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African Journal of Agricultural Research

Table of Contents: Volume 12 Number 39, 28 September, 2017

ARTICLES

African Journal of Agricultural Research

Table of Contents: Volume 12 Number 39, 28 September, 2017

ARTICLES

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Review

Challenges to the efficacy of forestry and wildlife policies in Ghana for environmental protection: A review

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Despite the promulgation of various forest and wildlife policies aimed at ensuring the sustainability and conservation of the environment since 1874, Ghana continues to trail in issues regarding environmental control, especially in mitigating the upfront menace of deforestation and pollution of the environment. The desk review study, driven by qualitative research approach with document analysis, was undertaken to unearth the recurring challenges that have been the obstacles to achieving sustainability and conservation of the Ghanaian environment and resources. The study revealed that the main recurring challenges to the sustainability of Ghana's forest and wildlife policies are lack of stakeholder participation of local people, factorization of cultural practices, strong implementation strategies, proper documentation for equitable distribution of resources to the local people, and provision of sustainable alternative sources of livelihood for local residents. The study suggested pragmatic ways through which conservation planners could ensure the sustainability of Ghana's forest and wildlife policies for optimum environmental protection.

Key words: Environmental sustainability, ecosystem conservation, biodiversity, wildlife, policies.

INTRODUCTION

The forestry and wildlife resources in the environment offer immeasurable support to mankind (Ankomah, 2012). They provide humans with food, clothing, shelter and medicine while providing a protective shield for man against natural disasters such as flooding, hurricane and so forth (Adom et al., 2016a). The flora and fauna species in the environment take in the carbon dioxide that would have culminated in the formation of greenhouse gases, causing climate change (CBD, 2009). Economically, the forestry resources contribute largely to the economy of nations. For instance, Ghana generated 240.9 US dollars, which is 7.6% of the total export value and 3% GDP in 2009 (MLNR, 2011). However, due to strong abuse and unsustainable ways on the part of some people and agencies toward the forest and wildlife resources, there has been the promulgation of formulated forestry and wildlife policies and strategies. Ankomah (2012) contend that these policies define the specific

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rules to mitigate negative impacts on the ecosystem such as deforestation while increasing the benefits derived from them. Various governmental agencies responsible for the conservation of biodiversity, such as the Ministry of Environment and other Non-Governmental Organizations have spearheaded the formulation of policies and strategies for the management and the sustainable use of the forest and wildlife resources in Ghana (Adom, 2016b).

Historically, Ghana's formal attempt for the development of a formal forestry policy was in 1906, when legislation was enacted to regulate the felling of commercial tropical tree species (Attuquayefio and Fobil, 2005). In 1927, eighteen years after the establishment of the Forestry Department in 1909, the Forest Ordinance was passed and it authorized the colonial government at that time to establish forest reserves to aid in conserving the fast depleting forest resources in Ghana (Teye, 2008). After two decades the Forest Ordinance was operational, there was still the recurring canker of deforestation of the flora species. As a result, the 1948 Forest policy came into force to monitor and regulate the seemingly lawless forestry activities in Ghana. Unfortunately, as Gyampoh (2011) noted, the policy turned out to accelerate the abuse of the forestry resources, though, it was meant to conserve it, with many scholars branding it with the accolade 'timberization'. The policy somehow endorsed the illegal logging and exploitation of the timber resources, leading to a massive decline in the forest resources. GFW (2006) revealed a rocketed increase in the volume of industrial logs from 0.56 to 1.4 million cubic meters in 1955. The forty year implementation period was marred as a great failure in the forestry sector in the country. To provide a lasting cure to arrest the high decline in the forestry and wildlife resources, the Ministry of Environment, Science and Technology (EPA Act 490) was set up in 1994 together with the establishment of the Environmental Protection Agency. The new agency then developed the 1994 policy that doubled as a policy for both the forest and wildlife resources management in Ghana (Ntiamoa-Badu et al., 2001). It achieved some successes, but failed to annihilate the menace of exploitation of both the flora and fauna species in Ghana. Its diverse ills called for its numerous remedial actions in the form of introduction of some Acts and their associated amendments. Attuquayefio and Fobil (2005) mentioned of the 1997 Timber Resource Management Act (Act 547) and the 1998 Timber Resource Management Regulations which were all aimed at bringing sanity into the granting of timber rights with the sustainable management and utilization of forestry resources in view. The next year, the Forestry Commission Act (Act 571) was passed and as grounds for the establishment of the Forestry Commission in the same year, thus, 1999. They were charged with the Herculean task of saving the worsening condition in the management of the forestry and wildlife resources in Ghana. Seventeen (17) years after the passing of the 1994 Forest and Wildlife Policy, the Ghana News Agency (2012) said of the policy that it failed to bring a standstill to the massive degradation of the forest and wildlife resource base. Recently, the 2012 Forest and Wildlife Policy were passed. It promises to be an improvement on the long serving 1994 Forest and Wildlife Policy. The Chief Executive Officer of the Forestry Commission, Mr. Afari Dartey, was sanguine that the policy would arrest all the lapses in the 1994 Forest and Wildlife Policy. However, a few months into full force, the new 2012 Forest and Wildlife Policy is still in anticipation of the same old poor performance as the prior forest and wildlife policies.

Thus, there is the need to investigate into the past and current forest and wildlife policies to ascertain the standing blocks that have been the underlying challenges to its efficacy. Three regulatory documents have been investigated thoroughly in this paper (Table 5). It is interesting to know that the underlying causes of the failure of the forest and wildlife policies and strategies in Ghana seem to be similar, if not the same. They keep on resurfacing and hunting the agencies responsible for the conservation of the forest and wildlife resources in Ghana. The purpose of the study was to thoroughly analyse some of these past and current forest and wildlife policies, especially those that defined the formulation of policies in the forestry and wildlife sector in Ghana. The thrust of the study was guided by three research questions:

1. What are some of the forest and wildlife policies in Ghana?
2. What are the recurring challenges associated with the forest and wildlife policies in Ghana
3. How can the identified recurring challenges of the forest and wildlife policies in Ghana be remedied effectively?

This understanding is crucial because developed policies and strategies have to be constantly analysed to ascertain their strengths and weaknesses with the aim of suggesting pragmatic solutions to curb the identified challenges (Nutley et al., 2007; Morestin, 2012). This would ensure that proactive strategies are implemented by the ministries and agencies responsible for environmental and biodiversity conservation to optimize the conservation and sustainability of the biodiversity resources in Ghana.

METHODOLOGY

Overview of the forest and wildlife resources in Ghana

Ghana has two predominant ecological zones: the high

Table 1. Forest land area of Ghana from 1990 to 2015.

Year	Closed forest (ha)	Open forest (ha)	Total forest area (ha)
1990	2,704,422.0	5,922,979.8	8,627,401.9
2000	2,317,165.7	6,591,441.3	8,908,607.0
2010	1,785,801.6	7,409,335.0	9,195,136.6
2015	1,556,146.4	7,780,853.6	9,337,000.0

Source: FPP Report (2013).

Table 2. Sector's contribution to Ghana's gross domestic product growth (prices in million Ghana Cedis).

Sector	2007	2008	2009	2010	2011	2012	2013	2014	2015
Forestry and logging	705.9	632.4	687	757	651	695	726	754	783
Cocoa	493.2	509.1	535	535	771	699	717	747.8	738
Mining	531.6	544.4	581	690	2,116	2,462	2,747	2,834.4	2,753
Oil and crude oil	-	-	-	65	1,372	1,669	1969	2,058	2076
Total GDP in purchased value	19,518	21,304	22,336	24,101	27,486	30,040	32,237	35,522	34,823

Source: GSS (2016).

forest zone mainly in the South-Western part (34% of the country's total land area) and the Savannah zone occupying the remaining 66% of the total land area of Ghana (Marfo, 2010). Seventy (70%) of Ghana's population resides in the rural regions and their livelihoods are largely depended on the natural resources in their jurisdiction (Teye, 2008). Ghana implements both in-situ and ex-situ conservation practices. Currently, there are 280 forest reserves in Ghana under the management of the Forest Service Division (Forestry Commission) covering a total area of about 23, 729 km^2 or 11% of the total land area of Ghana. Out of these reserves, 75% are production reserves while 25% are protection reserves (NBS, 2016). In the field of wildlife conservation, there are 21 legally constituted wildlife conservation areas comprising of six (6) Natural Parks, Six (6) Resource Reserves, Three (3) Wildlife Sanctuaries, One (1) Strict Nature Reserve and Six (6) Ramsar Sites (GFDMP, 2016).

The current forest assessment of the high forest zone of Ghana in 2015 totals the forest area at an estimated 9.337 million hectare consisting of 1.556 million hectare as closed forest and 7.781 million hectare as open forest (GFDMP, 2016). The sizes of the closed forests keep on reducing at an alarming rate while the open forests keeps increasing due to the forest plantation introduced in the year 2000 (Table 1).

Forest estimates today continue to reduce at an astronomical annual deforestation rate of 2% (65,000 ha) valued at an annual deforestation cost of 10% of Gross Domestic Products (MLNR, 2011). Since 1990, the forest degradation rate per annum in Ghana is estimated at 45,931.03 ha (FPP, 2013). This is a worrying situation because Eighty (80) years ago, 63% of the country's forests were in pristine or near pristine condition in the forest zone (Hackman, 2014). The responsibility of ensuring the management of forest resources in Ghana is centralized and vested in the authorities of the Ministry of Food and Agriculture (MoFA), Ministry of Energy (ME), Ministry of Environment, Science, Technology and Innovation (MESTI) and the Ministry of Lands, Forest and Mines (MLF) with its agencies, the Land's Commission, Forestry Commission and the Wildlife Division. The estimates show that these ministries and agencies must work extra hard at reversing the alarming degradation of the country's natural resources. This is essential because a greater part of the country's economy relies on the proceeds from the natural resources, especially the forest sector (Table 2).

In addition, Small and Medium Forest Enterprises (SMFEs) comprising of activities in wood products, non-wood products, and forest services contributed an income of three (3) million Ghana Cedis (MLNR, 2015). The total export of Ghana's wood products in 2015 was 267, 379.45 m^3 in volume contributed €135, 024, 200.64 to the economy of Ghana (GFDMP, 2016). Ecotourism services offer the country an annual revenue generation of 1.6 billion USD (Ghana Tourist Board, 2012).

There has also been various laws and regulations passed and amended as and when the need arises, to manage the forest and wildlife resources in the country (Table 3).

In terms of the level of afforestation in Ghana, there have been commendable increases since the year 2000 when the forest plantation development was introduced with an annual planting target of 20,000 ha (GFDMP, 2016). This was primarily aimed at restoring all the degraded forest lands in the country. This also opened up

Table 3. Forest legislation laws and reforms in Ghana.

Law/regulation	Act No.	Year passed	Purpose
Forest Plantation Fund Act	Act 583	2000	Revise some sections and plans in the forest plantation fund
Customs and Excise Duty Amendment Act	Act 2000	2000	Removal of duty on imported lumber for processing
Revision of Ghana Investment Promotion Centre Act, 1994	Act 478	2013	Regulations investment into the forestry sector
Forest Protection (Amendment) Act	Act 624	2002	Increase in the fines and punishment for abuse of forest resources
Timber Resource Management Act, 1997 (Amendment)	Act 547	2002	Introduction of competitive bidding in the allocation of timber and other forestry resources
Timber Resource Management Regulations, 1998 (Amendment) Act	L.I. 1721	2003	
Timber Resource Management (Amendment) Act	Act 617	2002	Adoption of a social responsibility assessment backed by law to provide residents in contract areas with social amenities
			Allow private plantation developers to harvest their planted trees without a TUC and the permission of landowners
Timber Resource Management (Amendment) Regulations, 2003	L.I. 1721	2003	Revised the sanctions under NRCD 243 and PNDCL142 to increase the resistance to cause harm to forest reserves while extending its liability under Act 547 and LI 649 to include drivers and the one who hired vehicles that transport illegal timber as well as the one who gave the instruction to 'carry, haul, evacuate or transport such timber.'
			Revised law to empower the Ministry of Lands, Forests and Mines to regulate new investments into the forestry sector and ensure that only the required plant and equipment for the country's needs are installed in the wood processing sector.

Source: Author's Construct from GFDMP (2016).

employment avenues for the rural poor living in the forest fringe communities in Ghana. It is estimated that the total planted forest in Ghana is 325,000 ha (Table 4).

Research design

The qualitative research approach was utilized for the study. This was due to the fact that the researcher collected and examined numerous secondary data such as previously published articles, public reports (Creswell, 2009; Narayanan, 2015) related to the forest and wildlife policies in Ghana. Multiple views from different scholarly materials to highlight the challenges and proposed suggestions were highlighted by the researcher, hence the adoption of the qualitative research approach for this research. Also, the visual texts (Denzin and Lincoln, 1994) related to the forestry and wildlife policies were analyzed to comprehend the challenges associated with their formulation and implementation.

The research was largely driven desk research and interpretative document analysis were the main research methods that guided the finding, selecting, and rigorous examination of the forest and wildlife policies in Ghana. Travis (2016) opines that desk research seeks to thoroughly analyze secondary data on forestry and wildlife policy reviews from the internet, online databases, published reports, information from government agencies and magazines. This is exactly what the researcher did to aid him in gaining a broader and deeper interpretation of the phenomena under study. The interpretative document analysis process (Hefferman, 2013) that is guided by the essential factors, namely, authenticity, credibility, and representativeness before the final stage of deducing the meanings illustrated in the examined documents.

The secondary data was analyzed quantitatively. The researcher commenced the analytical process by perusing the documents severally to gain the overall picture of their contents (Creswell, 1998). Summarization of the main ideas with the research questions of the study in focus was carried out by the researcher

Table 4. Afforestation levels in Ghana from 1990 to 2015 (000 ha).

Categories	1990	2000	2005	2010	2015
Primary forests	395	395	395	395	395
Naturally regenerated forests	8,182	8,454	8,498	8,540	8,617
Planted forests	50	60	160	260	325
Total	8,627	8,909	9,053	9,195	9,337

Source: FC (2015).

Table 5. Presentation of the three forest and wildlife policies in Ghana.

Regulatory document	Aims	Objective	Ministry involved	Period covered
1948 Forest Policy	Utilizing and conserving timber outside the forest areas before they were all converted to agricultural lands	1. Conservation of forest environments by protecting the major water catchment areas and providing a conducive climatic condition for the growing of major agricultural crops. 2. Management and sustenance of the permanent forest estate in the Ghanaian environment. 3. Promotion of research in all branches of scientific forestry. 4. Utilization of areas that were not dedicated to permanent forestry. 5. Provision of technical advice and cooperation in schemes for land use plans and avoiding soil erosion.	Ministry of Lands and Forestry	1948-1994
1994 Forest and Wildlife Policy	Conservation and sustainable development of the nation's forest and wildlife resources for the maintenance of environmental quality and perpetual flow of the optimum benefits to all segments of the society	1. Management and improvement of Ghana's permanent forest estate for preservation of soil and water, conservation of biological diversity, environmental stability and sustainable production of domestic and commercial products. 2. Promotion of efficient forest-based industries, in secondary and tertiary processing, to use timber and other products from forests and wildlife and satisfy domestic and international demand with competitively priced products. 3. Promotion of public awareness and involvement of rural people in forest and wildlife conservation to maintain life-sustaining systems, preserve scenic areas and enhance potential for recreation, tourism and income generating opportunities. 4. Promotion of research-based and technology-led forestry and wildlife management to ensure forest sustainability, socio-economic growth and environmental stability. 5. Development of effective capacity and competence at district, regional and national levels for sustainable management of forest and	Ministry of Lands and Forestry	1994-2011

Table 5. Contd.

2012 Forest and Wildlife Policy	Conservation and sustainable development of forest and wildlife resources for the maintenance of environmental stability and continuous flow of optimum benefits from the socio-cultural and economic goods and services that the forest environment provides for the present and future generations whilst fulfilling Ghana's commitments under international agreements and conventions	1. Management and enhancement of the ecological integrity of Ghana's forest savannah, wetlands and other ecosystems for the preservation of vital soil and water resources, conservation of biological diversity, while enhancing carbon stock for sustainable production of domestic and commercial produce. 2. Promotion of the rehabilitation and restoration of the degraded landscapes through forest plantation development, enrichment planting, and community forestry informed by appropriate land-use practices to enhance environmental quality and sustain the supply of raw materials for domestic and industrial consumption and for environmental protection. 3. Promotion of the development of viable forest and wildlife based industries and livelihoods, especially in the value added processing of forest and wildlife resources that satisfy domestic and international demand for competitively-priced quality products. 4. Promotion and development of mechanisms for transparent governance, equity sharing and citizens' participation in forest and wildlife resource management. 5. Promotion of training, research and technology development that supports sustainable forest management whilst promoting information uptake both by forestry institutions and the general public.	Ministry of Lands and Natural Resources	2012-Present

Source: Author's Construct from the Forest and Wildlife Policies of 1948, 1994 and 2012 (MLNR).

(Peshkin, 1993). Categories and themes were generated to give detailed comprehension of the secondary data reviewed (Denzin and Lincoln, 1994). The new understanding was subjected to verification with the aim of validating the claims, assumptions and theories made by scholars (Peshkin, 1993). Finally, the facts were evaluated and presented in a persuasive write-up to accentuate the challenges with the forest and wildlife policies in Ghana while suggesting proactive solutions to arrest them (Denzin and Lincoln, 1994). The 1948, 1994 and 2012 Forest and Wildlife Policies were also subjected to the interpretive policy analysis (Morestin, 2012) that involves assessing their effectiveness, equity, community and political acceptability.

RESULTS AND DISCUSSION

Analysis of some of the forest and wildlife policies in Ghana

The 1948 forest policy

The 1948 forest policy was the first formal and comprehensive policy that was formulated to guide forestry development in Ghana to replace the less effective 1927 Forest Ordinance (Cap. 157) (Teye, 2008). It was developed in 1946 and endorsed by the Governor-in-Council in 1948 during the era of colonialism (Gyampoh, 2011). According to the GFW (2006), the first policy was aimed primarily at regulating and monitoring the sustainable supply of industrialized timber. This was necessitated by the constant abuse of the forestry resources in both the protected reserves and off reserves.

Derkye (2007) highlighted some of the strengths of this policy as factorizing the developing trends in global forest management as well as capturing the colonial experience in forest management in the policy development. Also, the policy is credited as leading to the demarcation of tracts of forest lands as reserves to protect and conserve the permanent forest estates while intensifying scientific systems of forest management that boomed the output of timber production (Kotey et al., 1998). Despite the promising objectives of the policy as well as some of its successes, the 1948 forest policy is marred by several challenges. CFMU (1999) revealed that the forest policy was very exploitative in nature. Forfeiting its chief aim of conservation and sustainable management of forest resources and endorsing the depletion of forestry resources, the 1948 forest policy, thus, lacked efficiency and efficacy. Gyampoh (2011) admits that the early foresters called the policy ‘timberization’ because of the unbridled and illegal harvesting spearheaded by the chainsaw operators. GFW (2006) avers that the policy gave confidence to the conversion of off-reserve forests into non-forest land use forms, resulting in massive degradation of biodiversity. Derkye (2007) rightly blamed it on the policy’s emphasis on the protection and management of just 30% of the total forest tract as reserves while granting permission for the use of the remaining 70% of the forest tracts outside the forest reserves for agricultural activities. Teye (2008) adds that the policy placed great emphasis on exportation of timber to generate revenue for the newly independent state of Ghana. This only satisfied the selfish interests of the colonial administration. Also, there was an increased international demand for some tree species, particularly *Wawa (Triplochiton sclerexylon)* and this rose the exportation level high resulting in the wanton depletion of the forest resources. Kotey et al. (1998) regrettably stated that the colonial administration did not propose any or reinforce plans for replacement of the depleted forest resources through reforestation programmes.

Management and administration of forest resources in the 1948 forest policy: In terms of governance related to the management or administration of the forest resources, Ankomah (2012) contend that the 1948 forest policy gave exclusive and greater authoritative rights to the central government. This lead to the deliberate side-lining and exclusion of the local people and their communities from the administration of the forest resources in Ghana. As a result, the 1948 forest policy lacked community acceptability. Derkye (2007) concurs that the component of collaboration between the government and the local communities was somehow neglected in the administration of the forest resources though the clause four of the policy encouraged the formation of a native or local forestry administration. This policy dictates did not materialize since the hands of the local communities were figuratively tied and their lips sealed in relation to the management, administration, decision-making and formulation of the 1948 forest policy. Therefore, the acceptance of the policy by the numerous local communities became a great challenge, nullifying the efficiency of the policy.

Community acceptance of the 1948 forest policy: Another challenge to ensuring community acceptance of the 1948 forest policy was with its great emphasis on scientific forestry practices with research provisions made to that effect (Teye, 2008). The abrogation of the traditional forestry practices that heavily implemented cultural practices and beliefs in consonance with the scientific forestry practices relegated the equity in the 1948 policy document. The policy was truncated in its forestry approach to purely scientific methods focusing mainly on silviculture and regeneration (GFW, 2006). Derkye (2007) contends that the priceless skills and highly practical traditional knowledge and practices of the local people were shunned completely. This widened the gap of acceptability of the policy by local communities who are accustomed to these traditional forestry practices that hinges on their cultural beliefs and practices like taboos, totem, festivals and so forth.

Equity and fair distribution of forest resources in the 1948 forest policy: In terms of equity and fair distribution of forest resources, the 1948 forest policy performed poorly. GFW (2006) reported that the commercial plantation development was silent on any arrangement for local people’s inclusion. The local people in various communities were restrained from entering the forests while the token royalties were reluctant and under-paid to their chiefs (Teye, 2008). This deliberate marginalization led to the situation where due to the lack of equitable sharing of forest proceeds, the local communities supported illegal chainsaw operators and illegal felling of timber resources. Indeed, the active involvement of the local people in the forest management and benefit sharing was flouted by the top managers of the forestry commission (Tuffour, 1996; Ankomah, 2012). Derkye (2007) adds that the local communities were denied meaningful rights to the forestry resources and were banned from entering the forest reserves without seeking permission through prior writing. Entry was under the eagle supervision of impassionate foresters who paid little or no attention to the customary rights of the local people. Sadly, the local communities were denied access to fell the trees on their legitimate farms while no alternative sources of livelihood were provided by the government for the rural poor whose livelihood solidly rested on the forest resources (Teye, 2008). The 1948 forest policy thus became a policy of ‘bones without meat’ because it was merely a generalized statement of intent (Marfo, 2012).

Factors that led to the revision of the 1948 forest policy: Owing to these gigantic limitations of the 1948 forest policy, there was a public outcry both local and international for its nullification and possible revision (Gyampoh, 2011). The indiscriminate felling of timber resources, favouring the rich, famous and affluent foreign countries led to severe environmental threats such as droughts, deforestation and wildfires in the country (Derkye, 2007). Moreover, the intense international call for the recognition of the rights of local communities as well as their involvement in forestry administration and management proposed by the United Nations Conference on Environment and Development known as the Rio de Janeiro Earth Summit held in Brazil in1992. In addition, the World Bank Preparatory Mission for the Forest Resource Management Project (FRMP) reported that the 1948 forest policy was incapable of dealing with the illegal and the wanton depletion of the forest resources and thus needed a revision (GFW, 2006).

The 1994 forest and wildlife policy

The 1994 forest and wildlife policy is the second formal policy in Ghana and the first formal document to highlight the management of both forest and wildlife resources in Ghana. It was to arrest the deficit of the 1948 forest policy that was only about the management of the forest resources in the country with wildlife not seen as an integral part of the forest resource management (GFW, 2006). The policy was formulated to halt the 'timberisation' of the 1948 forest policy by attempting to strike a balance between preservation and utilization of forest resources (MLF, 1994). Alhassan (2010) adds that the policy stressed on the need to boost the involvement of the private sector and local communities in the forest management, while assuring the forest-dependent communities access rights and benefits from the forest resources in their jurisdiction (MLF, 1994).

