubação orgânica. R. Bras. Eng. Agríc. Amb. 14(7):747-754.

Oliveira FA, Oliveira Filho AF, Medeiros JF, Almeida Júnior AB, Linhares PCF (2009). Desenvolvimento inicial da mamoneira sob diferentes fontes e doses de matéria orgânica. R. Caat. 22(1):206-211.

Paiva Sobrinho S, Tieppo RC, Silva TJA (2011). Desenvolvimento inicial de plantas de girassol em condições de estresse hídrico. Enciclop. Biol. 7(12):1-12.

Usda- United States Department of Agriculture. Keys to Soil Taxonomy (2014). 12 ed. Washington. pp. 1-353.

Rivera-Cruz MC, Trujillo NA, Córdova GB, Kohler J, Fuensanta C, Roldán A (2008). Poultry manure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability

in banana crops. Soil Bio. Biochem. 40(12):3092-3095.
Santos ACV (1992). Biofertilizantes líquidos: o defensivo agrícola da natureza. 2 ed. P. 162.
Silva ARAS, Bezerra FML, Sousa CM, Pereira Filho JV, Freitas CAS (2011). Desempenho de cultivares de girassol sob diferentes lâminas de irrigação no Vale do Curu, CE. R. Ciên. Agro. 42(1):57-64.
Silva Júnior JP, Siqueira JO (1997). Aplicação de formononetina sintética ao solo como estimulante da formação de micorriza no milho e na soja. R. Bras. Fisio. Veg. 9(1):35-41.
Silva MLO, Faria MA, Morais AR, Andrade GP, Lima EMC (2007b). Crescimento e produtividade do girassol cultivado na entressafra com diferentes lâminas de água. R. Bras. Eng. Agríc. Amb. 11(5):482-488.
Silva TJA, Folegatti MV, Silva CR, Alves Júnior J, Silva BEM (2007a). Balanço de energia e estimativa da evapotranspiração em culturas irrigadas de maracujazeiro pelo método da Razão de Bowen. R. Eng. Agríc. 27(2):392-403.
Souza RM, Nobre RG, Gheyi HR, Dias NS, Soares FAL (2010). Utilização de água residuária e de adubação orgânica no cultivo do girassol. R Caat. 23(2):125-133.
Taiz L, Zeiger E (2013). Fisiologia vegetal. Porto Alegre: Artmed, 5.ed. P. 918.
Tiessen H, Sampaio EVSB, Salcedo IH (2001). Organic matter turnover and management in low input agriculture of NE Brazil. Nut. Cycl. Agroeco. 61(2):99-103.
Vazin F, Hassanzadehdelouei M, Kheradmand M (2011). Effect of Defoliation and Feand Mn Spraying on Quality and Quantity Characteristics of Sunflower (*Helianthus annuus* L.). Not. Sci. Biol. 3(3):113-119.
Zobiole LHS, Castro C, Oliveira FA, Oliveira Junior A (2010). Marcha de absorção de macronutrientes na cultura do girassol. R Bras. Ciên. Solo. 34(2):425-433.

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Vol. 10(41), pp. 3921-3929, 8 October, 2015
DOI: 10.5897/AJAR2015.10089
Article Number: 0A5546355853
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Temporal variation in weed occurrence and biomass under conservation agriculture and conventional farming practices

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Received 30 June, 2015; Accepted 10 September, 2015

Conservation agriculture (CA) is advocated as a management system for sustainable productivity, while preserving the environment simultaneously. CA has many advantages, but weed management is regarded as one of its biggest challenges. This study reports on the temporal variation in weed occurrence and biomass under conservation and conventional farming practices. The treatment design was a split-split plot, with a randomised complete block design with three blocks as replicates. Tillage was the main plot factor (reduced tillage [RT] and conventional tillage [CT]), and treatments (a combination of cropping systems and fertilizer levels) were treated as the sub-plot factor. Only cultivation year ($F(2.48) = 9.12$, $p < 0.001$) and the cultivation year and tillage interaction ($F(2.48) = 22.41$, $p < 0.001$) significantly affected weed biomass. Weed biomass and species diversity increased under RT from cultivation year 3 to 5. Under CT weed biomass had a slight downward trend and species composition was similar across the three years with two dominant weeds representing between 87.2 and 75.1% of total weed biomass. The results suggest that tillage practices can affect both the biomass and diversity of weeds. It is therefore important that practitioners understand such variation and apply weed management practices accordingly.

Key words: Conventional tillage, reduced tillage, weed composition, weed dynamics.

INTRODUCTION

Conservation agriculture (CA) is advocated to improve soil health, optimise crop yields and reduce input costs when effectively applying three principles: (i) Minimal soil disturbance (including practices such as no-till or reduced till); (ii) Permanent soil cover (including crop residues or cover crops); and (iii) Crop diversification (inclusion of various crops, especially legumes, and introduction of rotation or intercropping system) (Dumanski et al., 2006; Hobbs et al., 2008; Wall, 2008). In recent years, CA has been promoted under small-scale farmers in sub-

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Saharan Africa to improve food security (Mazvimavi and Twomlow, 2009), as conventional agricultural practices are difficult and costly to implement for many of these farmers. For example, it is estimated that up 35% of the world's population do not have access to high input technologies (such as intensive soil preparation and use of fertilizers and chemicals) associated with conventional farming practice (Pretty, 1999). Africa has been slower in adopting CA compared to other developing countries, and one of the major limiting factors is the number of necessary weeding events (Mashingaidze et al., 2012; Andersson and D'Souza, 2014). Most weeding is done manually, and labour is reported to be a constraint on many small-scale farms. Therefore, despite many successes achieved in the adoption of CA practices, weed management remains one of the biggest challenges (Rockstrom and Steiner, 2003; Hobbs et al., 2008; Giller et al., 2009; Andersson and D'Souza, 2014; Nichols et al., 2015).

Weed biomass, density, composition and temporal variation are closely associated with management practices, especially tillage (Teasdale et al., 1991; Garcia de Leon et al., 2014; Nichols et al., 2015). For example, conventional tillage practices may effectively control weeds by burial (Froud-Williams et al., 1984; Hobbs et al., 2008; Wall, 2008), or stimulate weed germination by raising soil temperature (Froud-Williams et al., 1984; Teasdale et al., 1991; Murphy et al., 2006). Alternatively, minimal or reduced tillage can shift weed composition from broadleaf to grass species (Swanton et al., 1999) or perennial weeds (Vogel, 1995), or increase weed species diversity when specific habitats for certain weeds are created (Murphy et al. 2006). Mulch or soil cover may reduce or inhibit weed germination through the release of allelopathic compounds (Christoffoleti et al., 2007) or smothering of weeds (Teasdale et al., 1991; Thierfelder and Wall, 2010). Furthermore, weeds can be influenced by location, time, nitrogen management (Swanton et al., 1999), timing of cultivation, rainfall (Teasdale et al., 1991), crop residue management, crop rotations, harvest procedures and other aspects of the production system (Wall, 2008).

Conventional tillage practices have many advantages, such as preparation of the seedbed, uniform placement of seeds, temporary relief from compaction, and effective removal of all weeds prior to planting (Hobbs et al., 2008), all of which level and clean the soil and simplify subsequent farming operations. On the other hand, reduced tillage lessens the use of fossil fuels, decreases runoff and erosion, thereby conserving soil organic matter (SOM) and preventing soil physical degradation (Hobbs et al., 2008; Wall, 2008). All three of the CA principles are intended to increase SOM, which, in turn, results in improved physical, chemical and biological properties of the soil that are associated with practising CA (Bot and Benites, 2005). High input cost, decrease in soil productivity and depletion of SOM could motivate a farmer to change from conventional to conservation agriculture. However, this change could also present new challenges regarding weed management (Rockstrom and Steiner, 2003; Murphy et al., 2006).

It is therefore important to understand not only the effect of agricultural practices on weed dynamics, but also the temporal variation in weed species expected under different agricultural practices. This study aimed to understand (a) the effects of conservation and conventional agricultural practices (tillage, crop rotation, soil cover) on weed abundance and biomass, and (b) temporal variation in weed community composition in a medium-term field trial at Zeekoegat, Gauteng, South Africa. The effect of tillage on weeds was the most evident, and therefore discussed in more detail compared to other CA aspects, such as permanent soil cover and multi-cropping.

MATERIALS AND METHODS

Experimental site

To evaluate the effect of CA on soil and plant properties, the Agricultural Research Council (ARC) initiated the Zeekoegat on-station, dryland field trial. The trial started in October 2007 and ended, after 6 growing seasons, in May 2013. The trial was conducted north of Pretoria at Zeekoegat, Roodeplaat (25°36'55"S, 28°18'56"E), in Gauteng Province, South Africa. The soil is moderately fine to medium structured with a clayey texture (45% clay). The long-term, annual mean daily minimum and maximum temperatures are 10.8 and 27.1°C, respectively, and the mean long-term rainfall for the area is 704 mm year^{-1} (ARC-ISCW, 2006). An automatic rain gauge (Texas 525 TE) was installed adjacent to the field trial, providing site specific rainfall measurements for the duration of the trial (Table 1).

Trial layout

The trial was designed to compare conventional farming practices (ploughing and monoculture) with various CA aspects (reduced tillage, permanent soil cover and multi-cropping systems). The treatment design was a split-split plot, with experimental design, a randomised complete block, with three replicates as blocks. Each replicate was split into two tillage systems (main plots) with each main plot (reduced tillage [RT] and conventional tillage [CT]) further subdivided into six treatments (three cropping systems x two fertilizer levels (subplots)), giving a total of 36 plots. Repeated measurements were taken over years and regarded as sub-sub-plot factors (Little and Hills, 1978).

The three cropping systems were: Maize (*Zea mays*) monoculture (MM)), maize/soybean (*Glycine max*) rotation (MS) and maize/cowpea (*Vigna unguiculata*) intercropping (MC)). The maize in the MC treatment was planted in 1.8 m tramline rows to accommodate the intercropping of cowpea between maize rows. Cowpea and soybean were planted in 30 cm rows. Plot dimensions were 7.2 m × 8 m with 0.9 m planting rows for maize.

Fertilizer was applied at two levels: an optimal level to represent ideal nutrient supply, and a low level (50% of the optimal) to represent situations of reduced inputs from small-scale farms. The optimal level was calculated according to the fertilizer application guidelines and soil analysis, using a target yield of 4 ton ha^{-1}. Fertilizer was band applied during planting (60% of total) and top-up fertilizer (40% of total) was surface-applied 6 to 8 weeks later.