A rigorous look at the 1994 Forest and Wildlife Policy document shows promising signs of averting the challenges in the 1948 forest policy while mapping up effective strategies to curb the wanton depletion of the forest and wildlife resources in Ghana. Okrah (1999) mentioned that the document accentuated almost all the general apprehensions of the Ghanaian populace regarding the management of the forest resources. He cites the policy statements regarding the rights of local communities' access to some of the forest resources and collaborative and participatory management of all stakeholders especially the rural people. Alhassan (2010) believes that the 1994 Forest and Wildlife Policy is the 'most pragmatic policy' that turned the face of stakeholder involvement in forest management. The greater mention of participatory involvement of local communities may be because of the global call for the recognition of the rights of people, especially the rural poor and the promotion of their involvement (Derkye, 2007). The senior planning officer and coordinator of the policy review process of the 1994 Forest and Wildlife Policy highlighted one of the strengths of the policy that it brought transparency into the bidding method for Timber Utilization Contracts (TUC) as well as an efficient monitoring system for the granting of concessions to timber companies when compared to the opaque, lawless large concessions which was apparent before the 1990's. Donkor and Vlosky (2003) also mentions that the 1994 forestry and wildlife policy revised the low fines paid by abusers of the forest resources and heightened the legal sanctions barring against the wanton degradation of the forest resources in the country. Due to the low fines cited in the 1948 forest policy, timber companies and chainsaw operators deliberately flouted the sanctions and illegally logged the timber resources in great impunity. Also, initiatives for the reforestation of the degraded forests were proposed by the policy which was absent in the 1948 forest policy. In addition, there was the introduction of some of the stumpage fees paid to local communities as well as Social Responsibility Agreements (SRA) between timber concessionaires and companies to undertake a developmental project or make funds available for such purposes to compensate the local communities (Wiggen et al., 2004). Moreover, the policy added more value to timber before they are exported (Teye, 2008).

Despite these strengths of the policy, The Natural Resources and Governance Programme of the Ministry of Lands and Natural Resources in Ghana revealed that the 1994 Forest and Wildlife Policy with all its major reforms could not prevent the depletion of the forest resources base since there were massive illegal chainsaw and mining operations in the country. The then Minister of Lands and Natural Resources, Hon. Mike Hammah said of the 1994 forest and wildlife policy that it was ineffective in putting to rest the illegalities in the forestry sector. These revelations bring to fore the question on the challenges and weaknesses of the policy irrespective of its highly hopeful paper document face value? Ankomah (2012) feels that this is a paradox in that the document had all the shining traits of potential success in its implementation processes. Unfortunately, this was not the case.

Planning, development and management decisions of the 1994 forest and wildlife policy: Tuffour (1996) contends that some level of consultation went on prior to the formulation of the 1994 forest and wildlife policy. He avers that there was an under-representation of the traditional rulers, farmers and residents of the forest-fringe communities (Kotey et al., 1998). It is very evident that their suggestions were not factored into the policy development in practicality. Bonye (2008) concurs that the traditional authorities and local communities were least consulted in the policy planning stages and neither was their traditional experiential knowledge in resource management sought. Okrah (1999) points out that the isolation of the major stakeholders, especially the forest owners and the traditional authorities in the consultation stage of the policy development was a great defect of the 1994 Forest and Wildlife Policy. Teye (2008) adds that though the policy advocated for the rights of local people in forest resource management, in practicality, they did not enjoy any of such user rights since all authority and control of timber and non-timber resources were stiffly in the grip of the Forestry Department. He admits that the local people still need to obtain written permissions before they are allowed to extract even non-forestry products from the reserves even dead trees for fuel. The formation of the Forest Service's Division Collaborative Forest Management Units in the local communities as an initiative to involve the local people in forest management so that they could help in the implementation processes failed because the decision-making process was not supposed to be part of their involvement (Amanor, 2000). Again, the partnership between the Forestry Commission and the local communities is marred with mistrust and conflicts due to the often flouting of agreements, especially on the part of governmental agencies (GFW, 2006).

Equity and fair distribution of forest resources in the 1994 forest and wildlife policy: Also, the equity that the 1994 forest and wildlife policy promised remained a mirage (Alhassan, 2010). This is because the royalty are paid to the paramount chiefs and not the common farmers whose trees were cut. Thus, the delayed and under-paid royalties do not reach the rural poor who rightly deserve the payment and compensation. GFW (2006) estimates that as at 2006, the Forestry Commission owed an amount of 300 million Cedis stumpage fees to local communities. The same can be said of the social responsibility agreements with the timber concessionaires and companies. Mostly, these elements are often abrogated and if they are honoured, timber operators and companies execute the amenity or project in isolation without consulting the local communities, thus, rendering it useless to the local people (Young, 2005). Therefore, the seeming involvement of local communities to benefit from the forest resources indeed remained a 'myth' as Guri (2006) rightly opined. As such, there was lack of commitment on the part of the local communities to support the implementation of the policy. Amanor (2000) highlights that the lack of stakeholder incentives such as the equitable distribution of forest benefits to the local communities hindered the successes of the 1994 forest and wildlife policy. When the local people sense that they are unjustly cheated regarding the equitable sharing of the forest resources, many turn their back on the policy and wantonly abuse the resources in the environment.

Sanctioning and implementation of the 1994 forest and wildlife policy: Another general challenge associated with the 1994 forest and wildlife policy is with its weakness on the part of the relevant sanctioning agencies in enforcing the sanctions for the abuse of the forest resources (MLF, 2007). MSE (2002) mentions that the weaknesses of the legislations and implementing agencies which is blamed on the lack of cooperation, collaboration and networking of the policy development agency and the implementing agencies. There seems to be a discord between the agencies who are supposed to work in consonance for the better good of the country. It was clear in 1998 that the implementation of the policy became difficult due to the lack of cooperation of the local people as a result of their poor involvement (Teye, 2008).

Moreover, GNA (2010) highlights that the 1994 Forest and Wildlife Policy lacked institutional and legal systems to implement or support the activities of the local communities who solely depended on the forest resources for their livelihood. Alhassan (2010) concurs that there was no legislation to support collaborative forest management between forestry agencies and the local communities. Owing to this, the former usually flouts agreements and escapes punishment. The statutory laws always override the customary rights in such instances.

Factors that led to the revision of the 1994 forest policy: Wiggens et al. (2004) contends that the policy statement on barring the wanton depletion of the forest resources in the off reserves achieved minimal success since illegal chainsaw and mining operations still abounded in Ghana. Teye (2008) revealed that the 1994 forest and wildlife policy still faced the challenge of resource exploitation that favoured the political elite, influential timber operators and top officials of the Forestry Commission. Consequently, GFW (2006) views the policy document as a commendable material, but useless because of its implementation flaws that clearly exposes the gap between intentions and reality. Thus, many individuals and agencies called for the immediate cessation of the 1994 forest and wildlife policy, yearning for a revision.

The 2012 forest and wildlife policy

The 2012 forest and wildlife policy was necessitated by the alarming rate of illegal harvesting, which is estimated by the over 80% of the domestic lumber gotten from illegal chainsaw operators (GFIP, 2012). Also, it was to strengthen and make the best use of the products and services of the forestry resources in the country (GNA, 2012). It is a paradigm shift from the consumptive spirit referred to as 'timberisation' of the 1994 forest and wildlife policy to non-consumptive values while creating an equilibrium between the production and marketing of timber to satisfy especially the domestic wood demands (MLNR, 2012). The GNA (2012) mentioned that the introduction of the 2012 forest and wildlife policy was necessary due to the emerging global trends in the forest sector, such as the voluntary partnership agreement, issuance of forest certificate, Reducing Emissions from Deforestation And Forest Degradation (REDD), European Union's Forest Law Enforcement, Governance and Trade (FLEGT) as well as the Non-Legally Binding Instruments (NLBI) project.

The policy directions from the objectives of the 2012 forest and wildlife policy looked very promising. This view was expressed by the Chief Executive Officer of the Ghana Forestry Commission, Mr. Afari Dartey. He was convinced that the 2012 forest and wildlife policy will ensure that culprits of biodiversity degradation will be appropriately disciplined by stringent sanctioning and prosecution procedures advocated in the document (Modern Ghana Online Radio News, 20th December, 2013). The age-long forestry problem on the laxity of sanctioning measures which was due to the lack of the judiciary being abreast with the forest laws has been catered for in the policy. The strategic direction 6.1.3 mentions the factorization of forest law into the judicial law and enforcement training programmes. This would equip the law enforcing agencies on how to prosecute the culprits of the forest and wildlife regulations. Thus, Abdul-Baql (2015) is convinced that the policy has achieved the enforceability required to enhance its implementation. This in the long-term would largely contribute to the efficiency and efficacy of the policy.

Political and community acceptability of the 2012 forest and wildlife policy: Moreover, for the policy to win political and community acceptability as well as equity and fairness, there is room for the involvement of civil society organizations and the local people in the decision-making and resource management of the forest and wildlife resources in Ghana (GFWP, 2012). Thus, the Forestry Commission has changed the focus of its management system from government-led system to a collaborative management approach. This is to ensure greater consultation with stakeholders, especially local communities that are dependent on the forests and are willing to ensure its maintenance (Abdul-Baql, 2015).

Forestry governance system in the 2012 forest and wildlife policy: The forestry governance system has been decentralized with the sole aim of ensuring transparency, accountability and equity in the management of the forest and wildlife resources in the country. The strategic direction 6.1.2 of the 2012 forest and wildlife policy mentions that the roles of traditional authorities, District Assemblies, Non-Governmental Organizations and Community Based Organizations in forest and wildlife management have been well defined. Abdul-Baql (2015) argues that the recognition of multi-stakeholder interests from members of the Ghana's Civil Society Organization as well as multi-sectoral approaches in forest and wildlife planning and management with the common aim of protecting, managing and ensuring collaborative resource management among societies, governments and other stakeholders has heightened the fairness and equity of the 2012 forest and wildlife policy (Abdul-Baql, 2015).

Generally, there was no proper legislation backing the participation of local people in the previous forestry and wildlife policies. However, the strategic direction 4.1.1 of the 2012 forest and wildlife policy recognizes the need to ratify all necessary legislation and regulations to facilitate and enhance local participation and control through decentralization of forestry operations at the district levels. To avert the lack of equity and fairness in the distribution of resources to the local people in the past forest and wildlife policies, the 2012 forest and wildlife policy proposes the passing of a legislation that would allow the local people to enjoy the forestry proceeds on their farmlands. The strategic direction 4.1.1a indicates that the local people have the right to the trees on the farms. Therefore, they are to be compensated individually for any forest proceeds taken from their farmlands.

Provision of alternative sources of livelihood in the 2012 forest and wildlife policy: The 2012 forest and wildlife policy makes provision for alternative sources of livelihood for the local people living in the forest fringe communities. The strategic direction 2.1e states the support of the creation of employment opportunities and sustainable livelihoods for the residents in the forest fringe communities through forest plantation development. The coordinator of the Ghana Forest Watch Ghana, Mr. Kingsley Bekoe admitted that the policy has ensured the creation of forest-based employment avenues such as tree planting and protection of boundaries. What has often been the setback to the cooperation of the local communities in helping in the implementation of forest and wildlife policies is with the unfair sharing of forest proceeds. As has been a common trend, royalties and stumpage fees due the local communities are on many occasions not paid, reduced woefully or are in high arrears. The 2012 forest and wildlife policy has proposed the use of consultative processes in apportioning, recovering and distributing equitably and effectively forest rent or royalties among the resource owners, state and the users of the resources (Strategic direction 5.1.1c, of the GFWP, 2012). This would bolster the interest and cooperation of local people in helping in the implementation of the policy.

Incorporation of indigenous knowledge in the 2012 forest and wildlife policy: Interestingly, the 2012 forest and wildlife policy had made provisions for the factorization of indigenous knowledge of local communities in the management of forest and wildlife resources in Ghana. This includes the religious beliefs, customs and values of local people that enhances and promotes resource management. To illustrate, the strategic direction 1.5.1c of the policy mentions the documentation of the biological, spiritual, religious, cultural and heritage values of natural sacred sites while maintaining their secrecy where it is required. Also, the strategic direction 1.5.1a of the policy called for a review of all relevant legislation to recognize the rights of local people and the customs and belief systems that led to the management of the sacred sites. Thus, the policy respects and recognizes the religious and cultural elements that are used for the management of the forest and wildlife resources in the local communities. This includes the cosmological belief systems, taboos, totemic practice and others that were shrouded in secrecy, but were powerful tools for promoting conservation and sustainable use of forest and wildlife resources. Often times, when conservation planners and foresters visit the local communities, they reveal to the people that those beliefs are pagan, satanic and superstitious. As a result, many local people are no more respecting these cultural and religious elements that promoted biodiversity conservation. Thus, it is a step in the right direction if the policy espouses the maintenance of the secrecy regarding these cultural elements that advance the course of conservation and sustainability of the forest and wildlife resources. This position taken by the Forestry Commission is in consonance with the global directive of the UNDRIP (2006) that stressed that the indigenous people have the right to maintain the spiritual connections that they have regarding the natural resources in the environment which must equally be respected by the larger community (Adom et al., 2017).

In terms of research into the traditional or indigenous knowledge systems for resource management, the strategic direction 5.1 of the policy indicates that the enlightenment of indigenous knowledge when used in conjunction with the scientific knowledge can enhance the management of wildlife and forest resources. Therefore, the policy encourages higher learning institutions to pursue research activities in indigenous knowledge systems that utilize the cultural elements like totem, taboos, festivals, cosmological beliefs and so forth in the management of biodiversity with conservation and sustainability in view. Abdul-Baql (2015) believes that the synergy of the indigenous knowledge of natural resource management evident in the cultural beliefs and practices of local communities would provide an incentive for the sustainable development of the forestry sector. The 2012 forest and wildlife policy have called for an institution of an annual forestry forum where all stakeholders will meet and deliberate on the efficiency of the implementation processes of the policy (Strategic Direction 6.1.1 of the GFWP, 2012). This annual policy review would help in arresting the identified challenges with the implementation in an early stage to avert dire implications.

Efficacy of the 2012 forest and wildlife policy: Despite these giant efforts and strengths associated with the current 2012 forest and wildlife policy, there are some weaknesses that challenge its efficacy. Irrespective of the fact that the policy offers some sort of alternative sources of livelihood for the local people living in the forest fringe communities in terms of offering job avenues in forest plantation and the protection of boundaries, Abdul-Baql (2015) is of the view that these alternative sources of employment are not better economic opportunities for communities depending on the forest and wildlife resources. He adds the livelihood opportunities of non-timber forest products for local communities were ignored entirely. He further argues that the employment avenues offered as alternative sources of livelihood cannot be strong deterrents for the many unemployed rural poor in the forest fringe communities. Derkye (2007) as well as Adom et al. (2017) argue that the absence of proper sustainable sources of livelihood for the rural people is due to the poor documentation of the agreements with the local people as assured by the Forestry Commission, timber firms and timber contractors.

Management decisions of the 2012 forest and wildlife policy: A daunting challenge related to the 2012 forest and wildlife policy is still with the recurring problem of local community involvement in the management of the forest and wildlife resources. Though the policy highlights greatly on the participation of all stakeholders, especially the rural people in the forest fringe communities, Sarpong and Inkoom (2015) contend that recent policy review on the 2012 forest and wildlife policy revealed that the traditional authorities admitted that they were somehow engaged by the Forestry Commission in the management of the forest resources but in a minimal degree. The traditional authorities regrettably said that many concessions were given out by the Forestry Commission without their permission or knowledge. This implies that the participation of local communities is still a challenge of the 2012 forest and wildlife policy. GFIP (2012) also mentioned that the forestry and wildlife sector still need to fight the challenge of inequitable benefit sharing and poor community involvement in the management and decision-making processes regarding the forest and wildlife resources. The document hinted that the forestry and wildlife sector still experiences poor accountability in resource exploitation. There is also the lack of cost-effectiveness in the use of resources and creation of appropriate benefits in a transparent and accountable manner. It adds that there is still more than 1.7 million meter cube of timber harvested, which is not accounted for, resulting in greater revenue loss to especially landowners in the rural communities, District Assemblies and the state. Moreover, the forestry and wildlife sector is still confronted with weak sectoral institutions due to poor capacity in technical skills in the forestry and wildlife personnel (GFIP, 2012; Abdul-Baql, 2015). This has accounted for poor work delivery with poor output in service delivery, such as the high rise in illegal forestry activities and mining operations with poor sanctioning measures.

Publicity of the 2012 forest and wildlife policy: Another recurring challenge is with the lack of publicity of the 2012 forestry and wildlife policy to the local communities. The Daily Graphic (December 13, 2012) reported that the Forestry Commission was not doing enough to educate local people living in the forest-fringe communities on the new initiatives or developments outlined in the new 2012 forest and wildlife policy.

Recurring challenges associated with the forest and wildlife policies and their suggested remedies

The analysis of the three forestry and wildlife policies namely the 1948 forest policy, the 1994 forest and wildlife policy and the 2012 forest and wildlife policy has revealed some recurring challenges associated with their implementation that needs redress. These include:

1. Lack of effective stakeholder participation of local people
2. Lack of proper factorization of cultural beliefs and practices
3. Lack of strong implementation strategies
4. Lack of and/or proper documentation of equitable distribution of forestry and wildlife resources to local people
5. Lack of proper provision of sustainable alternative sources of livelihood for residents in the forest fringe communities
6. Poor dissemination of forestry and wildlife policies to the local communities

Lack of active stakeholder participation of local people

The active involvement of local people in the administration and management of the forest and wildlife resources has been a persistent weakness to the implementation successes of all the forest and wildlife policies in Ghana. The 1948 forest policy assigned all managerial duties to the central government making the management approach government-led. The local people were forced to be aliens of the policy, weakening its smooth implementation. Successful countries with high peaks of biodiversity resources attribute their success largely to the active participation of local people in the forest and wildlife resources administration. Adom (2016b) noted of the Brazilians that they have granted the local communities' full participation in the decision-making and management of their country's biodiversity. This is not casual consultations with the local people, but a giant representation of them in the management decisions regarding the sustainable use and conservation of biodiversity. The 1994 forest and wildlife policy, however, made some efforts at including the voices of local people in the management of the biodiversity resources in Ghana. That may be the reason why Alhassan (2010) reckoned that the 1994 forest and wildlife policy was emphatic on the inclusion of local communities in the management and decision-making processes of the forest and wildlife resources in the country. However, this was not very effective because of the drivers that made the policy makers. Derkye (2007) argues that the mention of local communities' participation was just a paper presentation to satisfy the demands of global conventions that Ghana had ratified so as to keep enjoying the funds provided by the international bodies. Classical example is the UNCED Agenda 21, Rio de Janeiro, Brazil, 3-14 June, 1992 that called for a grass root approach to the seeking of the views of local communities during policy consultations on the planning and management of biodiversity (Adom et al., 2017). The adherence to this global directive was probably the cause of the under representation of the local communities in the consultations that led to the formulation of the policy (Tuffour, 1996; Kotey et al., 1998). As a result, the aftermath was an eventual low impact of local community voice in the policy directives, thereby reducing the support of local people for the implementation of the policy. The participation of local communities in the 2012 forest and wildlife policy is heightened in comparison to the 1994 forest and wildlife policy. The wheel of management of forest and wildlife resources has been changed from the government-led approach to a collaborative approach between the government and the local people. This is very commendable. Though the policy is in its young state, many scholars suggest that the inclusion of local people in consultations that led to the policy development was not enough. There are many operations of the forest and wildlife sector that still excludes or is absent with the views of the local communities. For instance, concessions are often times given to timber contractors without the consent of the traditional authorities who are the leaders of the local communities (Sarpong and Inkoom, 2015).

This problem of local community participation, which has been a recurring challenge in the forest and wildlife policies in Ghana can be remedied if the UNCED Agenda 21 directive that disseminates managerial power to the grass root level is implemented. Powerful lessons can be learnt from China and Japan who have established and improved their local management systems in each community or society to better handle the unique environmental challenges in their jurisdiction (Adom, 2016b; NBS of China, 2011). Ghana made progress in establishing CFCs in forest fringe communities to aid in protecting the forest and wildlife resources in their communities. Yet, their powers are limited and are not to engage in the decision-making processes regarding the managerial procedure adopted by the Forest and Wildlife Sector (Asare, 2000). Moreover, the formation of CFMU's tasked to increase the active participation of local communities (GFW, 2006) has been ineffective since the selection of representatives of the local communities on the team has not been fair. Adom (2017) warned strongly against the selection of the often advantaged in the society such as the traditional authorities, educated elders at the neglect of the vulnerable in the society such as the poor, aged and disabled. Usually, the elites in the local communities are offered this privilege by the Forest and Wildlife Commission, ignoring the often marginalized poor farmers, experienced elderly men and women, unemployed rural youth (Convention to Combat Desertification, 1995; Kotey et al., 1998) probably on the grounds of illiteracy which is very unjust. Of course, if such ones show great potentials of possessing rich experiential knowledge in forestry and wildlife management, they must be selected to sit at the decision-making and management table. The consequence of their intentional sidelining is the poor articulation of the ‘real’ voice of the people in the management of the forest and wildlife resources. The solution to the problem of local peoples’ participation rests on allowing the genuine or true representation of the local communities which can be ascertained in a communal election or endorsement of their representatives on the CFMU. Moreover, there should be a legislative backing the activities of the CFMU’s in the local communities to ground their suggestions in the management decisions of the forest and wildlife resources in Ghana. Also, the local communities’ participation must pass informative and/or consultative involvement. They must be streamlined onto the highest decision-making level so that their popular requests could be realized. This is what successful countries with rich environmental resources such as Brazil, China, Japan, India and Kenya have done (Adom, 2016b). Representatives of their local communities are part of the high level stakeholders and thus, play active roles in the planning and decision-making schemes regarding the environment. As such, policy implementation becomes supportive and easy in their local communities (Pretty et al., 2009). If these practical and efficient strategies discussed are implemented, Ghana stands a better position to permanently annihilate the recurring challenge of lack of active participation of local communities in the planning, decision-making, and management of forest and wildlife resources.

Lack of proper factorization of cultural beliefs and practices

The 1948 forest policy was silent on the incorporation of traditional ecological knowledge systems such as the promotion of the cultural beliefs and practices of local communities that barred against resource abuse. The policy emphasized on the research and application of purely scientific forestry practices. However, as Adom (2016a) posits, addressing the contemporary challenge to biodiversity abuse cannot achieve any recognizable success if just a ‘one-faced approach’ is utilized. His assertion agrees with Golo and Yaro (2013) as cited in Adom et al. (2016) who admitted that a truncated approach to using the scientific conservation strategies in combating the wanton depletion of biodiversity while sidelining the indigenous cultural beliefs and practices would accomplish less if not fail entirely. This was the case of the 1948 forest policy which, though popular in scientific models could not curtail the massive degradation of the forest cover and wildlife resources in Ghana. On the other hand, one of the guiding principles of the 1994 forest and wildlife policy mentions the need for an incorporation of traditional methods of resource management where appropriate in resource management where it deems necessary. However, the document was silent on which of the traditional methods are appropriate and in what capacities they could be implemented in this contemporary times. The 2012 forest and wildlife policy mentions the incorporation of indigenous knowledge of local communities in the management of the forest and wildlife resources as well as respecting the secrecy associated with the religious beliefs while promoting research into the indigenous knowledge systems by higher institutions of learning that could conserve biodiversity. This improved recognition of indigenous knowledge systems in the management and conservation of biodiversity is commendable. Indeed, the traditional conservation practices evident in the cultural beliefs and practices play significant roles in restraining resource abuse. Bonye (2008) argues the use of religious beliefs and cultural practices for environmental resource management have shown beyond all reasonable doubt to be more effective and sustainable in comparison to other forms of management.

Yet, the emphasis placed on the indigenous knowledge systems as conservation strategies in the 2012 forest and wildlife policy is less in the document, though the scientific conservation practices are well emphasized and implicitly explained. Therefore, there is the need for explicit explanations of which of the indigenous knowledge systems must be used as conservation strategies to complement the scientific conservation models. Thus, to solve the recurring problem of inclusion of cultural practices and beliefs (indigenous knowledge systems) in the forest and wildlife policies in Ghana, they must be a clear roadmap showing which of the cultural elements and in what capacities they must be utilized as conservation strategies. This can be effectively carried out if the unique indigenous knowledge systems of particular societies are identified and formulated into traditional biodiversity conservation strategies for that local community or ethnic society. Moreover, the traditional conservation strategy must not be implemented in isolation but in conjunction with the scientific conservation models.

Lack of strong implementation strategies

The issue on implementation strategies adopted by the forest and wildlife policies in Ghana has been a recurring challenge to the Forestry Commission. The sanctioning measures and agencies have not been up to their task. The 1948 forest policy's implementation seems weak because the allowable cut proposed in the policy was not enforced properly, resulting in a vast depletion of the timber resources in the country with an estimate of 65,000 ha (MLF, 2001; Ankomah, 2012). The World Bank Preparatory Mission for the FRMP concluded after a review of the 1948 forest policy that it had weak implementation strategies and institutions (GFW, 2006). The enforcement of the policy that is solely in the government institutions resulted in the lack of support from the local communities due to their neglect in the management of the policy (Teye, 2008). The same implementation challenge is associated with the 1994 forest and wildlife policy. Okrah (1999) contends that the lack of participation of stakeholders, especially the local communities in the implementation of the policy was the cause of its failure. In another lens, Amanor (2000) avers that the lack of support from the local communities in helping with the implementation of the 1994 forest and wildlife policy was due to failure on the part of the Forestry Commission to offer incentives in the form of fair and equitable distribution of forest proceeds. The 2012 forest and wildlife policy is the most efficient in terms of ensuring better representation of the stakeholders in the consultations that led to the development of the policy, especially, the inclusion of local people. Thus, the improvement of collaborative management approach is intensified in the policy. However, the sustainability of such forest management approach of involving the local communities while maintaining profit is questioned by the Forestry Commission (GFWP, 2012). Probably, the agency fears that making the local communities active players in the management of the resources may incur great loss to them because of having to pay them for the services they would render. This should not be the case if the Forestry Commission would follow the policy's strategic plan 4.1.1.b. which talks of allocating greater proportions of forest proceeds to the local people.

This may have been the cause of the little involvement of the local communities represented by the traditional authorities were not involved very well in the policy development as asserted by Sarpong and Inkoom (2015). Therefore, to remedy the recurring challenge of weak implementation strategies for developed policies in Ghana, there has to be a major support from the local communities and members of the general public. Thus, measures must be put in place to include them in the planning, decision-making and more importantly, the implementation processes of the policy. Moreover, the equitable and fair sharing of the forest proceeds with the local communities must be heightened and promoted to earn the support of the local people. In addition, the Forestry Commission as an institution needs to be strengthened. This can be achieved by the introduction of workers in the commission to workshops, short courses and seminars on effective implementation strategies. There is the need for the Commission to periodically upgrade the knowledge base of its staff for them to function more expediently. This would develop, strengthen and equip the human resource of the Commission in handling implementation challenges of the forest and wildlife policies.

Lack of and/or proper documentation for equitable distribution of resources to the local people

The local people have often times neglected in terms of fair distribution of forest and wildlife proceeds. This challenge is noticed in the 1948 forest policy as noted by GFW (2006) and Teye (2008), the local people were exempted from entering the forests and royalties were not properly paid. Equitable sharing of proceeds from the forests was lacking (Tuffour, 1996; Ankomah, 2012). However, Adom et al. (2017) mentioned of the ITPC (1989; Article 15) that said that local people have the legal rights to participate in the use of the forest resources. This is mainly because they are the landowners and as such must be catered for in the resource allocation. The 1994 forest and wildlife policy partly addressed this challenge of fair distribution of the forest proceeds to the local people by introducing stumpage fees and social responsibility agreement (Wiggen et al., 2004). But unfortunately, this became a paper document because due to poor documentation, the forestry officials and timber contractors flouted the agreements with the local communities regarding the equitable and fair allocation of forestry and wildlife proceeds (Derkye, 2007). This is a breach of the rights of indigenous people. For instance, UNDRIP (2006) stated in its article 37(1) that the local people owe the right to enforce the treaties and agreements that they enter with the forestry officers and timber contractors. Also, it tasks policy makers, forestry personnel and timber contractors to also respect and honor the agreements regarding the equitable sharing of the forest and wildlife proceeds. Therefore, if this is well cater for in policy development, the problem of unjust sharing of proceeds of forest and wildlife resources in local communities.

Another proactive way that can remedy the recurring problem of the lack of proper documentation of equitable sharing of forest and wildlife proceeds is providing a strong legislation nationally into policy development. Countries must incorporate international conventions related to the rights of local people, particularly, the UNDRIP (2006) that mentions that legislation must be passed by countries to respect all forms of agreement with local communities. The 2012 forest and wildlife policy advocate the passing of legislation to legitimize the rights of local people to the proceeds of their own farms. Yet, there are still challenges as it was noted by the GFIP

(2012). The document mentioned that there are poor structures put in place by the Forestry Commission to ensure proper accountability of the proceeds from the forests and thereby affects the quantity and the right compensation due the local communities. The absence of an elected member or representative from the local communities on the committee that oversees the fair and equitable distribution of the forest proceeds in the Forestry Commission is partly the cause of the situation. Moreover, there is weak supervision of the committee resulting in a potential foul play in the fair distribution and payment of the right royalties to the local communities. The Forestry Commission must ensure that proper structures are put in place to ensure that the right proportions of forest proceeds and revenue to the local communities to maintain fairness and equity in resource distribution. This committee set up must include representatives from the local communities so that they would fight for their due when the resources are shared. Also, they would guarantee that monetary gains such as stumpage fees and social responsibility agreements with timber concessionaires and companies are not flouted.

Lack of proper provision of sustainable alternative sources of livelihood for local residents

The members of the local communities rely heavily on the forest for their life sustenance. The GSS (2012) reports that a greater percentage of the local people engage in small-scale farming, logging and hunting as their main sources of livelihood. Therefore, with the shifting of total managerial powers of the forests to the government, it would mean the denial of the local people from enjoying the resources in the forests. Cobbina et al. (2015) contend that this situation has soared up the poverty rates in the majority of local communities in Ghana. Most governments failed to make proper provision for alternative sources of livelihood that are sustainable for members of the local communities so that they would not be rendered jobless. If their livelihood needs are not addressed, local people can be a nuisance and may abuse the forest resources any time they are privileged to them whether by fair or foul means. Teye (2008) concurs that the 1948 Forest Policy’s blind eye to the provision of alternative sources of livelihood for the poor local people propelled some of the local people to support illegal logging operations. They may sit on the fence and watch unconcerned as foreigners and other unscrupulous persons abuse the resources which they are customarily owners. This was what happened after the promulgation of the 1948 forest policy that gave close fisted hands to the provision of alternative sources of livelihood for the local communities while giving exclusive management rights of the forest proceeds to the government and other private timber producing agencies. The results were the deplorable state of Ghana forests and a scraping off of all its resources, giving birth to the new term ‘timberization’ for that generation (Gyampoh, 2011; Marfo, 2012).

The 1994 forest and wildlife policy somehow gave some attention to the provision of some source of alternative livelihood for the local people living in the forest fringe communities, though it was still the use of forest proceeds. The local people were granted the rights to tend some wildlife in personal pens so that they could sell them as bush meat or export them for monetary gains. However, this was woefully inadequate and it is not an efficient and sustainable form of alternative sources of livelihood since all local people cannot engage in this venture. Also, it would even result in an over exploitation of the wildlife resources. In addition to this, the GNA (2010) reported that there was no legislation in place to support the activities of alternative sources of livelihood by the local people who depended on the forests. In a related study by Cobbinah et al. (2015) at the Kakum Conservation Area in Ghana, they realized that when alternative sources of employment were created for the local people, few of them enjoyed the opportunity because the appointments favored many people who were not from the fringe communities around the Kakum Conservation Area. The site was properly developed into an ecotourism site and this generated employment avenue for the local people. The Kakum Conservation Area’s development interestingly coincides with the suggestion of Okra (1999). He suggested that if protected areas are well developed sustainably, they would be capable of meeting the basic needs of the local people living in the forest fringe communities without tying the hands of these poor people from the use of forest resources. This would ensure that the livelihood needs of the local people are met. Also, the 2012 forest and wildlife policy mentioned the creation of forest-based employment avenues like tree planting and the protection of boundaries for the local communities close to the forests. While these efforts are commendable, the allocations of the employment avenues to the local communities have often been thwarted to other persons from the cities as was the case in the Kakum Conservation Area. Therefore, strict measures and a possible legislation must be passed that authorize only or majority of the local people living in the forest fringe communities to enjoy the employment avenues. In cases where an employment avenue may require special skills, there must be skill training sessions organized for some local people who can occupy the best positions. This would greatly curb the impoverished state of most persons in the local communities, thereby ensuring proper provision of sustainable alternative sources of livelihood for the local people residing in the forest fringe communities.

Moreover, other employment prospects must be explored by the Forestry Commission with the aid of other Non-Governmental Organizations. For instance, organizations may not address the peculiar needs of buyers, eventually collapsing them. For instance, the Commission must improve and enhance the non-wood forest resource base and yield as fertile grounds for alternative sources of livelihood for the rural poor living in the forest fringe communities. This includes searching for avenues to explore the trading in non-forest products like the gathering of mushrooms, snails and so forth. Small-scale enterprises in forest and other non-forest products and services that are economically friendly and address the distinctive needs of members in the local community or adjacent communities can be set up as sources of alternative livelihood. These small and medium forest enterprises (SMFEs) added three million alternative source of income to the Ghana's economy in 2015 (NBS, 2016). Consultations with the local people about the appropriate business enterprise to set up as an alternative source of livelihood would prove very beneficial in that they would know the kind of good or service that sells and would result in gains; otherwise, setting up business enterprises based on only the discretion of the Forestry Commission and the non-governmental.

Poor dissemination of forestry and wildlife policies to local communities

Information on forestry policies and strategies in Ghana has always not been properly communicated to the local communities. It has already been established that policy makers usually neglect or least involve the local communities in discussions and consultations that lead to the formulation of forestry policies. Unfortunately, after the development of the policy, public education about the contents of the document is not popularized, especially, in the forest fringe communities. Ankomah (2012) regrettable reported that the situation of poor communication of developed forestry and wildlife policies in Ghana is even among the conservationists working in the Forestry Commission. He realized in his research that 20% of his respondents from the Forestry Commission were not familiar with the content of the 1994 forest and wildlife policy. This underscores how poor forestry policies and strategies redisseminated in the country even among elites, how much more the local communities in Ghana. The same challenge was admitted at the launching of the 2012 forest and wildlife policy. The coordinator of the Ghana Forest Watch Ghana, Mr. Kingsley Bekoe hinted that the dissemination of the policy content of the local people residing in the forest fringe communities was woefully inadequate (Daily Graphic, December 13, 2012). Therefore, he called for an intensive education of the forest and wildlife policies to the general public, especially, the local communities to assist them in making informal decisions regarding the sustainable use of forest and wildlife resources in their environment. Of course, effective communication of forest and wildlife policies beefs up the successes in their implementation (Adom, 2016b).

The Forestry Commission is the agency tasked to carry out such education, according to the Forestry Commission Service Charter. Therefore, the Commission must intensively strategize and plan on how to educate the general public, especially, the local people. The Commission must beef up education, extension and awareness programmes of people's participation in forest and wildlife issues. The commission must take advantage of the numerous social media outlets to broadcast the developed policies. More importantly, they must organize a series of workshops, seminars and forums in towns and villages rather than focusing on only the national and district levels. Moreover, the traditional and locally accepted means of communication such as through the use of storytelling, proverbs and folklores to instruct the people on the content of the policies. Modern Information Centers must be adopted as communication vehicles for the policies. Biodiversity experts and policy makers must take advantage of taboo days within local communities to enlighten the local people on the policy document. During such sessions, the forestry officer must break down the contents of the policy to the level of understanding of the largely illiterate local people. This will deepen their knowledge and understanding of the policy and adjust their thinking as well as farming methods to suit the directives in the policy.

CONCLUSION AND RECOMMENDATION

The study was undertaken with the primary objective of identifying the recurring challenges associated with the forest and wildlife policies in Ghana. This was to provide potent remedies to arrest those challenges that have perpetually been associated with the forest and wildlife policies. This would enhance their efficacy in halting the high rise of deforestation and pollution of the environment in Ghana. The 1948 Forest Policy, the 1994 Forest and Wildlife Policy and the 2012 Forest and Wildlife Policy in Ghana were rigorously analysed using the interpretive document analysis and the interpretive policy analysis. The study revealed the recurring challenges included the lack of effective stakeholder participation of local people in the planning, and decision-making processes in the Forest and Wildlife Policy formulation in Ghana. It was proposed that policy makers and conservation planners of forestry and wildlife policies must actively involve the members in the local communities in all phases of the policy development processes since they are the land owners and as such major stakeholders in the forestry resources in their environment. Also, the lack of strong implementation strategies for enforcing the dictates of the policies were seen as another recurring challenge associated with the reviewed forest and wildlife policies. This was realized through the rigorous analysis that it is as a result of the neglect of the local people and the sidelining of traditional authorities living in the forest fringe communities to assist in the implementation processes of the policies. Therefore, it was suggested that the traditional authorities must be involved in the implementation processes of the forest and wildlife policies in Ghana for them to serve as agents in their local jurisdictions.

Moreover, the lack of effective factorization of cultural beliefs and practices of local communities in the management of the forests and wildlife resources was a recurring problem that has progressively been improved but still requires significant improvements. This was seen in the lack of clear-cut guidelines on which of the cultural beliefs and practices are to be utilized as well as the capacities for their implementation. It was argued that some of the cultural beliefs and practices of local communities are potent and beneficial in assisting in the conservation and sustainable use of the biodiversity resources in nature. Owing to this, it was suggested that forestry and wildlife policies must incorporate them in the management strategies while showing clearly the forms of the cultural beliefs and practices that should be utilized in local communities in Ghana. Furthermore, it was noticed that the other lapses in the forest and wildlife policies in Ghana are the lack of and/or proper documentation of equitable distribution of forestry and wildlife resources for local people as well as lack of proper provision of sustainable alternative sources of livelihood for residents in the forest fringe communities and the poor dissemination of forestry and wildlife policies to the local communities. The absence of proper documentation of agreements regarding the fair sharing of forest proceeds between the government represented by the Forestry Commission and the local communities, the payment of royalties and the provision of appropriate social amenities as compensations to the local communities are intentionally breached by the Forestry Commission and timber companies. Therefore, it was advised that due to the legal rights those local communities have with respect to the forest proceeds indicated by international conventions and by virtue of their status as landowners require that agreements with them are honored. Thus, local communities are to insist on gaining written and signed copies to formalize all agreements to curtail the recurring challenge of flouting of agreements especially on the part of the government officials.

Local communities have often been banned from entry into the forests that customarily belongs to them and have been the main source of livelihood for the people. It was realized through the analysis of the forest and wildlife policies that most often the policies do not offer any source of alternative livelihood for the local people as it was evident with the 1948 Forest Policy. Sometimes, the policy offers some form of alternative sources of livelihood as was realized in connection with the 1994 and 2012 forest and wildlife policies. Yet, it was noted that these options of alternative livelihood provided were not sustainable and multi-faceted to address the diverse employment and/or economic needs of the diverse fringe forest communities. Therefore, recommendations on how to generate diverse forms of alternative sources of livelihood for the local communities such as using the development of the forest reserves and conservation sites as eco-tourism sites to generate employment avenues that prioritize the employment of the local folks as well as assisting the local people in setting up economically viable enterprises in both forest and non-forest products and/or services have been suggested. Finally, the recurring challenge of poor dissemination of forest and wildlife policies to the general public, especially the local people were noticed in all the forest and wildlife policies reviewed. It was suggested that local means of communication such as through folklores, folk tales and proverbs, taboo days, community meeting days and forums must be utilized. Also, social media and Information Centers in local communities were suggested as powerful avenues for relaying the developed forest and wildlife policies to the general public as complements to the seminars and workshops that are usually organized at national and district levels by the Forestry Commission.

The research has sternly taken the firm stand that if the suggestions offered to curtail the recurring challenges of the forest and wildlife policies in Ghana are taken seriously and implemented, it would mitigate the deleterious situation of Ghana's forest and wildlife resources while mitigating all forms of environmental pollution. The study is tasking the Ministry of Lands and Natural Resources and their functionaries especially the Forestry Commission, Timber Companies and Non-governmental Organizations whose interests are in the forest and wildlife resources, to implement the suggestions that issue from the findings of this rigorous research as a helping hand to the efforts they have put into saving these biodiversity resources that pivots the life of Ghanaians.

There is the need for an extensive review of the scientific conservation practices in the Forest and Wildlife Policy so as to know which of the traditional conservation practices in the light of cultural beliefs and practices can complement them. This would help create a synergistic approach to tackle the biodiversity challenge in Ghana.

CONFLICT OF INTERESTS

The authors has not declared any conflict of interests.

Abbreviations

CBD, Convention on Biological Diversity; **CFC,**

Community Forest Committee; **CFMU,** Collaborative Forest Management Unit; **EPA,** Environmental Protection Agency; **FRMP,** Forest Resource Management Project; **FPP,** Forest Preservation Programme; **GFDMP,** Ghana Forestry Development Master Plan; **GFIP,** Ghana Forest Investment Plan; **GFW,** Ghana Forest Watch; **GFWP,** Ghana Forest and Wildlife Policy; **GNA,** Ghana News Agency; **GSS,** Ghana Statistical Service; **ITPC,** Indigenous and Tribal People's Convention; **MLF,** Ministry of Lands and Forestry; **MLNR,** Ministry of Lands and Natural Resources; **MSE,** Ministry of Science and Environment; **NBS,** National Biodiversity Strategy; **TUC,** Timber Utilisation Contract; **UNCED,** United Nations Conference on Environment and Development; **UNDRIP,** United Nations Declaration on the Rights of Indigenous People.

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Full Length Research Paper

Influence of *Diatraea saccharalis* (Lepidoptera: Crambidae) infestation on sweet sorghum productivity and juice quality

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Ethanol production from sweet sorghum depends on the quality of the biomass and sugars in the juice extracted from the plant. However, quality may be compromised by *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) or sugarcane borer infestation. This study evaluated the effects of infestation by the pest on sweet sorghum juice productivity and quality. Sorghum variety BRS506 was planted in an experimental area with 4,800 m^2. Samples were harvested at 115 days after planting and the following variables were evaluated: physical injury caused by the pest in the stalk, juice yield, total soluble solids in the juice and chemical composition. Additionally, the presence of *Fusarium* sp. in the stalks was checked. A significant difference was detected for juice yield, with lower values found in bored stalks resulting in a 2.62% decrease in juice yield at an infestation intensity of 4.16%. Infestation of *D. saccharalis* also caused a reduction of 34% in the plant sucrose content and significantly decreased fiber, lignin and ash rates. Infestation by *D. saccharalis* in sweet sorghum caused a significant reduction of plant height, juice productivity, and was associated with the presence of *Fusarium* sp., and sugar and fiber reduction.

Key words: Chemical composition, integrated pest management, juice quality, sugarcane borer.

INTRODUCTION

The sugarcane alcohol and energy industrial sector in Brazil is increasingly seeking profitable and sustainable alternatives to maintain its competitiveness. Sweet sorghum, an alternative feedstock for the production of biomass and ethanol (Durães, 2014; Zegada-Lizarazu and Monti 2012), has important agronomic and industrial

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characteristics for the production of bioenergy (Wu et al., 2010; Monk et al., 1984). Further, ethanol may be produced from the juice of sweet sorghum at the same facilities used for sugarcane processing (Tomaz and Assis 2013; Durães et al., 2012). Sweet sorghum contains fermentable sugars in the juice (sucrose, glucose, and fructose), starch in the grain, and lignocellulose in the bagasse, which may be used in second-generation ethanol plants (Wu et al., 2010).

Since sweet sorghum is characterized by high photosynthetic activity, it may be grown in temperate and tropical environments (Kim et al., 2012), especially if cultivated in summer when there is supply of raw material during the sugarcane offseason (May et al., 2014). However, ethanol production from sweet sorghum may be jeopardized by infestation of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), also known as the sugarcane borer (Mendonça 1996), one of the principal insect pests in sweet sorghum production (Waquil et al., 1980). This pest has a polyphagous food habit and causes damage in several economic cultures, among these are, sugar cane, rice, corn and sorghum (Cruz, 2007).

D. saccharalis causes direct and indirect losses to crops due to its feeding on the stalk of plant. In fact, it bores holes in the stalks, reduces the flow of photoassimilates, causes plant lodging and decreases the juice productivity (Fuller et al., 1988). Once it gets into the stalk, its control is difficult. Holes left in the stalk by *D. saccharalis* trigger infection by fungi. Many species of the genus *Fusarium*, including *Fusarium verticillioides* Sacc. (Nirenberg) (synonym *Fusarium moniliform* Sheld.) [teleomorph: *Giberella fujikuroi* (Sawada)], *Fusarium thapsinum* Klittich, Leslie, Nelson & Marasas, and *Fusarium proliferatum* (Matsushima) Nirenberg are capable of infecting stalks. *Colletotrichum falcatum* (Went) is also an important fungus which promotes the inversion of sucrose and reduces juice purity in sugarcane, causing lower sugar yields and contamination during alcoholic fermentation and, consequently, low ethanol yields (Mendes et al., 2012; Tesso et al., 2010; Leslie et al., 2005; Leslie, 1991). However, there are no records of sugar inversion in sweet sorghum as a result of the presence of *D. saccharalis* and the genus, *F. verticillioides*. Nevertheless, it is known that the “red rot” symptom caused by the attack of pests and plant pathogens is due to phytoalexins in sorghum. These substances are the plants defense against pests and plant pathogens. Their accumulation adjacent to the affected spots is marked by a reddish-purple colour (hence the name of the symptom).

The use of sweet sorghum for ethanol production should considered due the impact of *D. saccharalis* infestation on the quality of the raw material since the infestation of this pest interferes with sucrose content, soluble solids, acidity of juice and fiber content (Clarke and Legendre, 1999). Studies developed by Copersucar® in the 1980s established parameters to determine the effects of *D. saccharalis* on sugar and alcohol production from sugarcane (Téran et al., 1986; Precetti et al., 1988; Arrigoni, 2002). Since the effects caused by the attack of this insect on sweet sorghum have not been established, it is highly relevant to determine the effect of *D. saccharalis* infestation on the agronomic and quality-related components of sweet sorghum production. This study assesses the effect of *D. saccharalis* on production of juice and bagasse from sweet sorghum for ethanol production.

MATERIALS AND METHODS

Experiment site

This study was conducted in sweet sorghum field at the *Embrapa Milho e Sorgo* Research Center in Sete Lagoas MG Brazil (19°28’ S and 44°15’08” W), under field conditions, in the 2013/2014 crop year, using sweet sorghum variety BRS506.

To simulate a realistic field situation as closely as possible, sweet sorghum was grown in a 4800 m^2 field, with 0.70 m spacing between rows and 10 plants per row; the area was divided in two 2400 m^2 fields with and without insecticide treatments. Inside the growing areas, the samples were obtained using completely randomized design, with each stem being considered a repetition. The field plots were not replicated in other years.

The insecticide, chlorantraniliprole was used as chemical insecticide, with spraying at 20, 37 and 42 days after planting, at 60 g per ha. After the last spraying, sweet sorghum yield was high. The other treatment consisted of no-control of *D. saccharalis* and the crop management was made according to May at al. (2012). The soil correction and fertilizing was made follow recommendations of Santos et al. (2014). In each area, three different sorts of 50 samples were collected: the first was used to verify physical injury; the second was used to evaluate juice quality and chemical composition, and the third was used to verify fungus infection.

Physical injury caused by *D. saccharalis* infestation

The injury caused by natural infestation of *D. saccharalis* was evaluated at 115 days after planting, or rather, during the plants physiological maturity. 150 stalks were collected randomly in each area (with or without insecticide). Each stalk was opened lengthwise and data on height, total number of internodes, bored internodes (with symptoms of injury or tunnel formed by the larva) and tunnel lengths were assessed. The number of healthy internodes was calculated as the difference between total and bored internodes.

Determination of juice yield, sugar profile and chemical composition of sweet sorghum stalks

On physiological maturity, additional 400 stalks were collected at random from each treatment and subdivided into four groups: 100

stalks for whole-plant samples; 300 stalks sectioned into 3 parts (basal, middle and apical). Each sample, weighing 500 g, was processed to form replicate samples. The number of samples varied from 6 to 40 (Table 3).

Samples were processed with a hydraulic press to extract the juice and total soluble solids were evaluated with digital refractometer in °Brix. Juice yield (%) was determined as the volume of juice extracted from 500 g of processed stalk (volume/mass). Further, an 80 ml aliquot was collected out of a total volume of juice extracted from each sample, and kept frozen at -4°C in polyethylene bottles for subsequent quantification of fructose, glucose and sucrose contents by HPLC with refractive index detection. A carbohydrate column (Waters Alliance 2695, Waters Corporation, Milford MA USA) was employed to analyze sugars. The mobile phase was a mixture of acetonitrile: water (75:25) with 1 mL per min flow rate. Injection volume was 25 μL. Refractive index (Waters Corporation) was the detector. Standards of fructose, glucose and sucrose sugars with high purity (99% Sigma-Aldrich) were used to construct the calibration curve. The juice samples were diluted, centrifuged and adjusted in the proportion of the mobile phase by comparing retention times of the standards.