Limestone ammonium nitrate and superphosphate were applied,

Table 1. Site-specific rainfall data for Zeekoegat experimental farm (South Africa) for the period 2009 to 2012 indicating rainfall as corresponding with crop planting dates and weed sampling dates.

Season	Planting start date	Weed sampling start date	Rainfall for period between planting and sampling (mm)	Total rainfall for season* (mm)
Season* 3 2009/10	18 November, 2009	13 January, 2010	294.2	1316.7
Season* 4 2010/11	29 November, 2010	25 January, 2011	544	1010.8
Season* 5 2011/12	29 November, 2011	10 January, 2012	200.1	571.5

*Season started in June and ended in May.

but no potassium fertilizer was needed, as the natural K content of the soil was very high (average of 475 mg kg^{-1}). For legumes, only superphosphate was used (assuming a target yield of 1 ton ha^{-1} seed).

Land preparation and weed management

At the onset of each season (October), all crop residues from the previous season were flattened and slashed. The CT plots were ploughed with a mouldboard plough and then disked with a disk harrow. Furrows for planting were drawn with a four tine cultivator frame. The RT plots were undisturbed, except for furrows, created similar to those in the CT plots. Crops were manually planted with a hand-held planter.

Weed control was applied consistent across the trial and comprised a combination of chemical treatment (before and after planting) and manual weeding (hand hoed 2 to 3 times after planting). Every year, during November, just before planting, a mixture of Roundup® (glyphosate) and DualGold® (S-metholachlor) was applied equally across the trial by using a tractor and sprayer. This was repeated the day after planting, before crop emergence. Crops were planted after significant rainfall, usually by the end of November or early December. After crop emergence, weeds between the crops were manually removed instead of chemically, to prevent negative interaction of chemicals with microbial populations which were sampled during January each year.

Data collection

The Zeekoegat trial was designed to quantify effects of CA on soil and plant properties, and since weed evaluation was not originally included in the aims of the trial, weed data was initially opportunistically collected. However, in the third trial season (2009/10) the obvious difference between weed composition under reduced and conventional tillage systems served as a motivation to start thorough weed data collection. Weed samples were thus collected for three consecutive years, in January 2010 (third cultivation year (Yr 3)), January 2011 (Yr 4) and January 2012 (Yr 5). Weeds were sampled before the first manual weeding, which only occurred in January of each year (Table 1). The first weeding was delayed due to the nature of the sampling (chemical weed control interfered with biological samples for microbial research), and due to the planting time (logistic constraints during December holidays). This situation is representative of most small-scale farming practices, where either labour for manual weeding or funding for chemical treatments is limiting and weed control remains a challenge.

Two 1 m^2 quadrants were destructively sampled for weed biomass from each plot. Samples were taken in a fixed grid to eliminate bias sampling. Individual weeds were removed at ground level (above-ground biomass) after which they were oven-dried at 40ºC and weighed. The weights of two dominant weeds, large thorn apple (*Datura ferox* L.*)* and purple nutsedge (*Cyperus rotundus* L.), were measured separately, to calculate their relative abundance. Other weeds, which included flax-leaf fleabane (*Conyza bonariensis* (L.) Cronquist*),* khaki weed *(Tagetes minuta* L.*),* khaki bur weed *(Alternanthera pungens* Kunth*),* common blackjack *(Bidens pilosa* L.*),* narrow-leaved ribwort *(Plantago lanceolata* L.*),* and devil's thorn *(Tribulus terrestris* L.*)* were grouped in a separate class 'Other'. The focus was initially only on large thorn apple and purple nutsedge as they were the obviously dominant weeds. By the fifth cultivation year, other weeds were also becoming dominant, albeit in the RT plots (such as flax-leaf fleabane and khaki weed), but since these species were not included from the start, they were not specified at the end.

Maize yield was determined by hand harvesting two 5 m rows from the centre of the plots. Plants and cobs were counted. Cobs were removed and grain was stripped and weighed. Values were adjusted by taking into account the moisture content of the grain.

Statistical analyses

Weed biomass data was subjected to analysis of variance (ANOVA), using a split-split-plot model, with tillage as main-plots, treatments as sub-plots, and year as sub-sub-plots (sub-samples) (Little and Hills, 1978). ANOVA was used to test differences between effects. The data was acceptably normal distributed and separated using Fishers' protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor and Cochran, 1967). This was done using Genstat Statistical package (Genstat, 2011).

Cluster analysis (vertical hierarchical tree plot) was used to statistically analyse data on weed biomass using STATISTICA 6.1 (StatSoft Inc. Tulsa, OK, USA). A dendrogram was constructed with Ward's clustering algorithm, and the Euclidean distance measured, that is, the geometric distance between variables in a multidimensional space. Since our study was not designed and set up to investigate the effect of weeds on yield performance, we could not formally test these effects. However, we used a linear regression model between weed biomass and grain yield as heuristic test to evaluate effect of weed biomass on grain yield. Finally we used a Shannon-Wiener diversity index and Evenness index to investigate effect of tillage on species richness and abundance (Magurran, 1988).