Samples of wet bagasse, retrieved from the juice extraction process, were weighed (fresh weight) and dried in an oven at 105°C (AOAC, 1990) to determine dry matter content. After drying, the material was analyzed to measure cellulose, hemicellulose and lignin contents according to the method of Van Soest et al. (1991). Ash rate was determined according to the methodology of AOAC (1990).

Presence of *Fusarium* sp. in stalks

The presence of *Fusarium* sp. in sorghum plants with and without injuries caused by infestation of the *D. saccharalis* was evaluated at physiological maturity using plants from plots treated and untreated with the insecticide. One hundred plants were collected at random in each treatment. Plants were opened lengthwise for symptoms of red rot *Fusarium* sp. by separating the bored from the healthy ones and fragments of internodes were taken from healthy and bored stalks. Fifteen random samples of approximately 1 cm^2 from basal, middle and apical third sections were collected. Samples were taken to the Plant Pathology Laboratory at *Embrapa Milho e Sorgo* for isolation and evaluation for the presence of *Fusarium* sp. Samples were disinfected in 2% sodium hypochlorite for 2 min, transferred to Petri dishes with PDA + antibiotic and kept in a growth chamber at 27 ±2°C, for 7 days. Colonies formed were examined with a magnifying glass and a microscope to detect the colour of the colonies and the fungal morphology, for the identification of the genus.

Data analyses

Data underwent analysis of variance and means were compared by Scott-Knott test ($P \leq 0.05$), with Sisvar software (Ferreira 2007). Quantities of fructose, glucose and sucrose were determined separately and by grouping averages of each part of the plant.

The intensity of infestation was determined by calculating the proportion (%) of internodes bored by the *D. saccharalis*: (number of bored internodes/number of total internodes) × 100, following Bates (1954) cited by Williams et al. (1969). For variance analysis, the intensity of infestation data and incidence of *Fusarium* sp. in the plant samples were transformed by the square root of rates.

RESULTS

Analysis of structural and physical characteristics associated with infestation of *D. saccharalis* in sweet sorghum revealed significant differences in all variables as compared to the less infested (insecticide-treated) plots (Table 1). The insecticide-treated plants had more internodes, more healthy internodes, lower levels of infestation, fewer infested internodes and a higher number of healthy internodes. Results provide an example of the potential damage that occurs when no action is taken to control *D. saccharalis* on the sweet sorghum crop, although infestation levels vary and the insecticide-treated plots were not completely pest free. It seems that potential benefits of insecticide application are slightly underestimated.

In addition to structural and physical effects associated with insect infestation, significant differences were reported for juice yield (%) between the treatments and the different parts of the plant analyzed (basal, middle and apical segments, and the entire stalk). Highest juice yields were detected in the stalks from insecticide-treated plot and in the basal region of both treatments. The middle third segment showed the largest difference in juice yield between insecticide-treated plants and insecticide-free plants (Table 2).

The concentration of total soluble solids showed significant differences between plant sections, with higher levels for insecticide-treated plots. Significant differences between plant sections were less evident in the untreated plots. The highest concentration of total soluble solids occurred in the apical segment of the plant, whereas the middle third segment displayed the greatest concentration of total soluble solids in no insecticide-treated plots (Table 3).

The concentration of fructose showed significant differences in the basal region between treatments. The only treatment-specific difference was observed in the middle region in the case of glucose concentration (Table 3). Moreover, sucrose concentration in the entire stalk was significantly higher where *D. saccharalis* were controlled (insecticide treatment), as was the sucrose level in the middle and apical third segment of the insecticide-treated plant. In the case of free insecticide plants, no significant differences were detected between plant segments (Table 3).

Few significant differences were observed for dry matter, although the dry matter level of the entire stalk in the insecticide-treated plants was higher than in the basal, middle and apical segments (Table 4).

Significant differences were reported between the treatments for cellulose, hemicellulose and lignin. Differences varied according to the plant segment analyzed for each component, with a greater concentration of cellulose

Table 1. Sweet sorghum plant height (cm), number of internodes per plant, number of undamaged and insect-damaged internodes per plant, proportion of damaged internodes, and proportion of plants that were infested by *D. saccharalis* when sampled from insecticide-treated and untreated plots.

Response variable	Treatment[1]	
	Insecticide	Untreated
Plant height	218.74±1.79^{a}	161.85±1.50^{b}
Total internodes/plant	12.72±0.10^{a}	11.71±0.09^{b}
Undamaged internodes/plant	12.65±0.10^{a}	11.21±0.12^{b}
Insect-damaged internodes/plant	0.07±0.03^{b}	0.50±0.08^{a}
Bored internodes (%)	0.53±0.21^{b}	4.16±0.62^{a}
Infested plants (%)	4.70±0.02^{b}	31.87±0.04^{a}

[1]Means followed by the same letter in the row do not differ significantly by Scott-Knott test (*P* > 0.05).

Table 2. Juice yield (mean % ± SD) from sweet sorghum in insecticide-treated and untreated plots.

Treatment	Stalk portion[1,2]			Entire stalk
	Basal	Middle	Apical	
Insecticide-treated	65.03 ± 0.21^{Aa}	63.13 ± 0.47^{Ab}	55.45 ± 0.43^{Aa}	61.95 ± 0.54^{Ab}
No insecticide	61.36 ± 1.09^{Ba}	56.76 ± 1.48^{Bb}	54.33 ± 2.22^{Ab}	59.33 ± 1.00^{Ba}

[1]Analyzed segments of plant: basal, middle, apical, and whole plant. [2] Means followed by the same capital letters in the row and small letters in the column, for the same variable, do not differ by Scott-Knott test (*P* ≤0.05).

Table 3. Concentration of total soluble solids (°Brix) and sugars (mg mL^{-1}) in healthy and *D. saccharalis*-bored sweet sorghum stalks.

Characteristics of the juice	Stalk segment[1]	Treatment[2]	
		Control[3]	Bored
Total soluble solids	Basal	14.50±0.24^{Ac} (40)[4]	13.90±0.29^{Bb} (22)
	Middle	15.50±0.20^{Ab} (40)	14.60±0.30^{Ba} (21)
	Apical	16.50±0.12^{Aa} (40)	13.40±0.43^{Bb} (6)
	Entire stalk	16.40±0.22^{Aa} (37)	14.80±0.16^{Ba} (18)
Fructose	Basal	28.39±2.22^{Ab}	17.93±1.07^{B}
	Middle	28.83±2.82^{b}	24.95±1.81
	Apical	35.39±2.50^{a}	27.44±4.96
	Entire stalk	23.97±2.11^{Ab}	16.15±0.82
Glucose	Basal	24.50±0.67^{b}	24.84±1.35
	Middle	22.67±0.67^{Bb}	28.18±1.13^{A}
	Apical	27.08±1.58^{a}	29.00±2.80
	Entire stalk	25.08±0.91^{b}	24.98±1.08
Sucrose	Basal	56.14±4.68^{b}	52.42±3.29
	Middle	76.10±3.98^{Aa}	44.16±6.20^{B}
	Apical	77.46±5.00^{Aa}	35.86±11.51^{B}
	Entire stalk	85.78±2.52^{Aa}	56.25±4.04^{B}

[1]Analyzed segments of plant: basal, middle, apical, and whole plant. [2] Means followed by the same capital letters in the row and small letters in the column, for the same variable, do not differ by Scott-Knott test (*P* ≤0.05). [3] Control: insecticide treatment. [4]n: sample number.

Table 4. Percentages of dry matter, cellulose, hemicellulose, lignin and ash in the basal, middle, and apical segments and the entire stalk of sweet sorghum sampled from insecticide-treated and untreated plots.

Characteristic (%)	Stalk segment[1]	Treatment[2]	
		Control	Bored
Dry matter	Basal	93.90±0.06[b] (40)[3]	93.90±0.15 (22)
	Middle	93.90±0.06[b] (40)	94.00±0.03 (21)
	Apical	93.80±0.02[b] (40)	93.90±0.06 (6)
	Entire stalk	94.10±0.08[a] (37)	94.10±0.11 (18)
Cellulose	Basal	37.61±0.34[a]	37.57±0.55[a]
	Middle	36.20±0.43[b]	35.74±0.65[b]
	Apical	34.21±0.21[c]	35.71±0.91c
	Entire stalk	37.22±0.34[Aa]	34.16±0.39[Bb]
Hemicellulose	Basal	33.28±0.24[Bb]	35.34±0.33[A]
	Middle	34.10±0.17[a]	34.85±0.51
	Apical	34.47±0.27[a]	35.68±0.67
	Entire stalk	34.36±0.28[a]	34.08±0.31
Lignin	Basal	4.10±0.15[B]	4.6±0.22[Ab]
	Middle	4.00±0.15	4.40±0.23[b]
	Apical	4.40±0.17[B]	5.90±0.47[Aa]
	Entire stalk	4.40±0.14	4.00±0.13[b]
Ash	Basal	2.21±0.06[b]	2.32±0.03[a]
	Middle	2.03±0.05c	2.02±0.03[b]
	Apical	2.38±0.02[a]	2.30±0.09[a]
	Entire stalk	2.30±0.04[a]	2.16±0.09[b]

[1] Analyzed segments of plant: basal, middle, apical, and whole plant. [2] Means followed by the same capital letters in the row and small letters in the column, for the same variable, do not differ by Scott-Knott test ($P \leq 0.05$). [3] n: sample number.

Table 5. Percentage of sweet sorghum stalks (mean ± SD) from insecticide-treated (control), without borer symptoms and untreated plots (with bored symptoms) with *Fusarium* sp. in the basal, middle or apical segment of the plants.

Treatment	Plant segment[1]		
	Basal	Middle	Apical
Control	93.3 ± 6.7[a]	100.0 ± 0.0[a]	86.7 ± 8.2[b]
Bored	100.0 ± 0.0[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]

[1]Means followed by the same letters in the column for the same variable do not differ by Scott-Knott test ($P > 0.05$).

observed in the entire stalk in the insecticide-treated plants. However, higher concen-trations of hemicellulose and lignin were reported in the insecticide-free treatment, the former in the basal segment and the latter in the basal and apical segments. Significant differences of ash were detected among the different regions of the stalk and in the entire stalk for each treatment (Table 4), but not between treatments.

The presence of *Fusarium* sp. was observed in all the bored stalks retrieved from insecticide-untreated plots (Table 5). In the case of samples from treatment with insecticides and in samples without bores by *D. saccharalis*, the presence of *Fusarium* was observed in 93.30, 100 and 86.70% of the basal, middle and apical

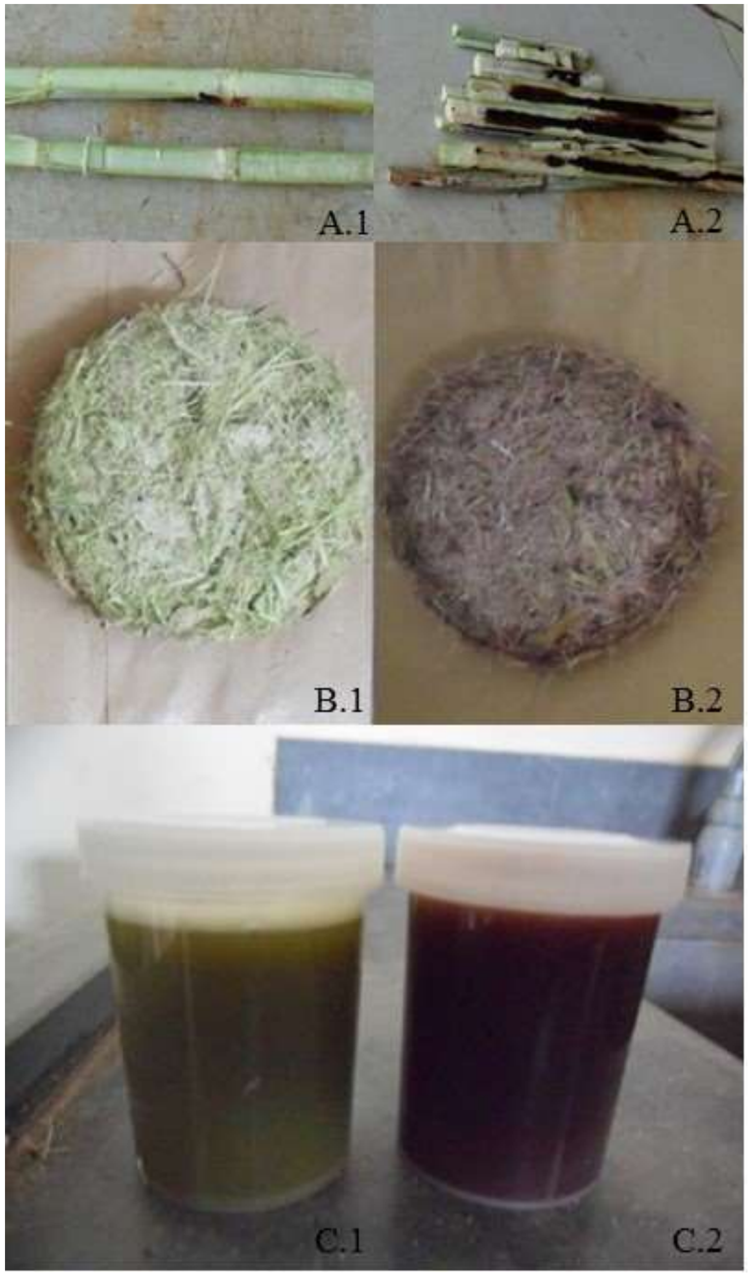

Figure 1. Samples of stalks (A), wet bagasse (B) and juice (C) from sweet sorghum in non-bored (1) and in the bored stalks (2).

segments, respectively (Table 5).

However, a statistically significant difference was only observed in the apical third segment. Moreover, the presence of the fungus changed the color of the extracted juice. A purple colour was observed in the wet bagasse of bored stalks. No red or purple colour was reported in samples of healthy stalks (Figure 1). *Fusarium* sp. was isolated from the two samples containing red, symptomatic (red rot) and asymptomatic segments (stalk with a normal colour).

DISCUSSION

Results of natural infestation and attack by *D. saccharalis* observed in sweet sorghum stalks demonstrate that the pest has a significant role in altering the quantity and quality of the extracted juice. The attack of the pest directly reduced productivity-related variables, since plant height and average number of internodes decreased, perhaps due to the reduction of the translocation of nutrients and photosynthates in the bored internodes and, consequently, reduction of juice yield, a product of industrial interest. Losses have also been reported in commercial production of other host plants of the insect, e.g. corn, where the attack of the borer directly affects grain production and yield (Cruz et al., 2010) and sugarcane, where plant height is reduced, among other issues (Souza et al., 2008).

In this study, 31.87% of plants were infested, with infestation intensity (considering total of internodes) of 4.16%, and a reduction of 2.62% in juice yield. This is the first study to show yield parameter reductions, from *D. saccharalis* injury in sweet sorghum cultivars. Studies conducted in the 1980s with sugarcane and published by Arrigoni (2002) demonstrated the borer´s capacity to decrease sugarcane production. According to the author, a 1% intensity of borer infestation causes 1.50% reduction in stalk production, 0.49% reduction in sugar production and 0.28% reduction in alcohol production. The relationship between juice reduction and occurrence of pest in the current study was higher than that demonstrated for sugarcane. These results demonstrated the need for improving integrated pest management (IPM) strategies, aiming at the maximization of juice and bagasse production.

In addition to quantitative effects, the borer also causes loss of juice quality in sugarcane (Dinardo-Miranda et al., 2012). This study reveals that high quality may be observed with the concentration of total soluble solids and sugars in the juice, influencing the production of ethanol. As a rule, the occurrence of tunnels in all parts of the plant reduced the concentration of total soluble solids. In the whole plant, a reduction of 1.6 °Brix units was observed when symptoms of damage were detectable. This corroborates data published by Gomes (2014), who used bored materials of sweet sorghum (CVSW80007) and obtained total soluble solids below 16 °Brix, whereas the rate was higher in healthy materials.

Fuller et al. (1988) obtained a significant negative correlation between the percentage of internodes bored by *D. saccharalis* and the concentration of total soluble solids in sweet sorghum, demonstrating the same impact on sugar reduction. Likewise, Rossato Júnior (2009) detected a significant difference of 1.58 °Brix units in total soluble solids in sugarcane between healthy materials and materials attacked by *D. saccharalis*. Consequently, the reduction of sorghum juice quality parameters demonstrates the pest´s effects.

Gomes (2014) showed that sweet sorghum has similar technological characteristics as unripe sugarcane, which include soluble sugars, reduced sugars, total reduced sugars and others. However, according to the above author, the end product will depend on the concentration of sugars available in the medium; thus, at higher concentrations of sugar (16 °Brix), yeast will transform sugar into ethanol and CO_2 (fermentation) more efficiently.

According to this study, infestation of *D. saccharalis* reduced significantly total soluble solids. Due to significant Brix reduction, there was a 34% sucrose reduction in the plant. Dinardo-Miranda et al. (2012) observed that the infestation of the pest in sugarcane reduced sucrose and increased the amount of reduced sugars (fructose and glucose), also reported in the current study, in which the glucose content increased in bored stalks as compared to stalks from control treatment.

However, the best strategy for industries is the maintenance of juice quality, that is, a higher concentration of sucrose relative to the concentration of glucose and fructose (Wu et al., 2010), as observed in the current study. The above emphasizes the need for stalks free of infestations by *D. saccharalis*. Thus, constant monitoring of the pest in the production of sweet sorghum and the adoption of appropriate IPM strategies are recommended regardless of the development of plant development.

Insoluble materials such as cellulose, a fraction of the plant´s fiber, may be transformed into glucose through physical and chemical treatments. Ethanol obtained from this process, or second-generation ethanol (Pereira Jr. et al., 2008), may be produced from agro-industrial materials such as sweet sorghum bagasse. However, in this study, differences were detected between healthy stalks and stalks with symptoms of infestation only for cellulose when the whole plant was evaluated. This corroborates observations by Milano (2012) who isolated bacteria that degraded cellulose in the digestive tract of the species. Although, Saldarriaga (2009) and Dantur et al. (2015) reported that bacteria isolated from the intestinal tract of *D. saccharalis* showed a positive response in *in vitro* degradation tests for cellulose, hemicellulose and lignin in sugarcane bagasse, they showed a greater efficiency in cellulose degradation.

Reduction in the quality of sweet sorghum juice was related to the presence of fungi of the genus *Fusarium*, which cause the red rot disease that infects stalks and reduces sugar content in the juice due to the inversion of the sucrose stored in the plant and its transformation into glucose and fructose, as described by Botelho and Macedo (2002) and Stupiello (2005). Furthermore, the contamination of the juice by the fungus inhibits fermentation, compromising industrial processes. Dinardo-Miranda et al. (2012) suggested that the infestation of opportunist and secondary fungi in sugarcane stalks reduces juice quality. The presence of *Fusarium* sp. in healthy sorghum tissues is common (Waniska et al., 2001) and indicates that the occurrence of *D. saccharalis* pest is not a *sine qua non* condition for the incidence of the pathogen in the plant. However, Schulthess et al. (2002) suggested that in maize, some Lepidoptera and Coleoptera were attracted or survived for a longer period in plants infected by *Fusarium verticillioides*. This relationship between the presence of *F. verticillioides* and insects was also reported by Cardwel et al. (2000). Rossato et al. (2013) demonstrated that juice quality decreased as the infestation intensity increased. Consequently, the effect of the borer-stalk rot complex on juice quality must be further elucidated to improve agro-industrial processes for ethanol production from sweet sorghum.

Results demonstrate that measures to ensure maximum growth and production of high quality raw material for ethanol production should be started by striving to keep pest damage at a minimal. Although studies such as that of Wu et al. (2010) were undertaken to understand the influence of the characteristics of sweet sorghum juice on fermentation for ethanol production, these authors focused their analysis on the end of the production chain. On the contrary, the quality of the raw material may be compromised from the beginning of the production cycle due to inadequate management of pests in the crop. As demonstrated in this study, infestation by *D. saccharalis* in sweet sorghum causes a significant decrease in plant height and juice yield, including a decrease in sugar content and fiber.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of deprivation of selected single nutrients on biometric parameters of cedar seedlings (*Acrocarpus fraxinifolius*) grown in nutritive solution

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The purpose of this work was to assess biometric parameters of cedar seedlings (*Acrocarpus fraxinifolius*) associated with visual symptomatology of macronutrients deficiency, growth rate, and content and accumulation of nutrients in aerial parts of seedlings grown in nutritive solution. The trial was established in completely randomized design with seven treatments, four replications, and one plant per pot. Treatments consisted of complete nutrient solution, Hoagland and Arnon, and deprivation of the following selected single nutrients: N, P, K, Ca, Mg and S. Micronutrients were provided to all treatments. The following biometric parameters were assessed 90 days after the transplant: shoot height, root collar diameter, root and shoot dry weight. The Dickson index and the ratio given by roots dry weight and shoot dry weight were obtained, as well as the content and accumulation of nutrients in aerial parts of seedlings. The Sisvar statistical analysis system was run for analysis of variance, and averages were compared by means of Scott-Knott test at 5% significance. Treatments consisting of omissions of macronutrients were found to be limiting factors of plants growth and obtaining of seedlings shoot dry weight in the following order: N > P > K > Ca > Mg > S. Deficiencies of these macronutrients caused visible morphological abnormalities, where common symptoms of deficiency of N, P, K and Ca appeared before symptoms of deficiency of Mg and S. The content of nutrients found in aerial parts of seedlings of *A. fraxinifolius* grown in nutritive solution was greater for treatments consisting of deprivation of N, P and K. The content of nutrients found for treatments consisting of deprivation of Mg and S was not significantly different from that found for the complete nutrients solution.

Key words: Nutritional requirements, forest nutrition, Indian cedar.

INTRODUCTION

The increased demand for forest products has led to constant search for novel silvicultural techniques to increase the productivity of forest plantations worldwide. Although, Brazil has the largest area of natural tropical forest in the world, there is a successful experience in introducing species from other countries, such as species of the genera, Eucalyptus and Pinus. In addition, a new species, *Acrocarpus fraxinifolius*, has recently aroused the interest of Brazilian researchers for its rapid growth (Gonçalves et al., 2012).

A. fraxinifolius is a large deciduous emergent tree native to the family, Fabaceae, subfamily Caesalpinioideae. This has been used as shade trees in the coffee plantations in India, as well as for wood production and/or forest enrichment. Besides, this species is found to be the best-suited tree for plantations in badly degraded areas which are not protected from cattle grazing (Gonçalves et al., 2012; Martínez et al., 2006).

A review is provided of the current state of understanding of *A. fraxinifolius*, focusing on silvicultural aspects, plantations management and utilization of the specie (Mishra et al., 2015; Martínez et al., 2006). However, there is no sufficient information regarding the nutritional requirements of the species so far, which can compromise the success of projects of reforestation and recomposition of native areas (Sorreano et al., 2012; Aquino et al., 2013). Thus, considering that fertilization practices are fundamental for the production process of high quality seedlings, and to enable forest plantations to reach adequate growth stages on field (Gonçalves et al., 2012; Mishra et al., 2015), a study on fertilizing of potentially producing species is found necessary.

Nutritional requirements refer to amounts of macronutrients and micronutrients that a given crop takes from soil, fertilizer and air, to attend its requirements, to grow up and produce adequately. The amount of nutrients required is described as function of the existing contents in the plant material, as well as from the total dry matter (Faquin, 1994). Regarding forest species, studies have demonstrated that fertilizing of such species increase productivity, quality and establishment of forest plantations over time (Braga, 1995).

One of the ways used to assess nutritional requirements of plant species is by means of the Minus One Element Technique, which determines soil nutrient deficiencies in actual field or greenhouse conditions, based on the law of minimum. This technique consists of testing a complete nutrient treatment along with treatments based on individual omissions of other crop nutrients (Silva et al., 2016) and provides semiquantitative nutrients-related data that may limit plants development (Malavolta, 1980; Vieira et al., 2008; Locatelli et al., 2007; Silva et al., 2005; Matheus et al., 2011; Silva et al., 2011; Andrade and Boaretto, 2012).

The rationalization of plants nutrition management in the seedlings production process may increase the knowledge on interactions between nutrients in higher plants, thus contributing to the productivity of forest plantations (Martínez et al., 2006). When one nutrient is missing or deficient for example, such deficiency causes anomalies due to changes made to the plant metabolism (Epstein and Bloom, 2006). The purpose of this study was to assess the effect of deprivation of selected single nutrients (N, P, K, Ca, Mg and S) on biometric parameters of cedar seedlings (*A. fraxinifolius*) grown in nutritive solution.

MATERIALS AND METHODS

The trial was conducted in a greenhouse in the Forest Nursery of the Federal University of Lavras (UFLA) located in Lavras, State of Minas Gerais, Brazil, at 21°14' South, 44°00' West and 919 m elevation. Seeds of *A. fraxinifolius* were collected from mother trees at the Historical Campus of UFLA, cleaned and scarified in the Laboratory of Silviculture of the Department of Forest Sciences, as outlined in Venturin et al. (2014). Then, seeds were sown in 55 cm^3 tubes containing vermiculite substrate, and wet using deionized water.

After reaching 5 to 10 cm height, about 30 days after sowing, seedlings were washed with deionized water in bare root and transplanted to a plastic tray containing 20 L of complete nutrient solution, Hoagland and Arnon (1950). The nutrient solution was maintained under constant aeration with compressed air to maintain the air flow and oxygenate the hydroponic nutrient solution. This solution was at 30 and 60% of its ionic force and seedlings were kept for 15 days in each solution as outlined in Marques et al. (2004). After these days, which are described here as the adaptation period, each seedling was transplanted in a 5 L pot and put on a stand under constant aeration. The pH rose to about 5.5 and very little precipitation occurred. Seedlings were then fixed by means of the stem with the help of polystyrene sheets of about 2 cm thick (Silva et al., 2016).

The trial was established in completely randomized design with seven treatments, four replications, and one plant per pot. Treatments consisted of complete nutrient solution Hoagland and Arnon, and omissions of selected single nutrients, namely N, P, K, Ca, Mg and S, based on the law of minimum. Micronutrients were provided to all treatments under study.

Analytical reagent and deionized water were used to prepare nutrient stock solutions. The nutrient solution was changed biweekly, since it was used for a fast-growing forest specie, and because is characterized by high concentration of nutrients (Epstein and Bloom, 2006). Plants were daily monitored and the solution volume was completed using deionized water whenever it was necessary. Seedlings were constantly monitored to diagnose nutrient deficiency symptoms under test and the first common symptoms of each nutrient were, in general, observed on seedlings about 45 days after the transplant, except symptoms of deficiency of N that appeared 20 days earlier.

About 90 days after the transplant, shoot height (H) and root collar diameter (D) were measured. After these assessments, plants were harvested, separated into shoots and roots, and washed in running water and deionized water. Then, plants were dried in a forced air heating system (hothouse) at 65°C temperature (Sorreano et al., 2011). Thus, the plant material was weighted on a

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Table 1. Biometric parameters obtained for cedar seedlings (*Acrocarpus fraxinifolius*) grown under nutrients omission, 90 days after the transplant.

Treatment	H (cm)	D (mm)	SDW (g)	RDW (g)	TDM (g)	SDR÷RDW	QDI
Complete	87.87[a]	16.07[b]	78.27[a]	12.18[a]	90.45[a]	6.59[a]	7.56[a]
minus N	16.62[d]	3.75[d]	1.36[b]	2.59[c]	3.95[b]	0.53[b]	0.80[c]
minus P	31.75[c]	4.25[d]	5.34[b]	2.28[c]	7.63[b]	2.53[b]	0.77[c]
minus K	48.37[b]	6.37[c]	16.68[b]	2.48[c]	19.16[b]	6.85[a]	1.32[c]
minus Ca	51.50[b]	7.37[c]	10.35[b]	1.48[c]	11.83[b]	7.57[a]	0.85[c]
minus Mg	96.62[a]	14.12[b]	79.80[a]	9.06[b]	88.86[a]	9.32[a]	5.64[b]
minus S	100.42[a]	18.37[a]	80.02[a]	12.76[a]	92.78[a]	6.34[a]	7.91[a]
CV (%)	13.54	13.12	20.36	24.68	18.95	32.01	22.04

Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. H = shoot height, D = root collar diameter, SDW = shoot dry weight, RDW = root dry weight, TDM = total dry matter and DQI = Dickson quality index. CV = coefficient of variation.

0.0005 g precision scale to estimate the following biometric parameters: shoot dry weight (SDW), root dry weight (RDW) and total dry matter (TDM). The ratio given by RDW and SDW was obtained, as well as the Dickson quality index (DQI) given by the following equation (Dickson et al., 1960):

$$DQI = TDM_{(g)}/[(H_{(cm)}/D_{(mm)}) + (SDW_{(g)}/RDW_{(g)})]$$

The shoot dry matter was ground in a Wiley-type laboratory mill and then subjected to sulphuric and nitric-perchloric digestion for the estimation of the content of macronutrients as outlined in Malavolta et al. (1997). The accumulation of nutrients was obtained by multiplying the content of dry matter by the content of each nutrient found in the matter and, then, divided by 1.000, either for macronutrients (g) or micronutrients (mg). Restrictive effects of treatments consisting of individual omissions of N, P, K, Ca, Mg and S were obtained by comparing the effect of each of these treatments with the effect of the complete nutrient solution.

The Sisvar Statistical Analysis System (Ferreira, 2011) was used for statistical analysis and the Analysis of Variance was carried out to determine the significance of differences of means of biometric parameters such as H, D, SDW, RDW, TDM, RDW÷SDW and DQI. Averages were then compared by means of Scott-Knott test at 5% significance.

RESULTS AND DISCUSSION

Growth of seedlings

Treatments consisting of omissions of selected single macronutrients, namely N, P, K, Ca, Mg and S were found to be the most limiting for all morphological parameters under study in the following order: N > P > K > Ca > Mg > S. In addition, statistically significant differences between treatments were found for all variables under study (Table 1).

The treatment consisting of omission of N showed the major restrictive effect on the seedlings growth. When the N was missing, biometric parameters reduced significantly as compared to the complete nutrient solution as follows: plants height (81.1%), root collar diameter (76.6%), shoot dry weight (98.3%), root dry weight (78.7%), total dry matter (95.6%), ratio given by shoot dry weigh divided by root dry weight (91.6%), and Dickson quality index (89.4%) (Table 1). According to Souza and Fernandes (2006), this finding was expected because this nutrient is commonly required in greater amounts and is found to be the most limiting factor for crops growth. Besides, when the N is missing, the synthesis of proteins and nucleic acids is compromised, causing a reduced plants growth (Marschner, 2012). For example, studies have shown that deficiency of N causes a significant reduction of shoot and root dry weight in many crops (Silva et al., 2016; Moretti et al., 2011; Silva et al., 2011; Camacho et al., 2014; Corcioli et al., 2014).

In the same context, the treatment consisting of omission of P affected significantly the plants height (63.8%), root collar diameter (73.5%), shoot dry weight (93.2%), root dry weight (81.28%), total dry matter (91.6%), ratio given by shoot dry weigh divided by root dry weight (61.6%), and Dickson quality index (89.8%) (Table 1). In general, the deficiency of P limits the plant growth because this macronutrient is found as part of key-molecules of cell metabolism such as ATP and nucleic acids (George et al., 1995). In this study, seedlings grown under deficiency of P showed a reduction in plant size, leaves and root weigh, and long roots and few lateral roots. P plays an important role in the synthesis of energy, so that its deficiency may be reflected in reduced plant growth (Taiz and Zaiger, 1998). Similar results were also found in many other studies (Benedetti et al., 2009; Moretti et al., 2011; Vieira et al., 2016).

Regarding the deficiency of K, a significant reduction was found for the following parameters: plants height (44.9%), root collar diameter (60.4%), shoot dry weight (78.7%), root dry weight (79.6%), total dry matter (78.8%), and Dickson quality index (82.5%) (Table 1). These findings were expected since K is commonly required by plants in greater amounts (Niu et al., 2013). In addition, this nutrient is associated with osmoregulation processes, cell extension, opening and closing of stomata, activation of enzymes and synthesis of proteins (Marschner, 2012; Pettigrew, 2008). Reduction in terms of root biomass was also found in many other studies performed with different crops under deficiency of K (Silva et al., 2009; Souza et al., 2012; Camacho et al., 2014; Carlos et al., 2014).

The treatment consisting of omission of Ca showed a significant restrictive effect on the plants height (41.4%), root collar diameter (54.1%), shoot dry weight (86.7%), root dry weight (87.8%), total dry matter (86.9%) and Dickson quality index (88.7%) (Table 1). Ca is also an essential macronutrient (Funk et al., 2013) found in association with the stabilization of the cell wall (White and Broadley, 2003). Plants growing under deficiency of Ca show a reduced number of leaves, early fall of folioles, and dying of the apical bud. Besides, roots may show less development, few lateral roots, and dark coloring. The deficiency of Ca occurs commonly in the growing points of shoots and roots due to its low translocation into plants. However, regions of greater cell expansion are the most affected by the deficiency of this macronutrient (Marschner, 2012). In the same context, Mendonça et al. (1999) found that deficiencies of Ca and P drastically limited the growth of seedlings of *Myracrodruon urundeuva*.

Treatments with deficiency of selected single macronutrients Mg and S promoted greater growth of seedlings, and increased the production of shoots biomass. This finding suggests that, based on the law of minimum, these macronutrients are not limiting for the growth of cedar seedlings (*A. fraxinifolius*), because the level of plant production was still greater than that allowed for limiting factors.

Symptoms of deficiency of macronutrients

Seedlings grown in nutritive solution, with a nutrient missing, showed visual symptoms of deficiency in different moments (Figure 1). However, the first common symptoms of each macronutrient were observed about 45 days after the transplant, except symptoms of deficiency of N that appeared 25 after the transplant. Then, seedlings were kept in the greenhouse for additional 45 days after the transplant for symptoms assessment and further tests.

Nitrogen (N)

The chlorosis appeared firstly on mature leaves about 25 days after the transplant (Figure 1A). Young leaves appeared already chlorotic, with size drastically reduced. After 90 days, there was a generalized chlorosis in the leaf blade, characteristic of deficiency of N, as well as in all leaves. Similar symptoms were described by Silveira et al. (2002), on hybrids of *Eucalyptus grandis* × *Eucalyptus urophylla*.

There was an interruption in the appearing of buds over time, as well as a great investment of plants towards the root system. According to Mengel and Kirkby (1987), visible symptoms associated with deficiency of N appear as a result of metabolic disorders, because N is found in the majority of organic compounds, including amino acids and nucleic acids.

Phosphorous (P)

Deficiency of P resulted in the shorter and narrow leaf blade. The inhibition of axillary buds caused a reducing growth and branching, resulting in smaller plants (Figure 1B). The leaf blade showed a dark green coloring at first and, then, chlorotic and withered folioles. Symptoms of deficiency of P occur because this macronutrient plays an important role in the metabolism of the energy of plants such as photosynthesis and respiration (Furlani, 2004). Thus, the growth of plants under deficiency of P is retarded (Mengel and Kirkby, 1987). Similar findings were described by Sorreano et al. (2012) for seedlings of *Astronium graveolens* Jacq. and *Enterolobium contortisiliquum* (Vell).

Potassium (K)

The deficiency of K was characterized by chlorosis on leaves edges, which developed to necrosis of the whole leaf blade (Figure 1C). The size of seedlings was reduced, either for shoots or root system, and the apical dominance was lost. The pronounced necrosis of the leaf blade occurred as a result of accumulation of chemical compounds coming from metabolic disorders such as accumulation of soluble or free nitrogen compounds. These compounds can be amino acids, amides, ammonia, amines, products that result from the decarboxylation of amino acids such as putrescine and agmatine (Malavolta and Crocomo, 1982; Epstein and Bloom, 2006). The chlorosis on mature leaves followed by the reduction of apical dominance in seedlings of *Croton urucurana* Baill was also found by Sorreano et al. (2011).

Calcium (Ca)

Plants grown under deficiency of Ca showed chlorosis on edges and tips of folioles of younger leaves (Figure 1D). Severe symptoms were described 45 days after the transplant, and developed from chlorosis to necrosis on edges of folioles, resulting in deformation, withering and, then, abscission of leaves. About 120 days after the transplant, the apical bud of the majority of seedlings

Figure 1. Symptomatology of deficiency of macronutrients on cedar seedlings (*A. fraxinifolius*) grown in nutritive solution. The left side of each picture shows plants grown in complete nutrient solution, and the right side shows solution formulated with deficiency of N (A), P (B), K (C) Ca (D), Mg (E) and S (F).

died, compromising the plant growth, and resulting in smaller plants. The appearing of symptoms on edges of younger leaves and other new tissues was due to the lack of this macronutrient in plant tissues (Malavolta, 2006). A similar growth pattern was described by Silveira et al. (2002) for hybrids of eucalyptus. On the other hand, the deficiency of Ca resulted in the interruption of the production process of new roots, and rottenness of secondary roots, as was also described by Barroso et al. (2005) for seedlings of *Tectona grandis* and by Muniz and Silva (1995) for seedlings of *Aspidosperma polyneuron*.

Magnesium (Mg)

The deficiency of Mg for seedlings of *A. fraxinifolius* resulted in chlorosis in spaces between nervures of folioles of older leaves (Figure 1E). The size and number of folioles per leaf were reduced in relation to plants of the treatment consisting of the complete nutrient solution. According to Taiz and Zeiger (2013), Mg is one of the main enzymatic activators in the photosynthesis and synthesis of DNA and RNA, and is part of the structure of the molecule of chlorophyll. Thus, its deficiency promotes necrosis at the leaves apex and chloroplasts; and

Table 2. Content of macronutrients ($g.kg^{-1}$) of shoots of cedar seedlings (*A. fraxinifolius*) grown under nutrients omission, 90 days after the transplant.

Treatment	Content ($g.kg^{-1}$)*					
	N	P	K	Ca	Mg	S
Complete	23.28^{c}	1.98^{b}	5.15^{c}	6.82^{b}	1.09^{c}	0.46^{d}
minus N	11.30^{d}	7.64^{a}	10.19^{a}	13.88^{a}	2.14^{a}	0.94^{c}
minus P	32.34^{b}	1.30^{b}	9.31^{a}	8.81^{b}	1.59^{b}	1.24^{b}
minus K	31.64^{b}	7.26^{a}	2.17^{d}	7.73^{b}	1.81^{b}	1.85^{a}
minus Ca	39.24^{a}	7.55^{a}	6.91^{b}	1.56^{c}	1.94^{a}	1.59^{a}
minus Mg	26.39^{c}	2.14^{b}	6.27^{b}	6.76^{b}	0.58^{d}	0.67^{c}
minus S	25.84^{c}	1.74^{b}	5.26^{c}	6.54^{b}	0.86^{c}	0.36^{d}
CV (%)	18.58	26.78	14.65	14.37	15.99	19.94

*Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. CV = Coefficient of variation.

Table 3. Accumulation of macronutrients ($g.plant^{-1}$) of shoots of cedar seedlings (*A. fraxinifolius*) grown under nutrients omission, 90 days after the transplant.

Treatment	Accumulation ($g.plant^{-1}$)*					
	N	P	K	Ca	Mg	S
Complete	1822.1^{a}	154.9^{a}	403.1^{a}	533.8^{a}	85.3^{a}	36.0^{b}
minus N	15.4^{c}	10.4^{c}	13.8^{b}	18.8^{b}	2.9^{d}	1.3^{c}
minus P	172.7^{b}	6.9^{c}	49.7^{b}	47.0^{b}	8.5^{d}	6.6^{c}
minus K	527.7^{b}	121.1^{a}	36.2^{b}	128.9^{b}	30.2^{c}	30.8^{b}
minus Ca	406.1^{b}	78.1^{b}	71.5^{b}	16.1^{b}	20.1^{c}	16.4^{c}
minus Mg	2105.1^{a}	170.7^{a}	500.3^{a}	539.4^{a}	46.3^{b}	53.5^{a}
minus S	2067.7^{a}	139.2^{a}	420.9^{a}	523.3^{a}	68.8^{a}	28.8^{b}
CV (%)	24.14	33.52	34.82	39.99	36.87	48.25

*Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. CV = Coefficient of variation.

deformation of structures of lamellas affecting the stability of thylakoids. Symptoms of deficiency of Mg were also described by Mendonça et al. (1999) for seedlings of *Myracrodruon urundeuva*.

Sulphur (S)

Symptoms of deficiency of S were the last to appear. At first, small whitish spots appeared on leaf blades (Figure 1F). Then, these spots became chlorotic and covered almost all young and old leaves. Deficiency of S resulted in small sized leaves, with edges and tips of folioles rolled up; however, the plant size did not reduce. Similar results were found by Wallau et al. (2008) for seedlings of *Swietenia macrophylla*, and Sarcinelli et al. (2004) for seedlings of *Acacia holosericea* grown in nutritive solutions.

Concentration of macronutrients

The external concentration of ions is one of factors that affect the absorption of ions by living roots (Marschner, 2012). In this study, by limiting a given nutrient in the solution, the content of such nutrient into plant tissues reduced (Tables 2 and 3).

Contents of P, K, Ca and Mg increased in the treatment consisting of omission of N for shoots of seedlings of *A. fraxinifolius* as compared to the complete nutrient solution (Table 2). The greatest content of P occurred probably as effect of the concentration of P absorbed to the lesser dry weight produced by plants. High concentrations may be related to the reduced growth that promoted the effect of N found in the dry weight. Therefore, data suggest that N is a significantly limiting factor for the growth of seedlings of *A. fraxinifolius*.

Treatment with omission of P resulted in the minor content of nutrients found in the shoots, probably because of the lower plants growth that reduced production of dry matter, with effect in the accumulation of P (Table 2). In addition, this lesser accumulation may be associated with the fact that phosphorus is a major nutrient, meaning that it is frequently deficient for crop production and it is found in every living plant cell involved in several key plant functions, including energetic metabolism.

The omission of K increased the content of P and S in the shoot as compared to the complete nutrient solution (Table 2). A significant content of S was found in this treatment. Besides, although N and P are potential limiting factors for seedlings of *A. fraxinifolius*, for example, the content of S found in treatments formulated with N and P missing was lesser than that found in the treatment formulated with potassium missing.

The treatment formulated with Ca omission resulted in significant contents of all nutrients under study in shoots as compared to the complete nutrient solution (Table 2). In addition, there was an accumulation effect of Ca due to the low biomass production recorded in this treatment. In the same context, similar results were found in many other studies (Mendonça et al., 1999; Marques et al., 2004; Barroso et al., 2005). Thus, the existing interaction effect between Ca, Mg and K was also found in this study. The increase of the content of Ca and Mg in the shoot is, according to Malavolta (1980) Marschner (1995) and Barroso et al. (2005), related to the deficiency of K, then, favoring the absorption of Ca and Mg by plants, and vice versa.

The treatment formulated with Mg missing reduced the content of Ca in shoots of seedlings of *A. fraxinifolius* as compared to the complete nutrient solution (Table 2). The reduced content of Ca in this treatment is described as result of the existing antagonism between these two nutrients (Mg and Ca), that is, Mg has a damaging effect on the Ca availability for plants uptake, and excessive amounts of Ca reduces the uptake of cationic macronutrients such as Mg (Epstein, 1975; Malavolta et al., 1997; Mendonça et al., 1999; Barroso et al., 2005).

In the treatment formulated with S omission, a reduction in the concentration of Ca in shoots when compared with the complete nutrient solution was found (Table 2). However, this treatment was characterized by the increase of biomass in the shoot, which was described as result of the dilution effect, from which, the reduction of the content of Ca did not affect the plant growth and accumulation of dry biomass.

Regarding the accumulation of nutrients in the shoot of seedlings of *A. fraxinifolius* (Table 3), a pattern similar to that described for dry mass obtained from shoots of the same seedlings was found (Table 2). However, treatments consisting of the complete nutrient solution, minus S and minus Mg provided a greater accumulation of nutrients for the majority of nutrients under study. In addition, by limiting a given nutrient in the solution, in general, the content of such nutrient into plant tissues reduced (Table 3).

The treatment formulated with N omission showed lesser accumulation of nutrients in the shoot of seedlings of *A. fraxinifolius* as compared to the complete nutrient solution (Table 3), probably due to the low-growing effect recorded in the trial. This result shows that the content of this macronutrient in the sample was highly affected by the amount of dry weight obtained per treatment. The accumulation of nutrients depends on the content of dry weight, thus, the accumulation of N in the shoot showed a direct proportionality with the total dry matter produced per plant.

Table 3 shows that the content of nutrients found in the treatment formulated with P omission was relatively low, especially as compared to the complete nutrient solution. This treatment (minus P) resulted in lower accumulation of nutrients in shoots of seedlings of *A. fraxinifolius*, this described by the low-growing effect and a pronounced reduction in the total dry matter produced per plant.

Therefore, as these macronutrients (N, P, K, Ca, Mg and S) are found to be essential elements for plant growth, there is a pre-determined ratio of them that is required by the plant system, depending on its life cycle, environment and its genotypic characteristics. Amounts found in this study show that the ratio of these macronutrients is more critical than the actual concentration of the individual elements, and macronutrients balancing is an important indicator of a synergistic and/or antagonistic relationships between them; which determines the effective uptake and utilization of a given macronutrient by plants.

Conclusions

Formulated treatments with deficiency of selected single nutrients N, P, K and Ca limited plants growth (shoot height and root collar diameter) and accumulation of shoot dry weight of cedar seedlings (*A. fraxinifolius*) in the following order: N > P > K > Ca > Mg > S. The deficiency of these macronutrients caused visible morphological abnormalities. However, common symptoms of deficiency of N, P, K and Ca appeared before symptoms of deficiency of Mg and S. The content of nutrients found in aerial parts of seedlings of *A. fraxinifolius* grown in nutritive solution was greater in treatments consisting of deprivation of N, P and K than in treatments consisting of deprivation of Mg and S. There was no statistically significant difference between treatments consisting of deprivation of Mg and S and the complete nutrients solution.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Oil palm Fusarium wilt distribution and incidence in Southern Region of Ivory Coast

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Fusarium wilt of oil palm is caused by *Fusarium oxysporum* f. sp. *elaeidis* (Foe) and is particularly prevalent in Africa. This work shows prospect in the industrial and village oil palm plantations in the Southern region of Ivory Coast, used to assess the incidence and distribution of this disease. The method used entailed taking an inventory of all the plants in a given plot, noting the presence or absence of symptoms of the disease and then calculating the incidence in each locality. The results showed that this is the first time Fusarium is identified in some plantations in the Southwest of Côte d'Ivoire such as plantations of Sassandra and Grand Bereby. But Fusarium wilt of oil palm was not observed in Iboké Region. The disease is still present in other localities (Divo, Dabou, Anguédédou, La Mé, Eloka and Ehania). The incidence of fusarium is higher in Sassandra (5.13%) than in other areas. These results should be taken into account in future palm plant replanting projects in South-west Ivory Coast, where fusarium has emerged.

Key words: Fusarium wilt, oil palm, distribution, incidence, Southern Ivory Coast.

INTRODUCTION

The oil palm (*Elaeis guinnensis* jacq.) is the world's first oil crop. It produces 3.5 to 9 tons of oil per hectare yearly (Cochard et al., 2001; Gasselin et al., 2002a). Palm oil and palm kernel oil, derived from the drupes of this plant, are highly valued by the agro-food industry (Ataga and Van Der Vossen, 2007), with an annual output of 450,000 tons of raw palm oil. Ivory Coast is the leading exporter of palm oil in Africa, the second largest producer after Nigeria (Anonymous, 2016) and the 5th exporter in the world. In Ivory Coast, this plant is cultivated in the southern part of the forest zone from east to west. Oil palm cultivation in the country covers an area of 370 000

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hectares (Carrere, 2013) and the production of palm oil represents 3.73% of Gross Domestic Product (GDP) (Anonymous, 2016) and more than 40% of that of the West African Economic and Monetary Union (WAEMU). The Ivorian market consumes 45% of the national produced oilseeds and the remaining 55% is exported, mainly in the countries of WAEMU and the Economic Community of African States (ECOWAS), which are still largely in deficit. This oilseed deficit is estimated at about 500,000 tons in the WAEMU area and just over 1,800,000 tons within ECOWAS (Palmafrique, 2013). To fill this gap, Ivory Coast has decided to increase its annual production of palm oil.