RESULTS AND DISCUSSION

During the three sampling years, rainfall was poorly distributed and resulted in low grain yields and low biomass production with subsequent low, ineffective soil cover (Figure 1). Soil moisture has a significant effect in delaying or restricting crop and weed emergence, which

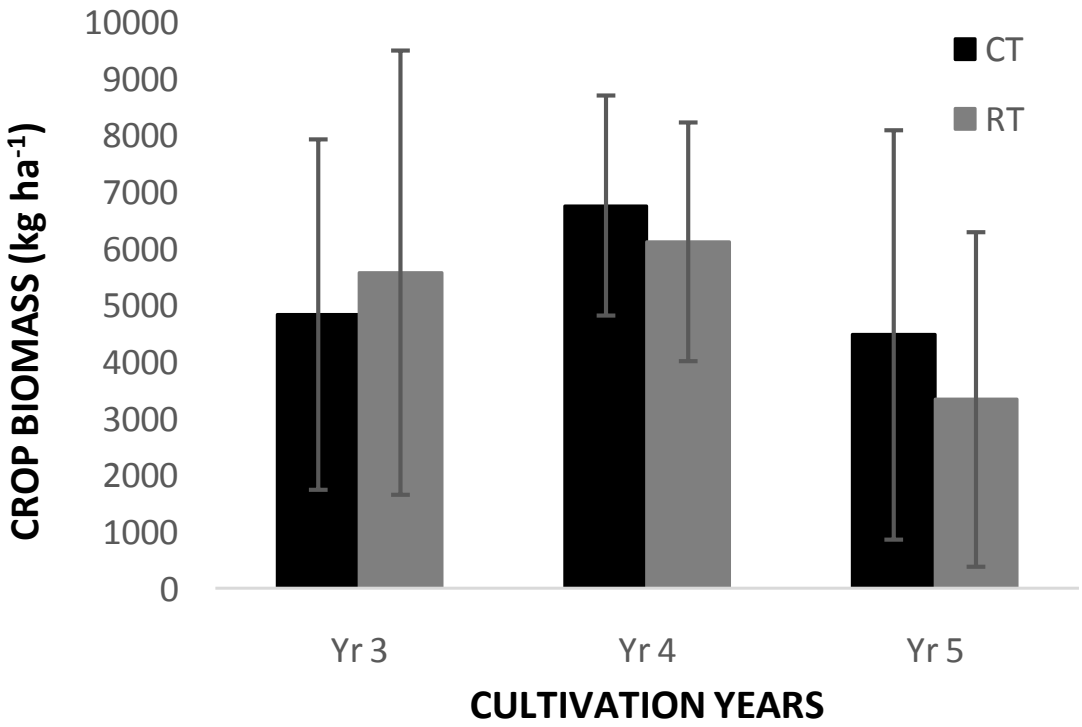

Figure 1. Crop biomass under reduced (RT) and conventional tillage (CT) for three consecutive years during weed sampling.

can affect the outcome of interference between crops and weeds (Roberts, 1984). The low soil water could delay weed seedlings, which could result in possible insignificant responses of weeds to cropping systems (Roberts, 1984). However, the comparative results from weeds under different tillage systems were more enhanced, as well as comparisons across three consecutive years. For this reason, the focus of the results is on the tillage aspects of the two farming systems (CA vs. conventional agriculture), and how this changed over the three sampling years.

Weed biomass

Weed biomass was significantly affected by cultivation year ($F(2,48) = 9.12$, $p < 0.001$), as well as the cultivation year and tillage interaction ($F(2.48) = 22.41$, $p < 0.001$). No other main effects or interactions were statistically significant. Weed biomass under RT systems was low in the third cultivation year (weed biomass for cultivation years 1 and 2 was not determined), but increased significantly by the end of the fifth cultivation year (Figure 2). Under CT, however, weed biomass was initially high, but it stayed more constant, possibly showing a tendency to decrease (Figure 2).

The results suggest that a temporal variation can be expected; with an increase in weed biomass under RT practices, while under CT practices weed biomass was more stable over cultivation time. Similarly, we detected a temporal trend in weed species diversity, where species diversity increased under RT but decreased under CT (Figure 3). Following this trend we would expect a time effect on species composition, with higher diversity the longer the trial continues. Indeed species diversity for the last year (5^{th} year of treatment) suggests that RT had a higher species diversity than CT (Table 2). CT also had a low Evenness index (E), which suggests that CT is dominated by a few weed species, but that these species occurs in high abundance, while RT had a lower E value and hence higher diversity, but at lower abundances (Table 2).

The interaction between tillage and cultivation year is illustrated in the cluster analysis (Figure 4). Cluster analysis assigns treatments into groups, thus clustering similar treatments together. Three distinctive clusters were identified, with a RT-only cluster on the left (exclusively from cultivation years 3 and 4), a CT-only cluster on the right (mainly from year 3 and 5), and thirdly, a mixed cluster (consisting of both CT and RT) closely linked to the CT-only cluster. It is interesting to note that the RT treatments included in the mixed cluster are all from cultivation year 5, where the biomass was, contrary to previous cultivation years, exceptionally high. The dendrogram successfully illustrated the shift in weed biomass dynamics under CT and RT from the third to the fifth cultivation year.