However, one of the major constraints to the development of oil palm cultivation is Fusarium wilt. This disease can cause the death of more than 70% of oil palm trees in plantations (Cooper, 2011; Rusli et al., 2013). It is a cryptogamic disease, caused by a telluric fungus specific to oil palm, *Fusarium oxysporum* f. sp. *elaeidis* (Foe) (Ascomycetes) (Wardlaw, 1946). It was observed and studied in Dabou Savanna by Bachy and Fehling (1957) for the first time in Ivory Coast. Despite the enormous efforts to select oil palm tolerant to this disease, high-yielding Fusarium-tolerant hybrids have been obtained, but others with high production potential are susceptible to this disease (Durand-Gasselin et al., 2003; Durand-Gasselin et al., 2010) and are propagated in Côte d'Ivoire. These disease-sensitive seeds are preferred by farmers because of the high cost of Fusarium wilt tolerant seeds. Therefore, the disease has spread and is observed throughout the traditional zone of oil palm cultivation. However, the cartography of the disease dissemination has not been updated to take into account in campaigns, to raise awareness on the use of seed tolerant to the disease.

The aim of the present study is to define, from field surveys in the traditional areas of oil palm cultivation, an updated map of Fusarium wilt in Côte d'Ivoire and to assess the disease incidence level in the traditional zone of oil palm.

MATERIALS AND METHODS

Prospecting area

Nine locations are considered in this study. They are located in the traditional zone of oil palm cultivation in the South of Ivory Coast (Figure 1). This study area was divided into three main sites: First, the southeast, which contains plantations of Ehania, Eloka and La Mé; the south-center which is composed of plantations of Divo, Dabou and Anguédédou and finally, the southwest which covers the plantations of Sassandra, Grand Bereby and Iboké. The surveys were carried out in 2014 and 2016. A GPS was used to record the coordinates of each prospecting site in order to update the disease dissemination map.

The biological material consists of different plots of oil palm present in the prospected localities. For technical equipment, a GPS, survey sheets, a digital camera, a survey questionnaire developed for this study, paper, pencils, machetes, dabas, chainsaws and sickles were used.

Observation of symptoms of Fusarium wilt in plantation

For each locality, a total of 15 plots of all ages were visited. The method of prospection set up in each plot is that adopted by Diabaté et al. (2015). This method consists of making an inventory of all the plants in each plot by monitoring the presence or absence of the symptoms of the disease. The parcel area was on average between 10 and 20 hectares. Concerning the internal symptoms which are characterized by the presence of brown fibers in the infected tissues, they are identified by making a cross section in the rachis, stip or roots.

Average incidence of Fusarium wilt in different localities

The incidence of Fusarium wilt at each site was determined according to the formula of Aka et al. (2009).

$$IM(\%) = \frac{NPI}{NTPC} \quad X \quad 100$$

IM (%): Average impact; NPI: number of infected palm during prospecting period; NTPC: total number of palm trees cultivated in the plots visited.

In each plot, the number of diseased plants and the total number of plants grown were determined by simple enumeration. The incidence of the disease at the community level was calculated by averaging the total number of plots visited per locality.

Statistical analysis

An analysis of variance (ANOVA) was performed to compare the mean incidence of the disease in the localities. When a significant difference is detected ($p<0.05$), the variance is complemented by the comparison of the averages using the least significant difference (LSD) test for the formation of homogeneous groups. Statistica 7.1 software was used for the analyses. The map of the Fusarium wilt was made using ArcGis 10.0 software.

RESULTS

Observation of symptoms of Fusarium wilt in oil palm

Analysis of the results shows that Fusarium wilt is present in all localities except Iboké where it has not been observed (Figure 2). This is the first time that symptoms of this disease are observed in the Southwest of Ivory Coast, particularly in Sassandra and Grand Béréby. It remains endemic to Divo, Dabou, Anguédédou, La Mé, Eloka and Ehania.

Symptoms of Fusarium wilt in plantation

Monitoring of oil palm trees in plantations in relation to external symptoms confirmed by internal symptoms after cross-section of the rachis, stip and roots was done. The presence of these symptoms showed that the fungi attacked several oil palm trees in the same place (Figure 3) or plants distributed at several points of the plantation.

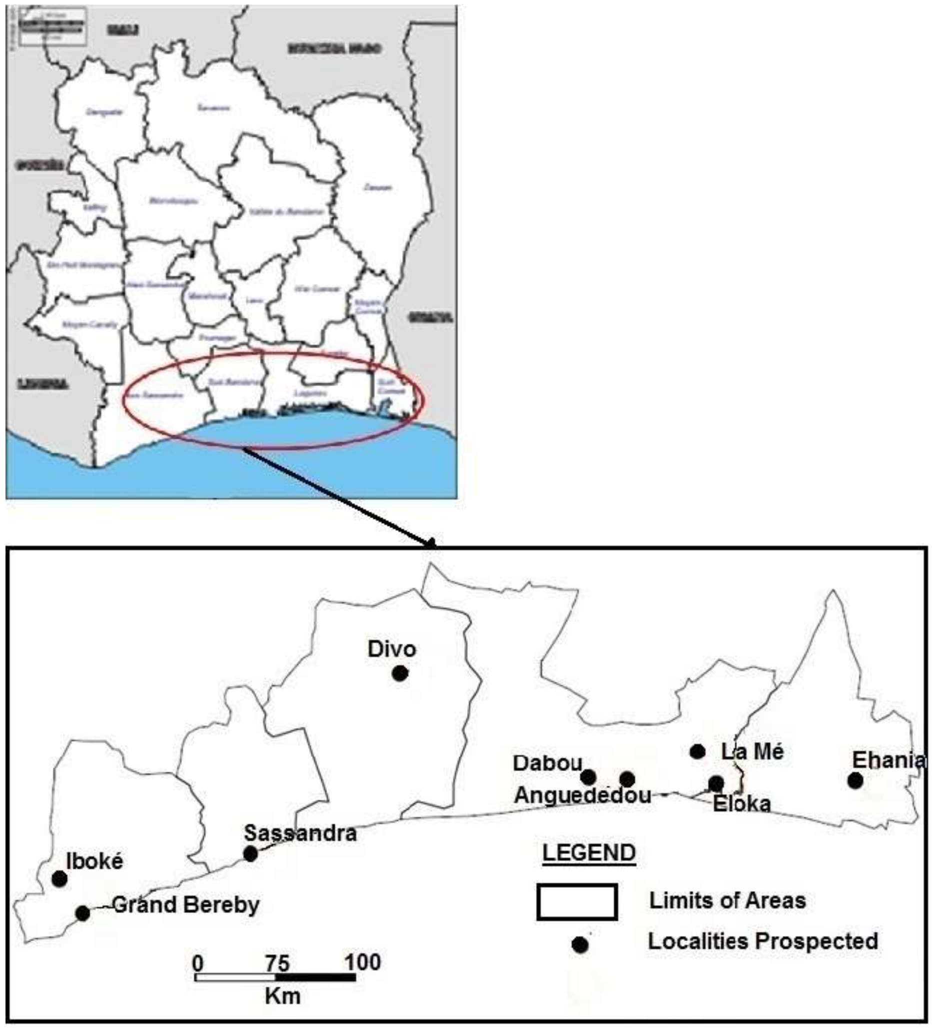

Figure 1. Study area (localities prospected).

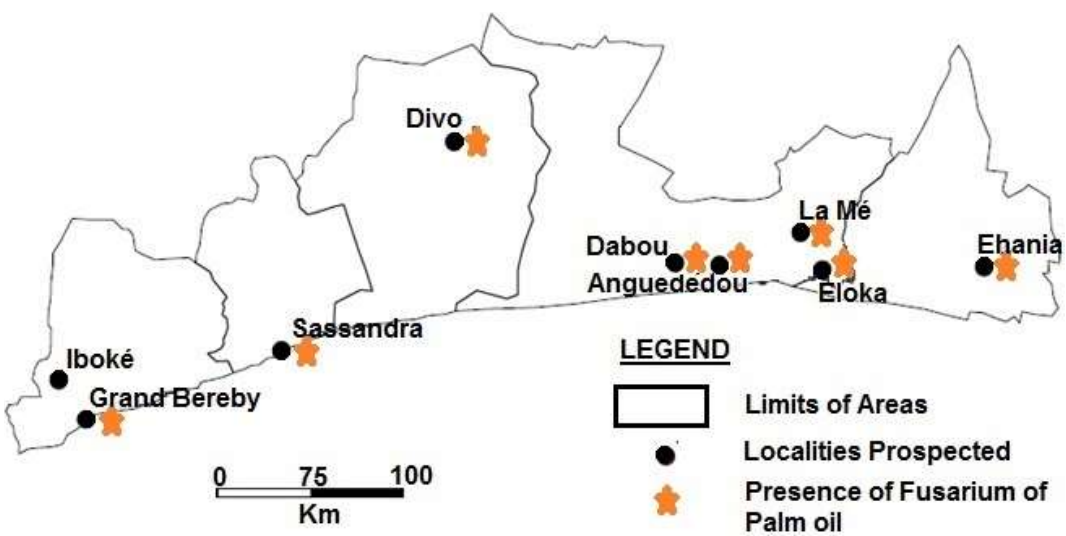

Figure 2. Distribution of oil palm Fusarium wilt in southern Ivory Coast.

Figure 3. Outbreak of Fusarium wilt in an oil palm plantation.

Figure 4. External and internal symptoms of Fusarium wilt in oil palm. A. Symptom in young culture; B: first type of related planting symptom; C: internal symptoms in the petiole; D: second type of symptom in plantation; E: internal symptoms at the level of the stipe; F: third type of planting symptom.

When palm trees are young (1 to 4 years), one or two crown leaves of some plants show yellowing and some older leaves dry out (Figure 4A). A cross-section of the petiole of the yellowed leaves shows a browning of sap conductive fibers, characteristic of the internal symptoms of Fusarium wilt.

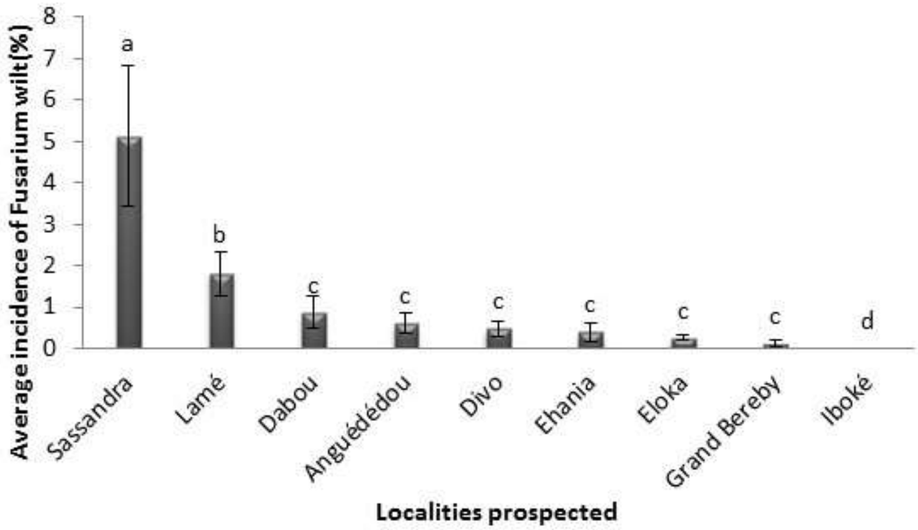

Figure 5. Average incidence of Fusarium wilt in oil palm tree in the localities surveyed.

In oil palm plantations of at least 5 years old, three types of symptoms were observed. The first types of symptoms are characterized by yellowing and drying of one or two of crown leaves.

Older leaves are dried and broken in the lower third part of the palm stem (Figure 4B). In addition, internal symptoms were observed in the petiole of the yellow leaves (Figure 4C). In the second group, some plants were completely stunted and others were medium in size but their stems were narrowed. These plants usually had green leaves with some dried out. None of these palms trees produce fruit (Figure 4D). A transverse section of the petiole did not show brown fibers in the plants. However, at the stipe level, brown fibers were observed (Figure 4E). Finally, in the third group, the crown of palm plants affected by fusarium was decoated or dried out (Figure 4F).

Incidence of Fusarium wilt in the localities surveyed

Data analysis showed that there was a significant difference (p = 0.01) between the average incidences of Fusarium wilts in the various localities (Figure 5). The highest average incidence was obtained in Sassandra area with 5.13% followed by La Mé with 1.80%. Those of the other localities, Dabou (0.87%), Anguédédou (0.60%), Divo (0.48%), Ehania (0.4 %) and Grand Bereby (0.11%) are statistically identical. On the other hand, in Iboké, the disease was not observed.

DISCUSSION

Fusarium blight of oil palm tree caused by *Fusarium oxysporum* f.sp *elaeidis* was observed in all the localities covered by this study, ranging from south-east to south-west of Ivory Coast except the locality, Iboké. For the first time, the disease was observed in the regions of Sassandra and Grand Bereby in the Southwest of Ivory Coast. These results show that the initial front that extended from the South-East (Ehania, Eloka, La Mé) to the South-Center (Anguededou, Dabou and Divo) (Mouyna et al., 1996) has reached Sassandra and Grand Bereby without reaching Iboké.

With regard to the manifestation of the disease in oil palm plantations, the infected plants presented typical and chronic symptoms and also dead plants as described by several authors (Bachy and Fehling, 1957; Renard and Ravisé, 1986; De Franqueville and Diabaté, 1995). Indeed, typical or acute symptoms are characterized by yellowing and drying of one or two crown leaves. Then, the older leaves were dried and broken in the lower third part of palm stem. As for chronic symptoms, they are characterized by stunting and shrinkage of the stipe. They generally had green leaves while some dried out. None of these palms tree produced fruit. When oil palm trees died because of the diseases, they were completely desiccated.

In the plantation, infected palms trees represent a source of infestation and are either grouped in a specific place or randomly distributed. The same observations were made by Renard and Meunier (1983) in the case of oil palm blight and Bani (2011) in the case of date palm blight.

The mean incidence of the disease in the different localities was estimated from the characteristic symptoms of Fusarium wilt in the oil palm. It varies from 0.0 to 5.13%. The highest average incidence was observed in Sassandra (5.13%), followed by La Mé with 1.8%. The other localities (Dabou (0.87%), Anguédédou (0.60%), Divo (0.48%), Ehania (0.40%), Eloka (0.26%) and Grand Bereby (0.11%) have low incidence and there is no significance difference in mean incidence of the disease. Concerning Iboké, currently, the disease is not observed in this locality. In the case of Sassandra, the high incidence of the disease could be explained by the fact that farmers have not yet adopted non-tolerant plant materials in the cropping systems. The same observations were made in the region of Grand Bereby where for the first time, the disease was observed during the surveys of 2016. Concerning La Mé Region, the incidence remained high, unlike other endemic areas. In fact, in this locality, most of the data monitoring was done in the plots used for the tests of introduction of the second cycle of selection of seeds from Nigeria and Cameroon. The other localities, such as Divo, Dabou, Anguédédou, Eloka and Ehania are recognized as endemic to Fusarium wilt. Therefore, Fusarium wilt tolerant seed is routinely used for replanting in these areas. As a result, the average incidence of the disease in these localities demonstrates that Fusarium wilt exists, but it is declining over the years (De Franqueville and Renard, 1990; De Franqueville and Diabaté, 1995; Allou et al., 2001).

However, it should be noted that in most village plantations of the visited localities, improvement of cropping system is not adopted. These observations are corroborated by studies done by Naï et al. (2000) and Akindes and Kouamé (2001). In fact, the surveys by these authors on farmers in the southern regions of Côte d'Ivoire showed that in Dabou and Anguédédou, almost 75% of farmers do not use improved seeds for their plantations and replanting. Thus, these plantations made up of a mixture of different categories of oil palms trees (pisifera, dura and tenera) do not guarantee good production.

Conclusion

The objective of this study is to update the map of Fusarium wilt in Ivory Coast and to assess the extent of the disease in the traditional areas of oil palm cultivation. The results show that this disease is observed in all the Southern region of Ivory Coast both in the traditional zone of the disease and new zone such as Sassandra and Grand Bereby in the southwest, except Iboké region. Concerning the incidence of the disease, it is higher in Sassandra despite the existence of endemic areas where the trend is declining. As a recommendation, the oil palm sector should carry out awareness-raising campaigns among farmers on the importance of the use of selected seeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Phosphorus, copper and zinc leached in lysimeters with red-yellow latosol subjected to different rates of reused swine water and irrigation water

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This study evaluates some micro- elements, phosphorus, copper and zinc leached in lysimeters with red-yellow Latosol under different rates of reused water and irrigation water. This was done for a period of 40 days (August to October 2014) at the initial cycle of cauliflower cultivar 'Verona CMS', in Sinop/Mato Grosso. Reused swine water (0, 50 and 150 m^3 ha^{-1}) was applied in one portion before transplanting. Irrigation water of 100, 125 and 150% with crop evapotranspiration potential (ETc) was used in a drip irrigation system daily. Leachate samples were taken at 10, 20, 30 and 40 days after applying the reused water. With balanced leached phosphorus (P), a higher percentage of P retained in the soil was observed, indicating low mobility of this element. The concentrations of copper in the leachate were low at 150 m^3 ha^{-1} rate after the reuse of swine water, at day 20. 150 m^3 ha^{-1} used in the irrigated lysimeters with 100% ETc is a good alternative for vegetable crops with a short cycle (less than 40 days), in Sinop/Mato Grosso, as it does not exceed the limits of the Conama Resolution No. 396/2008.

Key words: Cauliflower, evapotranspiration potential, lysimeters, irrigation.

INTRODUCTION

The use of swine wastewater in agriculture is an alternative source of nutrients and organic matter (Bertol et al., 2010); it grows combined with available elements such as phosphorus (P), copper (Cu) and zinc (Zn) (Smanhotto et al., 2010). Although these elements are essential for the growth and development of plants, when applied in excess, they can become sources of surface and groundwater pollution.

Phosphorus losses can occur via soil surface and subsurface, being potentially greater in sandy soils when subjected to high mineral or organic fertilizers. Another problem associated with P is the eutrophication of surface water that results from superficial runoff and leaching. When leaching occurs in groundwater it may

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affect surface waters (rivers and lakes) due to percolation.

However, the soil has a high phosphate adsorption capacity, and its mobility is lower compared to other nutrients; in some cases, phosphorus loss by percolation in croplands is considered insignificant. Bertoli et al. (2010) showed that phosphorus can be lost with greater sensitivity under reused swine water compared to fertilization with NPK formulation, thus demonstrating that the transport of P is higher under organic sources.

According to Campos (2010), the movement of Cu and Zn in the soil profile depends on the physical and chemical properties of each type of soil and the physicochemical properties of the metal ion. Cu and Zn metals have low mobility in soil profiles and therefore tend to accumulate on the ground surface, reducing its leaching potential. However, excessive use of wastewater can cause losses in the subsurface (Sistani et al., 2008). Campos (2010) emphasizes that pH variation, biological processes, and chemical toxicity of the element and environment also play a fundamental role in the availability and mobility of these metals.

According to Rieuwerts et al. (2006), pH has an influence on cationic metal ions, as these ions are more mobile in acid pH conditions. However, in pH higher than six, these would possibly lead to the dissociation of H^+ of OH groups of the organic matter and Fe and Al oxides. This would thus increase the absorption of the metals with subsequent precipitation, resulting in the reduction of its bioavailabilities.

In this sense, this study aims to evaluate phosphorus, copper and zinc transported in lysimeters with red-yellow Latosol, after the application of swine reused water and irrigation water at different rates.

MATERIALS AND METHODS

Experiment location

The research was conducted at the Federal University of Mato Grosso, Sinop University Campus, located in 11°51'S and 55°29'W, from August to October 2014.

Precipitation occurred during the experiment. The soil of the experimental area is classified as red-yellow Latosol. By the Köppen climate classification, the prevailing climate of the North Central region is Aw (hot and humid tropical). It is characterized by the presence of two well defined seasons: rainy (from October to April) and dry (from May to September); with low annual temperature range (between 24 and 27°C) and average annual rainfall of 1974 mm (Souza et al., 2013).

Lysimeters

For the leaching study, twenty-seven lysimeters were built, and arranged in plate. The distance between each lysimeter was 0.50 m; they were inserted in trenches of approximately 1.20 m depth and 0.30 m diameter (Figure 1). The structure of the lysimeters was hard PVC filled with soil, keeping the same sequence as in the original profile. The chemical and physical analysis of the soils was performed at two depths (0 to 20 cm and 20 to 40 cm) for subsequent filling of the lysimeters.

To fill the lysimeters, an isolated trench was opened at approximately 1.0 m depth; undisturbed samples were taken from every trench at a distance of 10 cm to determine the soil density. The soil profile in the lysimeter was rebuilt to keep the densities of the respective layers. The lysimeter was provided with a collection system (funnel and bottle) positioned at the bottom of the trench, and wherein leachate was stored for later collection.

Chemical and physical soil analysis

In the chemical analysis performed at soil layer of 0 to 20 cm, 2.46 to 32.00 mg dm^{-3} was determined for phosphorus and potassium, and 2.03 and 1.72 cmol dm^{-3} for calcium and magnesium, respectively. The micronutrients, zinc and copper concentrations were 3.90 and 0.59 mg dm^{-3}, respectively; the concentration of aluminum was zero, the pH of H_2O was 5.4; cation exchange capacity (CEC - pH7.0) was 6.98 cmol dm^{-3} and organic matter content was 38.22 g dm^{-3}; for the textural analysis, 462, 250 and 288 g dm^{-3} was identified for the clay, silt and sand, respectively.

At 20 to 40 cm layer, for the same aforementioned variables, 4.61 and 55.00 mg dm^{-3} was obtained; 3.75 and 1.30 cmol dm^{-3}, 85 and 0.84 mg dm^{-3} was obtained for P, K, Ca, Mg, Cu and Zn, respectively; pH (H_2O) was 5.9; cation exchange capacity (CEC - pH7.0) was 8.01 cmol dm^{-3} and organic matter content was 43.00 g dm^{-3}; for the physical analysis of the clay, silt, and sand contents, 483, 167 and 350 g dm^{-3} respectively was obtained.

Chemical and physical characterization of reused water

Swine reused water was collected from a farm in the municipality of Vera/Mato Grosso, after treating with biodigesters.

The chemical and physical characteristics of the waste (reused water) were determined. The following was obtained: 6.85 pH, 4.970 NTU turbidity, electrical conductivity of 1.1 S m^{-1}, total dissolved solids concentration of 7.0 g L^{-1}, biochemical oxygen demand (BOD) of 283.3, total Kjeldahl nitrogen (TKN) of 308.7, nitrite (NO_2^-) of 154.7, nitrate (NO_3^-) of 811.36, total phosphorus (P) of 150.29, zinc (Zn) of 35,90 and copper concentration (Cu) of 10.88 mg L^{-1}.

Implementation of cultivation

After the chemical and physical soil characterization, the supplement chemical fertilizer was calculated following the technical recommendations of Zanuzo et al. (2013) for cauliflower cv. Verona.

In this context, the fertilizer used for cultivation corresponded with 10 g of urea, 15 g of potassium chloride, 20 g of simple superphosphate and 12.5 g of dolomite lime added to the surface of each of the lysimeter before transplanting the seedlings. Transplanting of cauliflower seedlings was performed in (*Brassica oleracea* L.) Verona CMS variety, manually in each lysimeter, in 08/03/2014. The spacing was set at 0.50 x 0.50 m (between plants and rows).

After transplanting, drip irrigation system was installed. Daily irrigation was done for 40 days by using dripped polyethylene hose with 25 cm space between emitters, outflow rate of 7.5 L h^{-1} m^{-1} and 10 mwc working pressure.

Reused water and water irrigation rates

The reused water was applied once on the surface of the lysimeters, before transplanting the seedlings at three application rates of 0, 50 and 150 m^3 ha^{-1} yr^{-1}. The percentage rates of water

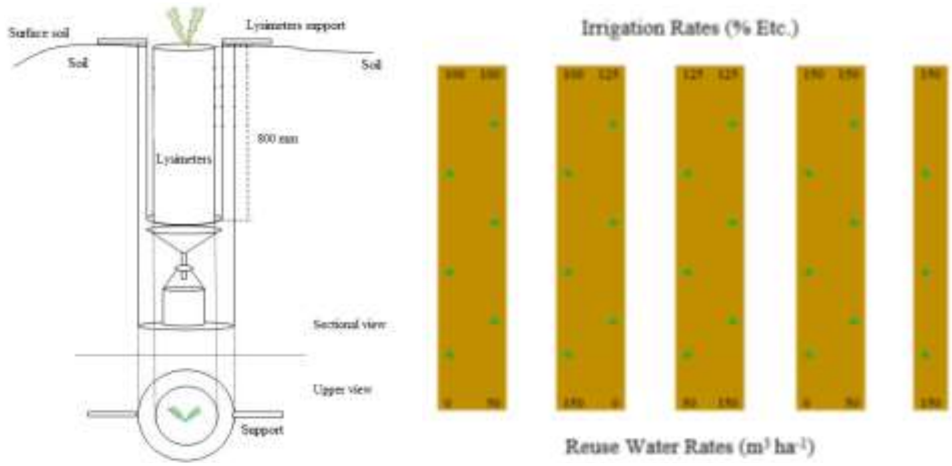

Figure 1. Construction scheme of the lysimeters and disposal in plat with a variation of water irrigation rates and reuse water rates.

used for irrigation were 100, 125 and 150% of crop evapotranspiration (ETc), obtained from Equation 2.