The results concur with other authors (Teasdale et al.,

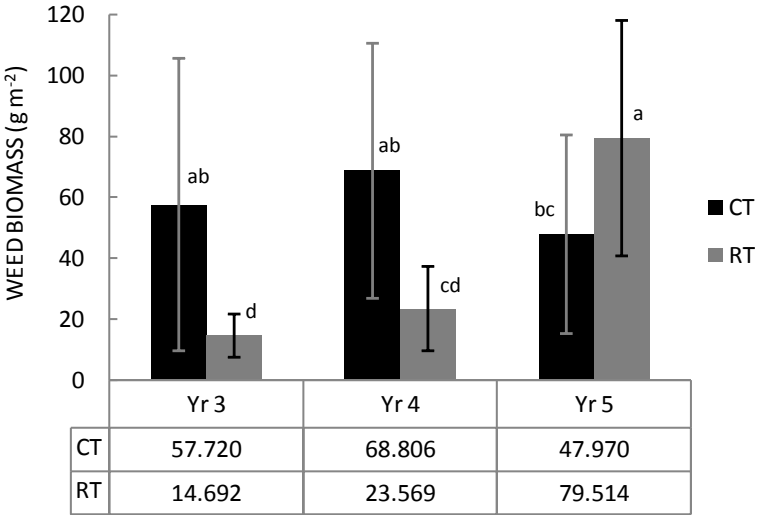

Figure 2. Effect of tillage practice (CT = conventional tillage; RT = reduced tillage) and cultivation year on weed biomass (g m^{-2}) at the Zeekoegat field trial, 2009-2012. * Significant differences are indicated by different letters (a,b, c and d).

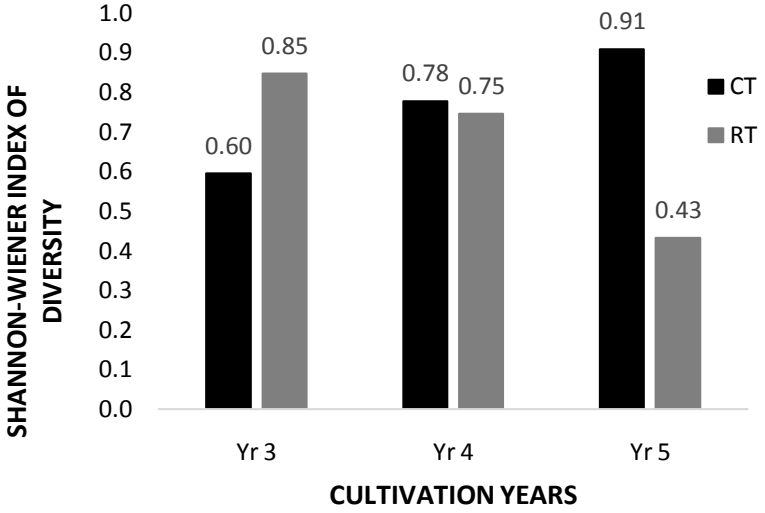

Figure 3. Shannon-Wiener diversity index of specie.

1991; Vogel, 1995; Mashingaidze et al., 2012) who also recorded increased weed biomass with cultivation time under RT practices. The relatively high weed biomass (14.69 to 79.51 g m^{-2}) corresponds with results from Vogel (1995), who reported values ranging between 20 and 220 g m^{-2} on small-scale farms, as well as Teasdale et al. (1991) with values between 48 and 623 g m^{-2} on a maize-based system. Furthermore, the temporal variation in weed biomass under different tillage practices concurs with Swanton et al. (1999) who reported that weed biomass varied between tillage practice and cultivation year. In conventionally tilled soils, this can be explained by the increase in environmental variables, such as temperature and moisture, as a result of tillage. Increased environmental variables could lead to more favourable conditions in certain years, and this in turn could lead to a large year-to-year variation in weed density (Clements et al., 1996). Additionally, tillage could increase the soil temperature and stimulate weed germination (Froud-Williams et al., 1984). Weed biomass in tilled soils could thus be a function of burial depth, periodicity of emergence, climatic conditions such as rainfall and temperature (Froud-Willliams et al., 1984; Teasdale et al., 1991; Mashingaidze et al., 2012) or

Table 2. Shannon-Wiener (H) and Evenness (E) index for the last cropping season (Yr 5).

	Conventional tillage (CT)	Reduced tillage (RT)
H	0.944	1.564
E	0.485	0.804

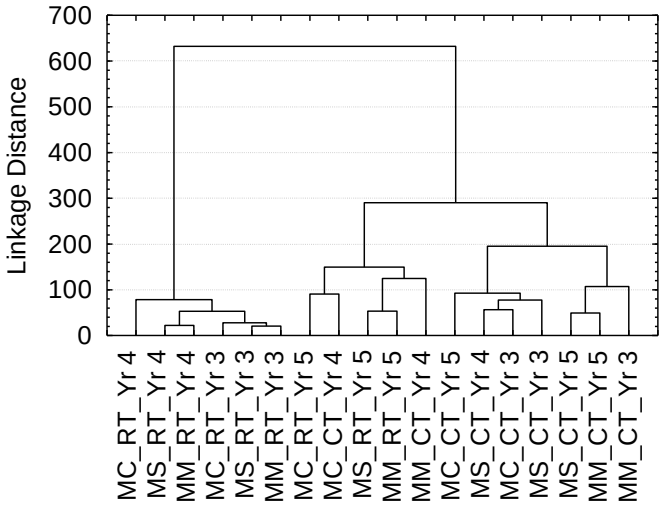

Figure 4. Dendrogram illustrating the clustering of weed biomass under CT and RT from the third to the fifth cultivation year.

density dependence due to change in spatial distribution (Garcia de Leon et al., 2014). In contrast, under RT soils, weed seeds are often buried shallower, which might lead to more weed germination. Weed germination under RT systems would more likely be linked to rainfall than temperature, as is the case in CT (Froud-Williams et al., 1984). Thus, weed biomass, emergence or diversity might be increased or decreased in both tillage systems, but for different reasons. It is therefore possible to see large year-to-year fluctuations between RT and CT as the specific weed seed bank changes, or certain climatic factors favour one or the other system (Menalled et al., 2001; Murphy et al., 2006).