The rates were determined according to the daily reference evapotranspiration (ET_0), obtained by the method of Class A Tank. It considers the product between the evaporation of Class A Tank (ECA) and the tank coefficient (Kp), depending on the tank type, weather conditions and its location. With an average value, Kp (0.7795) was estimated for the municipality of Sinop/Mato Gross. The value of the crop coefficient Kc (0.65) was used in the ETc calculation.

$$ET_0 = ECA * Kp \quad (1)$$

$$ET_c = ET_0 * Kc \quad (2)$$

Where, ET_0 is the daily reference evapotranspiration (L m^{-2}); ETc is the evapotranspiration of daily culture (L m^{-2}); ECA is the evaporation of the daily class A tank (L m^2); Kp is the coefficient of the tank; Kc is the crop coefficient depending on the development stage.

Collection and samples analysis

Four leachate collections were done (10, 20, 30 and 40 days after application of the wastewater) with the experiment. The leached elements evaluated were P, Cu, Zn ions and H^+ concentration, and the volume of water applied and collected was monitored. The analyses were carried out in the waste treatment and integrated laboratory for research in chemical sciences, following the methodology described in standard Methods of Water and Wastewater (APHA, 2012).

Evaluation and statistical analysis of data

The experimental design is a randomized block subdivided into a factorial plot of 3 x 3 x 4 (application rates x irrigation water rates x collection times), with three repetitions. The results obtained were statistically evaluated and submitted to analysis of variance and F test; the means were compared by the Scott Knott test at 5% significance. The statistical package used was Sisvar 5.5 Build 82.

RESULTS AND DISCUSSION

Volume of water applied and collected

The highest percentage of ETc generated higher volumes of water used for irrigation, regardless of the season; total volume of 179.73, 224.67, 269.61 L of water led to increments of 0.25 and 50% ETc. However, there was a tendency to reduce the volume applied daily throughout the experimental time for all slides due to the reduction of ET_0 (Table 1).

This research was conducted in an area with annual rainfalls of 2,000 mm yr^{-1}, in seven months, from October to April (Souza et al., 2013). Thus, using irrigation water rates (125 and 150%) higher than the 100% ETc is fundamental for the generation of leachate and understanding the movement of P, Cu and Zn, in periods of rainfalls higher than the demand for cauliflower.

As for the volume of water collected, the data showed a significant interaction between the irrigation rate and time, and also between water reuse rate and irrigation rate. In Table 2, it was observed that, the collection volume was greater with higher supply of water for irrigation. In all the collection times, there was a reduction in the volume collected at 125 and 150% ETc rates. This reduction is caused by irrigation since excess water promotes translocation of solid particles of soil (mainly clays), which in turn favors the process of soil storage within the lysimeters, reducing its permeability over time. However, even with the replacement of 100% ETc, the formation of leachate lysimeters was observed.

According to Barros et al. (2009), the determination of the reference evapotranspiration (ET_0) by Class A tank provides overestimation even when Kp is regionally calibrated. The total volume collected at 100, 125 and 150 ETc was 8.86, 10.72 and 12.72% of the applied volume, respectively.

Table 1. Volume of irrigation in liters subjected to different water irrigation rates and collection times.

Time (days)	Water irrigation rates (% ETc)*		
	100	125	150
10	59.85 ^{Ca}	74.81^{Ba}	89.78^{Aa}
20	55.18 ^{Cb}	68.98^{Bb}	82.77^{Ab}
30	30.91 ^{Cd}	38.64^{Bd}	46.37^{Ad}
40	33.79 ^{Cc}	42.24^{Bc}	50.69^{Ac}
Total (L)	179.73	224.67	269.61

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability.

Table 2. The volume of collected water in liters, subjected to different water irrigation rates and collection times.

Time (days)	Water irrigation rates (% ETc)*		
	100	125	150
10	5.14^{Ca}	8.19^{Ba}	11.96^{Aa}
20	3.61^{Ca}	5.87^{Bb}	8.51^{Ab}
30	3.50^{Ba}	4.89^{Bb}	6.89^{Ac}
40	3.68^{Ba}	5.14^{Bb}	6.94^{Ac}
Total (L)	15.93	24.09	34.30

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability.

Table 3. The volume of water collected in liters, submitted to different water irrigation rates and water reuse application rates.

Water irrigation rates (% ETc)	Reuse water rates (m^3 ha^{-1}) *			
	0	50	150	Total (L)
100	3.80 ^{Ac}	4.10 ^{Ac}	4.10 ^{Ab}	12.00
125	5.56 ^{Ab}	6.06 ^{Ab}	6.45 ^{Aa}	18.07
150	10.34 ^{Aa}	8.59 ^{Ba}	6.79 ^{Ca}	25.72

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability.

Table 3 shows the interactions of irrigation water rates and reused water rates, observing that the leached volume increased with an increase rate, regardless of the wastewater percentages applied. In the highest irrigated rate without wastewater, higher leached volumes occurred (10.34 L). The variation of the rates was significant only at 150%. In this case, there was a reduction in the volume collected with increased rates. The total collected volume was also higher for the greater rate (25.72 L).

The difference between the volume of water applied and collected indicates that the rest of the remaining water content was required by the atmosphere for evaporation and/or was stored in the soil pores.

pH

The pH of the samples was assessed by the concentration of H^+ ions. The transport of ions showed a significant interaction for rate x time and residual water rate x rate. The transport of H^+ ions was significant only for 40 days after the application of wastewaters; it was the highest concentration observed at 150% ETc rate (Table 4).

The pH is acidic throughout the experimental period, although it has been observed that the unfolding time results were only significant at 150% ETc rate. In this case, the mineralization of organic matter and nitrogen, as well as the reduction of soil CEC can cause

Table 4. Concentration of ion H^+ (L^{-1} mg) and pH values (in parentheses) submitted to different water irrigation rates and collection times.

Time (days)	Water irrigation rates (% ETc)*		
	100	**125**	**150**
10	0.56×10^{-06} Aa (6.45)	0.84×10^{-06} Aa (6.21)	0.61×10^{-06} Ac (6.38)
20	0.37×10^{-06} Aa (6.56)	0.42×10^{-06} Aa (6.47)	0.22×10^{-06} Ac (6.75)
30	0.75×10^{-06} Aa (6.20)	1.58×10^{-06} Aa (5.93)	1.91×10^{-06} Ab (5.96)
40	1.08×10^{-06} Ba (6.02)	1.42×10^{-06} Ba (5.92)	3.81×10^{-06} Aa (5.68)

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability. Note: pH values were transformed into H^+ ions using $[H^+] = 10$^(-pH).

Table 5. Concentration of H^+ ions (mg L^{-1}) and pH values (in parentheses) submitted to different water irrigation rates and reuse water application rate.

Water irrigation rates (% ETc)	Reuse water rates (m^3 ha^{-1})*		
	0	**50**	**150**
100	0.81×10^{-06} Ab (6.25)	0.78×10^{-06} Aa (6.18)	4.64×10^{-07} Aa (6.50)
125	0.67×10^{-06} Ab (6.31)	1.70×10^{-06} Aa (5.90)	8.21×10^{-07} Aa (6.19)
150	2.66×10^{-06} Aa (6.06)	1.49×10^{-06} Ba (6.26)	7.57×10^{-07} Ba (6.40)

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability. Note: pH values were transformed into H^+ ions using $[H^+] = 10$^(-pH).

solubilization of H^+ ions, causing its increase in leachate and reducing the pH over time. At 40 days, after the reuse of swine water it was observed that at 125 and 150% there was a higher concentration of H^+ ions.

Table 5 shows the data of the interaction rate x rate, corroborating the fact that the transport of H^+ ions was significant only at 150%, with higher and lower concentrations in the lysimeters that did not receive wastewater and with the highest rates, respectively (2.66×10^{-6} pH value 6.06; 1.49×10^{-6} pH value 6.26, and 7.57×10^{-6} pH value 6.40). The evaluation of the H^+ ion transport in the rate x rate interaction indicates it was significant only in the lysimeters withot waste water, which increased with increased rates.

Soil acids interfere with the proper development of roots; being necessary to carry out liming for acidity correction. Thus, acid in soils can be a concern considering that productivity can be affected. In this sense, the rate that provided higher transport of ions out of the zone of the root system of the plant was 150% ETc, observed in 0 rate, with a concentration of H^+ ions of 2.66×10^{-6}. Therefore, 50 and 150 m^3 h^{-1} rates were similar to the effect observed in this interaction with 150% ETc.

Phosphorus

Table 6 shows that phosphorus leached in irrigation water at days 10 and 30, after application of wastewater did not differ statistically. However, P leached significantly increased after 20 days with increased irrigation water percent. At day 40, this behavior differed from other samplings since there was an increase in the leaching of P between 100 and 125% ETc, with a further reduction from 125 to 150% ETc.

P mobility in soil is very low, thus justifying the fact that the losses caused by leaching in arable soils are considered insignificant. The available phosphorus content usually tends to decrease with depth, following the content of soil organic matter. P applied at concentration exceeding the culture of demand can lead to leaching of this element in soil profile.

According to Maggi et al. (2011), evaluating the leachate impacts on drainage lysimeters at different times of collection, under different swine wastewater rates during the soy crop cycle observed quadratic regression models for phosphorus concentrations in the leachate over time. This approach confirms the data found in this work, which despite not having adjusted regression models showed that the P concentrations in the leachate increased and soon after decreased.

At 100 and 125% ETc, after the application of wastewaters at day 20, there were lower concentrations of P in the leachate over 150% ETc. This result indicates that P concentrations subjected with diiferent rates were very heterogeneous with respect to the various collection times.

Table 7 shows the total average concentrations of the phosphorus obtained from the reused water and collected

Table 6. Concentration of P (mg L^{-1}) submitted to different water irrigation rates and collection times.

Time (days)	Water irrigation rates (% ETc)*		
	100	125	150
10	0.12^{Ab}	0.10^{Ac}	0.28^{Ab}
20	1.30^{Ba}	1.57^{Ba}	2.32^{Aa}
30	0.30^{Ab}	0.31^{Ac}	0.42^{Ab}
40	0.08^{Bb}	0.96^{Ab}	0.01^{Bb}

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability.

Table 7. Total P average concentration applied and leached (in 4 collections) and the average nutrient retained in the soil.

Water irrigation rates (% ETc)	Reuse water rates (m^3 ha^{-1})	P apllied (mg)	P leached (mg)	P in soil (mg)
L100	T0	0.00	1.82	-
	T50	13.53	1.68	11.85
	T150	40.58	2.44	38.14
L125	T0	0.00	3.89	-
	T50	13.53	3.58	9.95
	T150	40.58	5.87	34.71
L150	T0	0.00	8.37	-
	T50	13.53	6.96	6.57
	T150	40.58	4.48	36.10

Li: water irrigation rate; Ti: reuse water rates; P: phosphorus.

in lysimeters and average nutrient retained in the soil. From the results, a higher concentration of P was leached at 150% ETc; at 0 m^3 h^{-1} rate, there was a concentration of 8.37 mg L^{-1} and at 100 and 125% ETc, there was an increase of P rates in the leachate. At 150% ETc, a decrease was observed in P leachate concentration due to increased concentration of P.

The P concentration in the leachate was lower than the concentration applied, at a rate of 50 to 150 m^3 h^{-1} for the three evaluated rates. In the treatment without wastewater, P concentrations in the leachate were observed at the three irrigation water rates, resulting from leaching of existing sources in soil and additional chemical fertilization performed with superphosphate in the experiment.

The balanced P applied and leached in lysimeters showed that most of the nutrient was retained in the soil and, therefore, available for the culture and various irrigation water rates L2 to L3. This led to a reduction of the P leached only in T150, ranging from 5.87 to 4.48 mg, respectively. The results found by Chahal et al. (2011) corroborate with this research since phosphorus and potassium concentrations were observed in the leachate, and are lower than the wastewater concentrations used.

Copper

Table 8 shows Cu concentration in the leachate under various wastewater rates and collection times. Changes were observed in Cu concentration in the leachate only at 20 and 40 days after the application of wastewater. The mobility of Cu in this study was much reduced because the leachable concentrations did not exceed 0.044 mg L^{-1}, indicating that part of the metal applied by the effluent was retained in the soil particles inside the lysimeters and/or was absorbed by the used culture.

The copper concentrations obtained were lower as observed by Barros et al. (2003), in which swine effluents subjected to integrated treatment under soil deformed columns were applied. From their study, a leached maximum concentration of Cu with value around 0.06 mg L^{-1} was obtained. Messias et al. (2007) observed low movement of Fe, Zn and Cu with sewage sludge contents, and Cu concentrations observed in soil without wastewater proved to be uniform in depth. However, in the soils containing swine waste, the authors observed higher metal concentrations in surface layers (0 to 5.0 cm). According to Oliveira and Mattiazzo (2001), low movement may be related to mechanisms of

Table 8. Concentration of Cu (mg L^{-1}) submitted under different reuse water rates and collection times.

Time (days)	Reuse water rates (m^3 ha^{-1})*		
	0	50	150
10	0.012 ^{Ab}	0.013^{Ac}	0.014^{Ab}
20	0.015 ^{Bb}	0.028^{Ab}	0.021^{Bb}
30	0.015 ^{Ab}	0.017^{Ac}	0.012^{Ab}
40	0.031 ^{Ba}	0.039^{Aa}	0.044^{Aa}

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability.

Table 9. Zn concentration (mg L^{-1}) submitted to different water rates reuse and water irrigation rates.

Water irrigation rates (% ETc)	Reuse water rates (m^3 ha^{-1})*		
	0	50	150
100	0.139 ^{Bb}	0.322 ^{Aa}	0.228 ^{Bb}
125	0.201 ^{Bb}	0.306 ^{Aa}	0.376 ^{Aa}
150	0.486 ^{Aa}	0.385 ^{Ba}	0.349 ^{Ba}

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability.

adsorption/desorption, precipitation/dissolution, complexation, and redox.

The collection done at day 20 showed an increased concentration of the element at 50 m^3 ha^{-1}, while at day 40, the highest Cu concentrations occurred in soils containing wastewater. There was a noted trend of increased Cu concentration in all the three evaluated rates over time. In this case, the presence of Cu metal regardless of the rate was increased by fertilization cultivation.

According to Messias et al. (2007), from the evaluation of iron, copper, zinc and cadmium movement in soil treated with sewage sludge, higher sludge dose increased the leachates elements concentration, except for copper. In short, the lower Cu concentrations found in the leachate were observed at 0 rates in all collection times. At day 20, there were no differences between the rates of 0 and 150 m3 ha^{-1}, being characterized as the period and rates that provided lower losses of Cu leaching. With observed low concentration of Cu under the experimental conditions and considering that the use of waste can occur for decades, it is necessary to monitor groundwater to ensure metals do not concentrate on the water resource.

Zinc

The zinc concentration showed an increased changes with increasing irrigation rate at 0 and 150 m^3 h^{-1} (Table 9), ranging from 0.139 to 0.486 mg L^{-1} and 0.228 to 0.349 mg L^{-1}, respectively.

The variation of reused water rates had a significant effect on metal leaching in any of the observed rates, and control plots. Zn leaching was also observed, which might be due to fertilizer incorporated in the soil. Since the plots did not receive an effluent and have a lower organic matter content compared to other lysimeters, the lowest content of organic matter may have provided greater leaching of Zn. The presence of organic matter provides negative charges to the ground, and this in turn acts to maintain the positively charged elements adsorbed (as Cu and Zn), which consequently reduces mobility in the soil profile.

The affinity of metals with soil varies according to the type, amount of organic matter, cation exchange capacity (CEC), pH, clay quantity, mineralogy, and other characteristics; and it is also influenced by characteristics of metals. According to Paganini et al. (2004), a large amount of Zn can be fixed in the organic fraction of the soil, and may be temporarily fixed in microorganisms after the addition of organic matter in the soil. In this context, it is evident that treatment with a replacement of 100% ETc, at 0 rates and 150 m^3 ha^{-1} led to lower losses of Zn in the cauliflower.

Table 10 shows that the increase in reused water rates applied to the lysimeters was significant only for 40 days, with an increase in Zn losses in the lysimeters under effluent application. This behavior can be explained based on pH (Table 4), while Campos (2010) states that

Table 10. Zn Concentration (mg L^{-1}) submitted to different water reuse rates and collection times.

Time (days)	Reuse water rates (m^3 ha^{-1})*		
	0	50	150
10	0.201^{Ac}	0.213^{Ac}	0.183^{Ac}
20	0.217^{Ac}	0.268^{Ac}	0.279^{Ab}
30	0.402^{Aa}	0.349^{Ab}	0.397^{Aa}
40	0.280^{Bb}	0.521^{Aa}	0.412^{Aa}

*Means followed by the same lowercase letter in the columns and capitals in rows do not differ by the Scott Knott test at 5% probability.

low pH values favor lixiviation and availability of metals in the soil. Increase in pH increases the CEC which allows the formation of chelates from the organic material, decreasing its mobility in soil.

According to Messias et al. (2007), evaluating the mobility of micronutrients there were no observed variations in the leaching of Cu and Zn over time (60 days), even for soils with a higher concentration of sewage sludge (75 mg h^{-1}). In this sense, the rate of 150 m3 ha^{-1} reused water was a good choice for nutrient supply of cauliflower until 30 days. The data evaluated at 40 days showed higher Zn concentrations compared to other evaluated periods. It is noteworthy that the continued application of reused water in the soil as a source of nutrients for crops can cause groundwater contamination, due mainly to changes in the concentrations of Cu and Zn based on the provisions of Conama resolution No. 396/2008 (maximum values of 2.0 and 5.0 mg L^{-1} for copper and zinc, respectively).

Conclusion

The volume of leaching increased with increasing amount of water supplied by irrigation; however, it decreased with increased swine wastewater rates. At 100 and 125% ETc, there were higher concentrations of H^+ ions at day 40, after the application of swine wastewater.

P leached concentrations were higher at day 20 after the wastewater application, regardless of the irrigation water rates. The smaller Cu concentrations found in the leachate were observed at 150 m^3 ha^{-1} rates at 20 days after application of reused water.

The application of 150 m^3 ha^{-1} and replacement of 100% ETc can be indicated as a good alternative for short cycled vegetables (less than 40 days), as the concentrations of Cu and Zn did not exceed the limits of Conama resolution No. 396/2008, at this interval, for red-yellow Latosol.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Detection and identification of bacterial soft rot of potato *Pectobacterium carotovorum* subsp. *carotovorum* using specific PCR primers in Jordan

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Potato soft rot is one of the major destructive diseases affecting potato plants throughout the world. In a survey of different potato growing seasons in different regions of Jordan, samples of rotten potato tubers were collected and 131 isolates identified biochemically as *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*). The PCR primer pair (EXPCCR/EXPCCF) was used to detect these Jordanian isolates. The primer set amplified a single fragment of 550 bp in size from the total genomic DNA, which was extracted independently from 67 *Pcc* strains. In a nested PCR, the primer set (INPCCR/INPCCF) amplified the expected single fragment of 400 bp from the PCR product of first PCR amplification. The use of these primers was not reliable in detecting all isolates identified biochemically as *Pcc*. Different rots causal agents were detected by PCR amplification and further sequenced. The sequencing data revealed similarities to different genera; *Pseudomonas*, Enterobacteriaceae genera such as *Enterobacter* spp., *Serratia* spp. and *Klebsiella* spp., in addition to *P. carotovorum* subsp. *carotovorum*. So far this is the first study where *Pcc* has been identified by using PCR and sequencing approaches in Jordan.

Key words: *Pectobacterium carotovorum* subsp. *carotovorum*, specific primers, nested PCR, sequencing.

INTRODUCTION

Different bacterial diseases have been reported to attack potatoes around the world leading to high economic losses in yield and quality under favorable environmental conditions; of these are brown rot (*Ralstonia solanacearum*), common scab (*Streptomyces scabies*), ring rot (*Clavibacter michiganensis* subsp. *sepodonicus*), black leg (*Pectobacterium carotovorum* subsp. *atrosepticum*) and soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*).

However, potato soft rot is one of the most important diseases of potatoes; it causes a great reduction in yield resulting in economic losses in field and during transit. Hence, it is reportedly caused by various species like *Bacillus*, *Pseudomonas*, *Enterobacter cloacae* and

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Erwinia (Liao and Wells, 1987; Bishop and Davis, 1990; Agrios, 2005; Schroeder et al., 2009). However, *Erwinia carotovora* subsp. *carotovora* is reported as the most common causal agent of bacterial soft rot of potato and other commercially important crops (Perombelon, 2002; Agrios, 2005; Monilola and Abiola, 2011).

Bacterial soft rot is one of the destructive diseases prevalent in subtropical regions. It occurs worldwide wherever fleshy and stored vegetables are present (Bhat et al., 2010b). Soft rot disease causes greater total losses of produce than any other bacterial disease, hence it is considered among the factors contributing to yield losses, and among the most prevalent and destructive bacterial diseases that affect vegetables which is difficult to control (Perombelon and Kelman, 1980; Bhat et al., 2010b).

Soft rot disease caused by *P. carotovorum* subsp. *carotovorum* (*Pcc*) (Dye, 1969) is listed among the top ten plant pathogenic bacteria (Mansfield et al., 2012) with a wide host range in tropical and subtropical regions infecting vegetable species belonging to different major vegetable families including cabbage, cauliflower, lettuce, onion, pepper, carrot and potato (Rajeh and Khlaif, 2000; Bhat et al., 2010a).

In the last 30 years, Polymerase Chain Reaction (PCR) has been used for specific and rapid method for detection and identification of pathogen (Czajkowski et al., 2009). PCR technique greatly enhances detection sensitivity compared with other methods of detection (Toth et al., 1999; Kang et al., 2003; Czajkowski et al., 2009).

In Jordan, *Pcc* was identified as the causal agent of soft rot disease of vegetables, whose detection and identification was carried out through traditional techniques such as isolation on selective media and biochemical characterization. The pathogen infects and causes disease on a wide variety of hosts belonging to different families of vegetables either in field or in storage in different areas mainly Jordan Valley and Uplands. Soft rot of potatoes is a tuber borne disease where the contaminated mother tubers were reported to be the main source of inoculum. However, this bacterium was found to survive in the soil with population trends that vary with the fluctuation in soil temperature (Rajeh and Khlaif, 2000).

This research was conducted in order to isolate and identify the causal agent of potato soft rot from different potato growing areas and storages in Jordan using biochemical and physiological tests. We also aimed to characterize the obtained isolates by PCR technique using specific set of primer.

MATERIALS AND METHODS

Samples collections

Potato rotted tubers samples were randomly collected during the years 2013 to 2015 from different potato growing areas in Jordan. The collected samples were placed in an ice box for further work in the laboratory.

Bacterial isolation

Infected potato tubers were surface sterilized with 0.5% sodium hypochlorite; thereafter, 10 g of rotted tubers were cut into small pieces placed in sterile bottle with 90 ml of sterile distilled water placed on a shaker at 200 rpm at room temperature. Series of serial dilutions were then prepared up to 10^{-3} dilution, and 0.1 ml of the 10^{-3} dilution spread onto the surface of three Logan's medium plates (Schaad et al., 2001). The inoculated plates were incubated at 27±2°C and checked periodically. Appearance of bacterial colonies with wide pink centers within the first 24 h of inoculation was suspected to be *Pcc* (Fahy and Parsley, 1983). Single colonies were re-streaked onto new nutrient agar (NA) plates. The obtained bacterial isolates were kept as SDW suspension in sterile Eppendroff tubes and kept in refrigerator for further identifications.

Biochemical and physiological tests

In order to identify *Pcc,* the Jordanian isolates were grown at 27ºC for 24 h on nutrient agar plates and were then subjected to the biochemical and physiological tests; oxidase, catalase potato soft rot, oxidative fermentative, growth at 37ºC, sodium chloride tolerance, reducing substances from sucrose, urease production and acid production from carbohydrates as described by Schaad et al. (2001). The same tests were run against a reference culture of *Pcc* isolate NCPPB312 obtained from Food and Environment Research Agency (*fera*), United Kingdom and against sterile water as a negative control.