Weed diversity

The CT system was annually dominated by the two pioneer weed species, that is, large thorn apple and purple nutsedge, which when combined, contributed to 87.2% (yr 3), 75.1% (yr 4) and 78.5% (yr 5) of the total weed biomass (Figure 5). Ploughing seems to be conducive for these species, and one possible reason therefore might be the succession of a plant community that must start anew each year after ploughing, resulting in these dominant pioneer plants germinating first. However, under RT systems, there was a temporal increase in the relative weed diversity (Figure 5) largely due to the successional trajectory of the weed community (Murphy et al., 2006). Weed communities are indicative of ecological succession, and since a reduction in soil tillage leads to a reduction in agro-ecosystem disturbance, the associated change in weeds can be measured accordingly (Clements et al. 1996). Under RT the dominant weeds (large thorn apple and purple nutsedge) actually declined in biomass (34.8% [yr 3], 28.3% [yr 4], 13.4% [yr 5]; Figure 5). The decrease in pioneer weed species under RT coincided with an increase in other weeds, such as flax-leaf fleabane, khaki weed, khaki bur weed, common blackjack and devil's thorn (Figures 3 and 5).

Crop performance

Since this study was not set up to investigate the impact of weeds on crop performance, our results on crop performance here should be interpreted cautiously. For example, we did not have control sites, and as such we cannot investigate the effects of weeds on grain yields.

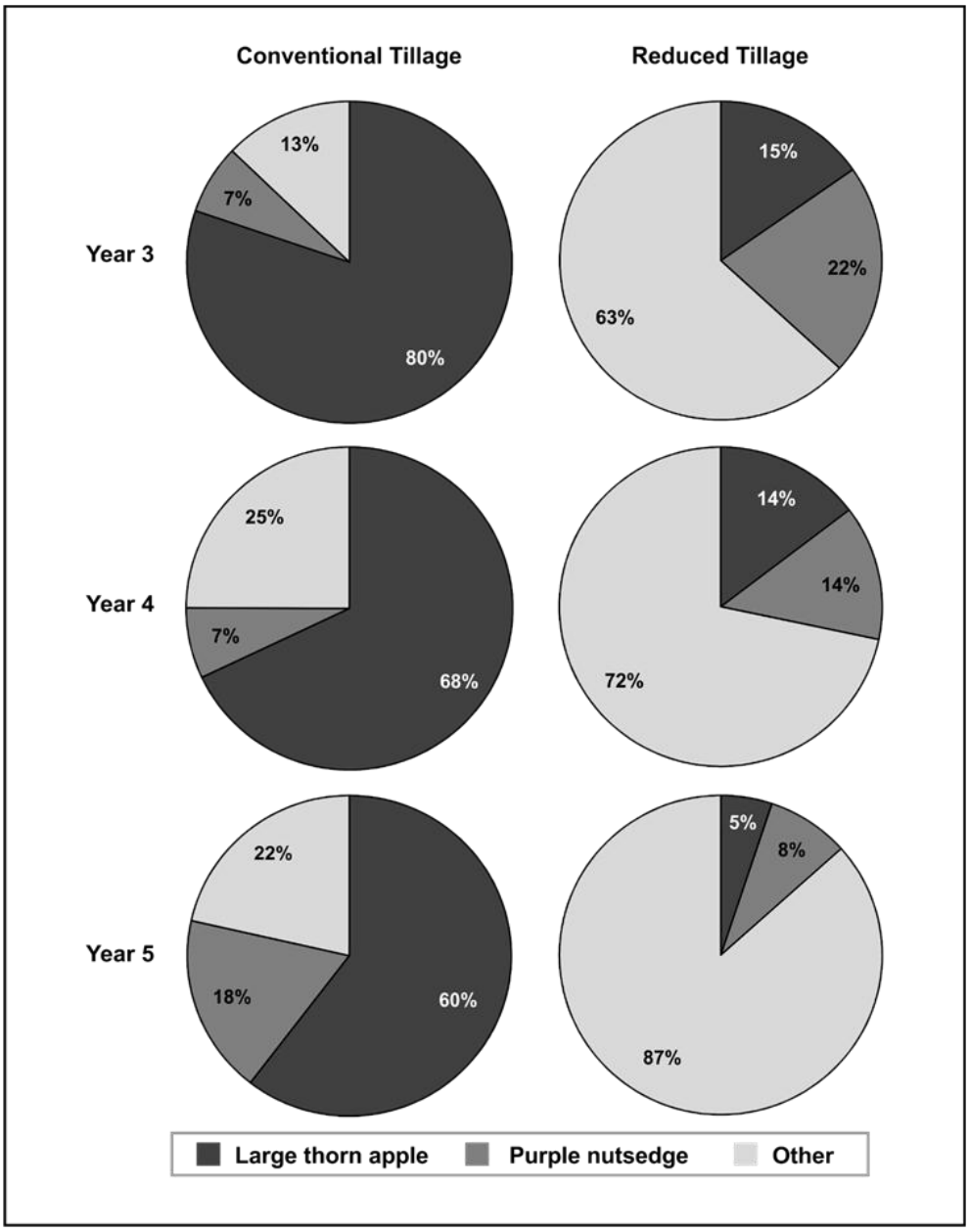

Figure 5. Temporal variation in weed species composition expressed in percentage of biomass, under conventional tillage (left) and reduced tillage (right) practices, for a long-term trial at Zeekoegat.

Nonetheless, using a linear regression model heuristically suggested that in this trial weed biomass had a negligible effect on grain yield (Figure 6). We provide two, not necessary mutually exclusive, hypotheses for this observation. First, rainfall during the trial was poorly distributed, especially during key grain growing periods. Such varying rainfall could have caused low grain performance, irrespectively of weed biomass (Roberts, 1984). Secondly, weeding was done timeously before interference with critical developmental stages. According to Hall et al. (1992), maize yields will not be reduced if weeding is done between the 3 and 14-leaf stage, depending on climatic and site conditions. Overall our observation concurs with Murphy et al. (2006), where increased weed species composition under commercial no-till systems did not result in significant crop losses. Nonetheless, in smallholder CA systems increased weed biomass and diversity could lead to an increase in the amount of labour required for weeding, or alternatively requires increased use of herbicides (Giller et al., 2009;

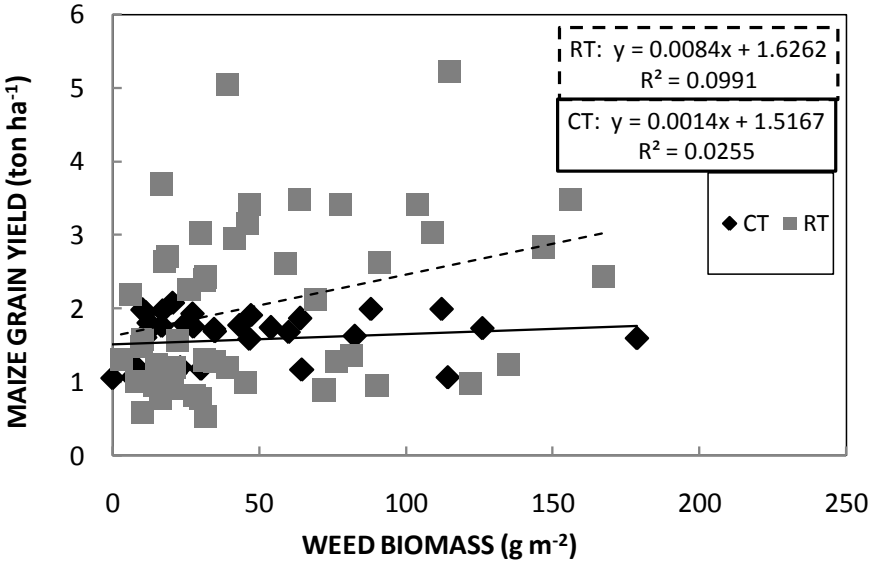

Figure 6. Effect of weed biomass on maize grain yield under conventional tillage and reduced tillage practices, for a long-term trial at Zeekoegat.

Andersson and D'Souza, 2014), which can be a major limitation in implementation of CA on small-scale farms.

CONCLUSION AND MANAGEMENT IMPLICATIONS

After five cultivation years of CA practices, a measurable shift in weed biomass and species composition was observed. Low weed diversity under conventional farming practices (ploughed soils), changed into a diverse weed population under CA practices in a relatively short time period. Cover crops and crop rotation did not have an effect on weed biomass, due to low and poorly distributed rainfall that resulted in low biomass production from previous years, in turn leading to ineffective soil cover and weed suppression. Tillage and cultivation year, however, did have an effect, and while weed biomass was initially low under RT in cultivation year 3, it had increased considerably by cultivation year 5. Species composition also changed; the two main pioneer weeds (large thorn apple and purple nutsedge) made up a decreasing fraction of the weeds under RT, while under CT, more than three quarters of the weed biomass consisted of these two weeds. Adaptable weed management (where continued monitoring of a system should influence the decision-making process and can be changed as needed, depending on available resources) or integrated weed management (combination of biological, chemical and mechanical weed management) should be practised for effective weed control. Such weed control programmes can include crop rotation and application of mulch (Teasdale et al., 1991; Swanton and Murphy, 1996). Cover crops or mulch only suppress weeds effectively when the cover is adequate, and should cover at least 30% of the soil surface (CTIC, 1999).

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The research was funded by the Maize Trust and the ARC-ISCW. The authors thank the staff from ARC-API who assisted with field maintenance, Mr Michael Kidson (ARC-ISCW) for assistance with weed sampling, and Dr Thomas Fyfield (ARC-ISCW) for editorial contributions.

REFERENCES

ARC-ISCW (2006). Agro-Climatology Long-term Reports. In: ARC-ISCW Climate Information System. Agricultural Research Council - Institute for Soil, Climate and Water, Pretoria.

Andersson JA, D'Souza S (2014). From adoption claims to understanding farmers and context: A literature review of conservation agriculture (CA) adoption among smallholder farmers in southern Africa. Agric. Ecosyst. Environ. 187:116-132.

Bot A, Benites J (2005). The importance of soil organic matter: Key to drought-resistant soil and sustained food production. FAO Soils Bulletin 80. Food and Agricultural Organization of the United Nations, Rome.

Christoffoleti PJ, De Carvalho SJP, Lopez-Ovejero RF, Nicolai M, Hidalgo E, Da Silva JE (2007). Conservation of natural resources in Brazilian agriculture: Implications on weed biology and management. Crop Prot. 26:383-389.

Clements DR, Benoit DL, Murphy SD, Swanton CJ (1996). Tillage effects on weed seed return and seed composition. Weed Sci. 44:314-322.

CTIC (Conservation Technology Information Centre) (1999). What's conservation tillage? CTIC Core 4 Program, Lafayette, IN, USA.
Dumanski J, Peiretti R, Benites JR, McGarry D, Pieri C (2006). The paradigm of conservation tillage. Proc. World Assoc. Soil Water Conserv. 1:58-64.
Froud-Williams RJ, Chancellor RJ, Drennan DSH (1984). The effects of seed burial and soil disturbance on emergence and survival of arable weeds in relation to minimal cultivation. J. Appl. Ecol. 21:629-641.
Garcia de Leon D, Freckleton RP, Lima M, Navarrete L, Castellanos E, Gonzales-Andujar JL (2014). Identifying the effect of density dependence, agricultural practices and climate variables on the long-term dynamics of weed populations. Weed Res. 54:556-564.
Genstat (2011). VSN International (2011). GenStat for Windows 14th Edition. VSN International, Hemel Hempstead, UK.
Giller KE, Witter E, Corbeels M, Tittonell P (2009). Conservation agriculture and smallholder farming in Africa: The heretics' view. Field Crops Res. 114:23-34.
Hall MR, Swanton CJ, Anderson GW (1992). The critical period of weed control in grain corn (Zea mays). Weed Sci. 40(3):441-447.
Hobbs PR, Sayre K, Gupta R (2008). The role of conservation agriculture in sustainable agriculture. Philos. Trans. Royal Soc. B. 363:543-555.
Little TM, Hills FJ (1978). Agricultural experimentation design and analysis. New York: John Wiley. pp. 125-137.
Magurran AE (1988). Ecological diversity and its measurement. Princeton University Press, Princeton.
Mashingaidze N, Madakadze C, Twomlow S, Nyamangara J, Hove L (2012). Crop yield and weed growth under conservation agriculture in semi-arid Zimbabwe. Soil Tillage Res. 124:102-110.
Mazvimavi K, Twomlow S (2009). Socioeconomic and institutional factors influencing adoption of conservation farming by vulnerable households in Zimbabwe. Agric. Syst. 101:20-29.
Menalled FD, Gross KL, Hammond M (2001). Weed aboveground and seedbank community responses to agricultural management systems. Ecol. Appl. 11:1586-1601.
Murphy SD, Clements DR, Belaoussoff S, Kevan PG, Swanton CJ (2006). Promotion of weed species diversity and reduction of weed seedbanks with conservation tillage and crop rotation. Weed Sci. 54:69-77.
Nichols V, Verhulst N, Cox R, Govaerts B (2015). Weed dynamics and conservation agriculture principles: A review. Field Crop Res. 183:56-68.
Pretty J (1999). Can sustainable agriculture feed Africa? New evidence on progress, processes and impacts. Environ. Dev. Sustain. 1:253-274.
Roberts HA (1984). Crop and weed emergence patters in relation to time of cultivation and rainfall. Ann. Appl. Biol. 105:263-275.
Rockstrom J, Steiner K (2003). Conservation farming – a strategy for improved agriculture and water productivity among small-holder farmers in drought prone environments. In: Beukes DJ, De Villiers M, Mkhize S, Sally H, Van Rensburg L (eds), Proceedings of the Symposium and Workshop on Water Conservation Technologies for Sustainable Dryland Agriculture in sub-Saharan Africa (WCT). 8-11 April 2003. Agricultural Research Council – Institute for Soil, Climate and Water, Pretoria. pp. 200-208.
Snedecor GW, Cochran WG (1967). Statistical Methods (6th edn). Iowa: The Iowa State University Press. pp. 272-273.
Swanton CJ, Murphy SD (1996). Weed science beyond the weeds: The role of integrated weed management (IWM) in agroecosystem health. Weed Sci. 44(2):437-445.
Swanton CJ, Shrestha A, Roy RC, Ball-Coelho BR, Knezevic SZ (1999). Effect of tillage systems, N, and cover crop on the composition of weed flora. Weed Sci. 47:454-461.
Teasdale JR, Beste CE, Potts WE (1991). Response of weeds to tillage and cover crop residue. Weed Sci. 39:195-199.
Thierfelder C, Wall PC (2010). Weed control in smallholder conservation agriculture. Bulletin 6. CIMMYT. International Maize and Wheat Improvement Centre.
Vogel H (1995). The need for integrated weed management systems in smallholder conservation farming in Zimbabwe. Der Tropenlandwirt 96:35-56.
Wall PC (2008). Tailoring conservation agriculture to the needs of small farmers in developing countries. J. Crop Improv. 19:137-155.

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