DNA extraction

Bacterial DNA was extracted from 24 h old pure bacterial cultures grown on NA plates at 27ºC, obtained and identified by biochemical and physiological tests as *Pcc* isolates. Pure bacterial colonies were picked with a sterile loop and mixed in 4 ml of nutrient broth in a sterile and labeled culture tubes incubated overnight at 37ºC, with shaking at 150 rpm.

Genomic DNA extraction was done using DNeasy Blood and Tissue Kit (Qiagen, Valancia, CA); the protocol was performed according to the manufacturer's instructions designed for purification of total DNA from gram-negative bacteria.

The quantity and quality of the extracted genomic DNA were measured using the spectrophotometer with DNA visualized by electrophoresis in 1.0% agarose gel in Tris-Acetate-EDTA(TAE) buffer stained with ethidium bromide (0.5 µg/ml). The extracted DNA was stored at -20ºC for further PCR work.

PCR amplification, purification and sequencing

Oligonucleotide primers EXPCCR (5-GCCGTAATTGCCTACCTGCTTAAG-3) and EXPCCF (5-GAACTTCGCACCGCCGACCTTCTA-3) were used in standard PCR (Kang et al., 2003; Mahmoudi et al., 2007; Palacio-Bielsa et al., 2009). The PCR reactions were performed in 25 µl PCR mixture containing 25 mM $MgCl_2$, 5X Crimson *Taq* buffer, 10 mM dNTPs, 10 µM primer, 5U/µl*Taq* polymerase. PCR amplification was carried out as follows: one cycle of 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C and a final extension for 7 min at 72°C.

After amplification reaction, 10 µl of PCR products were separated on 1.5% agarose gel in TAE buffer and visualized by staining with ethidium bromide; also, 100 bp was used as a molecular DNA marker. Electrophoresis was performed at 110 V for 35 m in gels which were photographed under ultraviolet (UV) light.

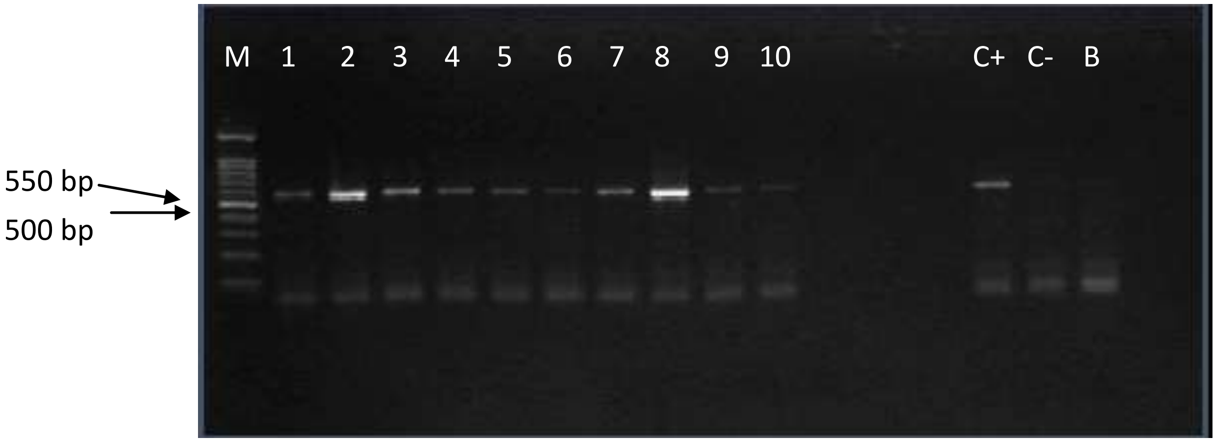

Figure 1. Agarose gel electrophoresis for PCR-amplified DNA of *Pectobacterium carotovorum* subsp. *carotovorum* isolates using EXPCC primers set with the expected amplified product of 550 bp. Lane M represents Ladder 100 bp (GeneDirex). Lanes 1- 10: isolates Jo-Q 16, Q19, A11, A2, Q14, Q21, Q29, A5, Q23 and Q27, respectively. Lanes C+: Positive control (reference isolate NCPPB312). C: Negative control isolate *Escherichia coli.* B: Buffer.

For nested PCR, primers INPCCF (5-TTCGATCACGCAACCTGCATTATC-3) and INPCCR (5-GGCCAAGCAGTGCCTGTATATCC-3) were used for amplification of an expected 0.4 Kb (Kang et al., 2003). Two microliters of the amplified template from the first PCR were used in nested PCR reaction under the same conditions as stated previously.

The PCR products were purified using the Wizard Purification System (Promega, Madison, Wisconsin) with the protocol performed according to the manufacturer's instructions. After purification, the DNA fragments were sequenced in both directions by Macrogen Korea (Seoul, Rep. of Korea) or Quintara Biosciences (South San Francisco, CA).

The DNA sequence data were analyzed for homology using Basic local alignment searching tool (BLAST) at NCBI server. Blast search was performed only for nucleotide sequence using BLASTn (Stephen et al., 1990).

RESULTS AND DISCUSSION

The bacterial isolates isolated from rotted potato from different regions were all identical in their cultural and biochemical properties and similar to that of reference culture of *Pcc* isolate NCPPB312. Logan's medium small circular bacterial colonies with pink to red purple centers were developed 24 h after incubation at 27ºC (Fahy and Parsley 1983).

However, bacteria from purified colonies were found to be oxidase negative, catalase positive, fermentation of glucose positive, rotting induced on inoculated potato slices, urease enzyme was produced, growth developed on nutrient agar plates incubated at 37±2ºC and on 5% NaCl. Also, all isolates were able to oxidize the alcoholic sugar and discharge it in the media to acidic reaction and were not able to reduce substances from sucrose. The above mentioned physiological reactions support the identification of these isolates as *Pcc*.

Pectobacterium species and subspecies have been increased over the recent years, and as a result, their identification and differentiation by classical methodology became more challenging (De Boer et al., 2012).

PCR assays

A total of 67 (51%) out of 131 isolates which were biochemically identified as *Pcc* showed a 550-bp bands with the specific EXPCC set of primers (Figure 1); also, bands of 380 bp were observed when PCR products amplified with EXPCC primers set were used as templates (nested PCR) using INPCC set of primers (Figure 2) (Kang et al., 2003).

Sequence analysis

Maximum nucleotide similarity results of BLASTn for selected *Pcc* isolates amplified with EXPCC species-specific set of primers are presented in Table 1, showing maximum similarity percentage with closely related reference strains, E-value and accession numbers in the GenBank.

BLASTn results showed that most of *Pcc* Jo-isolates showed high similarity with the strain *E. carotovora* subsp. *carotovora* from Korea (Acc. no. AF046928.1) and with *Pcc* strain PC1 from USA (Acc. no.CP001657.1). Maximum similarity percentage ranged from 86 to 98%; some *Pcc* Jo-isolates showed high similarity with the reference strain *P. carotovorum* subsp. *odoriferum* (Acc. no. CP009678.1) from China, whereas their maximum similarity percentage ranged from 85 to 98%.

In order to determine the specificity of EXPCC primers (Kang et al., 2003), eleven bacterial isolates sequenced on the bases of EXPCC primers and their sequences

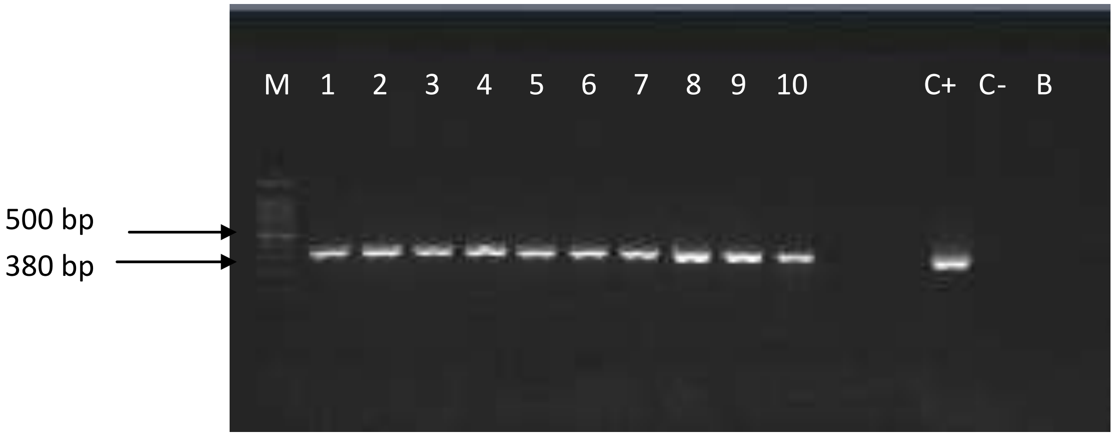

Figure 2. Agarose gel electrophoresis for PCR-amplified DNA of *P. carotovorum* subsp. *carotovorum* isolates using EXPCC primers set followed by nested PCR using INPCC primers set with the expected amplified product of 380 bp. Lane M represents Ladder 100 bp (GeneDirex). Lanes 1-10: Isolates Jo-Q16, Q19, A11, A2, Q14, Q21, Q29, A5, Q23 and Q27, respectively. Lanes C+: Positive control (reference isolate NCPPB312); C: Negative control isolate *E. coli.* B: Buffer.

Figure 3. Agarose gel electrophoresis for PCR-amplified DNA of *P. carotovorum* subsp. *Carotovorum* isolates using EXPCC primers set followed by nested PCR using INPCC primers set with the expected amplified product of 380 bp. Lane M represents Ladder 100 bp (GeneDirex). Lanes 1-11: Isolates Jo-S97/2/1, S97/2/2, Q111, G60/2/1, G60/2/2, G42/1/1, G42/1/2, M2/3, M2/4, G40/2/1 and G40/2/2, respectively. Lanes C+: Positive control (reference isolate NCPPB312). B: Buffer.

showed maximum similarity percentage with other closely related species. Interestingly, nested PCR gave the expected product size of 380 bp (Figure 3), although they were identified as *Enterobacter* spp., *Serratia* spp. and *Klebsiella* spp., based on their sequencing, rather than *P. carotovorum* subsp*. carotovorum*.

Specificity of PCR with the species-specific set of primers (EXPCCF/EXPCCR), which was designed to test specificity of *Pcc* isolates was more limited because they also amplified the expected 550 bp product from some isolates identified as species other than *Pcc* as shown in Figure 2 and did not amplify DNA from other isolates that were identified as *Pcc* on the basis of biochemical test (Table 2). Similar results were obtained by De Boer et al. (2012) where the specific primer set for *Pcc* amplify the expected size of strains identified as *P. wasabiae* and did not amplify other strains biochemically identified as *Pcc*.

Our results are also in agreement with previous finding of Azadmanesh et al. (2013) where none of the 12 Iranian *Pcc* tested isolates produced the 550 bp products in PCR in contrast to two standard *Pcc* isolates that produced the desired bands and they found that these

Table 1. Maximum nucleotide similarity (BLASTn) between *Pectobacterium carotovorum* subsp. *carotovorum* Jo-isolates amplified with EXPCC set of primers and the most closely related species/subspecies.

S/N	Isolate and accession no. in GenBank	Closely related species/subspecies	E-value	Maximum % similarity	Accession no.
1	Jo-G59 (MF535186)	*Pco*	5 e^{-42}	89	CP009678.1
		Ecc	2 e^{-35}	86	AF046928.1
2	Jo-S97 (MF535187)	*Ecc*	2 e^{-26}	97	AF046928.1
3	Jo-Q16 (MF535188)	*Pco*	2 e^{-79}	98	CP009678.1
		Ecc	8 e^{-30}	94	AF046928.1
		Pcc PC1 strain	1 e^{-157}	92	CP001657.1
4	Jo-Q19 (MF535189)	*Pco*	4 e^{-82}	98	CP009678.1
		Ecc	8 e^{-10}	94	AF046928.1
		Pcc PC1 strain	2 e^{-155}	92	CP001657.1
5	Jo-A11 (MF535190)	*Pco*	3 e^{-133}	98	CP009678.1
		Ecc	4 e^{-122}	94	AF046928.1
		Pcc PC1 strain	2 e^{-155}	92	CP001657.1
6	Jo-A2 (MF535191)	*Pco*	3 e^{-133}	98	CP009678.1
		Ecc	4 e^{-122}	94	AF046928.1
		Pcc PC1 strain	2 e^{-155}	92	CP001657.1
7	Jo-Q14 (MF535192)	*Pco*	3 e^{-133}	97	CP009678.1
		Ecc	4 e^{-122}	93	AF046928.1
		Pcc PC1 strain	2 e^{-155}	91	CP001657.1
8	Jo-Q29 (MF535193)	*Pco*	1 e^{-127}	98	CP009678.1
		E cc	2 e^{-116}	94	AF046928.1
		Pcc PC1 strain	4 e^{-147}	92	CP001657.1
9	Jo-A5 (MF535194)	*Pco*	7 e^{-125}	98	CP009678.2
		Ecc	9 e^{-115}	94	AF046928.2
		Pcc PC1 strain	2 e^{-155}	92	CP001657.1
10	Jo-Q23 (MF535195)	*Pco*	3 e^{-133}	97	CP009678.2
		E cc	4 e^{-122}	93	AF046928.2
		Pcc PC1 strain	2 e^{-155}	90	CP001657.1
11	Jo-Q27 (MF535196)	*Pco*	3 e^{-133}	98	CP009678.2
		Ecc	4 e^{-122}	94	AF046928.2
		Pcc PC1 strain	3 e^{-148}	91	CP001657.1
12	Jo-Q30 (MF535197)	*Pco*	5 e^{-126}	85	CP009678.2
		E cc	7 e^{-115}	88	AF046928.2
13	Reference strain NCPPB312	*Pco* BC S7	9 e^{-154}	98	CP009678.1
		Ecc	1 e^{-131}	94	AF046928.2
		*Pcc*1 PC1 strain	2 e^{-120}	91	CP001657.1

two isolates could not be identified by PCR using *Pectobacterium* subsp. specific primers. Also, Kang et al. (2003) found that only genomic DNA of 29 strains of *Pcc* out of 54 bacterial strains which is equal to about 54% yielded the expected 550 bp amplified product following PCR with EXPCC specific primers. These results could be due to sequence variation among strains of *Pectobacterium* isolated from different regions of the world (Azadmanesh et al., 2013). Whereas the species-specific EXPCC was generated from the nucleotide

Table 2. Polymerase chain reaction (PCR) of set of primers for all *P. carotovorum* subsp. *carotovorum* Jo-isolates collected from different regions of Jordan.

No.	Reference no.	Region	Result of biochemical tests	Primer set (Species-specific EXPCC)
1	A1	Amman	Positive	Positive
2	A2	Amman	Positive	Positive
3	A3	Amman	Positive	Positive
4	A4	Amman	Positive	Negative
5	A5	Amman	Positive	Positive
6	A6	Amman	Positive	Negative
7	A7	Amman	Positive	Negative
8	A8	Amman	Positive	Positive
9	A9	Amman	Positive	Negative
10	A10	Amman	Positive	Positive
11	A11	Amman	Positive	Positive
12	A12	Amman	Positive	Negative
13	A13	Amman	Positive	Positive
14	R123/1	AR Ramtha	Positive	Negative
15	R104/3	AR Ramtha	Positive	Negative
16	R105/2	AR Ramtha	Positive	Negative
17	R85/4	AR Ramtha	Positive	Positive
18	R105/4	AR Ramtha	Positive	Negative
19	R104/4	AR Ramtha	Positive	Negative
20	R105/3	AR Ramtha	Positive	Negative
21	R89/4	AR Ramtha	Positive	Negative
22	R89/3	AR Ramtha	Positive	Negative
23	R106/4	AR Ramtha	Positive	Negative
24	R83/3	AR Ramtha	Positive	Negative
25	R105/4	AR Ramtha	Positive	Positive
26	G29/1	Jordan Valley	Positive	Negative
27	G27/4	Jordan Valley	Positive	Positive
28	G72/2/4	Jordan Valley	Positive	Negative
29	G70/2	Jordan Valley	Positive	Negative
30	G6/4	Jordan Valley	Positive	Negative
31	G32/2/1	Jordan Valley	Positive	Negative
32	G4/4	Jordan Valley	Positive	Negative
33	G71/1	Jordan Valley	Positive	Negative
34	G68/1	Jordan Valley	Positive	Positive
35	G44/4	Jordan Valley	Positive	Negative
36	G43/1	Jordan Valley	Positive	Positive
37	G40/1	Jordan Valley	Positive	Positive
38	G59/3	Jordan Valley	Positive	Positive
39	G59/2	Jordan Valley	Positive	Positive
40	G71/2	Jordan Valley	Positive	Positive
41	G4/2	Jordan Valley	Positive	Negative
42	G68/4	Jordan Valley	Positive	Positive
43	G68/3	Jordan Valley	Positive	Positive
44	G68/2	Jordan Valley	Positive	Negative
45	G43\2	Jordan Valley	Positive	Positive
46	G60/1	Jordan Valley	Positive	Positive
47	G59/4	Jordan Valley	Positive	Positive
48	G60/2	Jordan Valley	Positive	Positive
49	G60/3	Jordan Valley	Positive	Positive
50	G60/4	Jordan Valley	Positive	Positive

Table 2. Contd.

51	G40/4	Jordan Valley	Positive	Positive
52	G40/3	Jordan Valley	Positive	Positive
53	G32/2/2	Jordan Valley	Positive	Positive
54	G32/2/3	Jordan Valley	Positive	Positive
55	G32/2/4	Jordan Valley	Positive	Positive
56	G32/3	Jordan Valley	Positive	Positive
57	G37/1	Jordan Valley	Positive	Positive
58	G37/2	Jordan Valley	Positive	Positive
59	G37/3	Jordan Valley	Positive	Positive
60	G37/4	Jordan Valley	Positive	Positive
61	G29/2	Jordan Valley	Positive	Negative
62	G42/1	Jordan Valley	Positive	Positive
63	G42/3	Jordan Valley	Positive	Positive
64	G59/1	Jordan Valley	Positive	Positive
65	G40/2	Jordan Valley	Positive	Positive
66	G29/3	Jordan Valley	Positive	Negative
67	G29/4	Jordan Valley	Positive	Negative
68	G18/1	Jordan Valley	Positive	Negative
69	G18/2	Jordan Valley	Positive	Negative
70	G18/3	Jordan Valley	Positive	Negative
71	G20/4	Jordan Valley	Positive	Positive
72	S24/3	Ma'an	Positive	Positive
73	S24/4	Ma'an	Positive	Positive
74	S98/2/2	Ma'an	Positive	Positive
75	S103/2	Ma'an	Positive	Positive
76	S97/2/4	Ma'an	Positive	Positive
77	S97/2/2	Ma'an	Positive	Positive
78	S103/3	Ma'an	Positive	Negative
79	S97/1/1	Ma'an	Positive	Negative
80	S97/2/3	Ma'an	Positive	Positive
81	S102/4	Ma'an	Positive	Negative
82	S24/2	Ma'an	Positive	Positive
83	S83/4	Ma'an	Positive	Negative
84	S99/4	Ma'an	Positive	Negative
85	S102/2	Ma'an	Positive	Positive
86	M2/2	Madaba	Positive	Negative
87	M117/2/4	Madaba	Positive	Positive
88	M113/2/3	Madaba	Positive	Positive
89	M114/2	Madaba	Positive	Positive
90	M2/3	Madaba	Positive	Negative
91	M112/2	Madaba	Positive	Negative
92	M113/2/4	Madaba	Positive	Positive
93	M113/1	Madaba	Positive	Positive
94	M114/2/1	Madaba	Positive	Positive
95	M126/4	Madaba	Positive	Negative
96	M117/2/3	Madaba	Positive	Negative
97	M114/2/4	Madaba	Positive	Positive
98	M114/2/2	Madaba	Positive	Positive
99	M113/4	Madaba	Positive	Positive
100	M126/2	Madaba	Positive	Positive
101	M2/4	Madaba	Positive	Negative
102	Q111/2	Mafraq	Positive	Negative

Table 2. Contd.

103	Q8/2	Mafraq	Positive	Negative
104	Q9/2	Mafraq	Positive	Negative
105	Q14/3	Mafraq	Positive	Negative
106	Q111/4	Mafraq	Positive	Negative
107	Q12/1	Mafraq	Positive	Negative
108	Q8/3	Mafraq	Positive	Negative
109	Q9/3	Mafraq	Positive	Negative
110	Q14/4	Mafraq	Positive	Negative
111	Q12/2/2	Mafraq	Positive	Negative
112	Q12/2/3	Mafraq	Positive	Negative
113	Q9/4	Mafraq	Positive	Positive
114	Q12/2/4	Mafraq	Positive	Positive
115	Q14	Mafraq	Positive	Positive
116	Q15	Mafraq	Positive	Positive
117	Q16	Mafraq	Positive	Positive
118	Q17	Mafraq	Positive	Negative
119	Q18	Mafraq	Positive	Positive
120	Q19	Mafraq	Positive	Positive
121	Q20	Mafraq	Positive	Negative
122	Q21	Mafraq	Positive	Positive
123	Q22	Mafraq	Positive	Positive
124	Q23	Mafraq	Positive	Positive
125	Q24	Mafraq	Positive	Negative
126	Q25	Mafraq	Positive	Positive
127	Q26	Mafraq	Positive	Negative
128	Q27	Mafraq	Positive	Positive
129	Q28	Mafraq	Positive	Negative
130	Q29	Mafraq	Positive	Positive
131	Q30	Mafraq	Positive	Positive
	Total positive		131	67 (51%)

sequence of a *Pcc* universal rice primer (URPs) PCR product, although URPs were developed from repetitive sequences in the rice genome that have been used to fingerprint genomes of diverse organisms (Kang et al., 2003).

Kang et al. (2003) presented that PCR used on the bases of EXPCC set of primers should be followed by using PCR product as a template for second run of nested PCR in order to confirm the detection of *Pcc* which will yield 380 bp bands. On the other hand, our results of nested PCR for some isolates which were confirmed as *Enterobacter* spp. and *Serratia* spp. using maximum nucleotide similarity (BLASTn) gave bands of 380 bp, where these results are in conflict with Kang et al. (2003) and indicated that EXPCC was not a species specific primer and in agreement with De Boer et al. (2012). Although specific DNA markers were commonly used for detection of bacteria at subspecies level as reported in many studies, specific detection of *Pcc* isolates using molecular techniques is faced by complexity among strains associated with other subspecies. In general, molecular approach that was used to evaluate microbial population revealed a more complex soft rotting population than is usually evident from evaluations based on isolation alone.

Homology search results for all Jordanian sequenced on the bases of EXPCC set of primers showed high similarity percentage with *Pc* subsp. *odoriferum* (*Pco*), and are in contrast with Kang et al. (2003), who reported that specific primers were able to differentiate *Pcc* strains among other subspecies. *P. carotovorum* subsp. *odoriferum* was reported as a typical *Pc* subsp. *atrosepticum* strain and it is pathogenic to chicory only and produce odorous volatile (Gallois et al., 1992), which consequently differs from *Pcc* known as widely distributed pathogen and has a broad host range (Kang et al., 2003). For all of the above, this confirms that all our isolates are *Pcc* rather than *Pco*.

CONCLUSION AND RECOMMENDATIONS

Soft rot disease is widely common in different potato growing areas in Jordan and the results of biochemical and physiological tests confirmed that the main causal agent of soft rot in Jordan is *P. carotovorum* subsp. *Carotovorum.* While using the PCR primer pair (EXPCCR/EXPCCF) was found to be not reliable in detection and identification of all soft rot Jordanian isolates of *Pcc*, DNA sequencing was found to be the most reliable way in specific detection and confirmation of the causal agent of soft rot. On the other hand, more studies needs to be implemented in order to study soft rot disease etiology and epidemiology.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Vibration levels emitted by agricultural tractors

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The objective of this study was to evaluate the vibration levels emitted by agricultural tractors with different capacities, comparing the results with the current norms in Brazil. The test was carried out with four tractors of different strengths - Massey Ferguson MF 4292 (engine power of 110 hp at 2,200 rpm, manufactured in 2011, and at the moment of the beginning of the tests has worked for 2,560 h), MF 4275 (engine power of 75 hp at 2,200 rpm, manufactured in 2011, and at the time of the beginning of the tests has worked for 3,036 h), MF 680 (engine power of 173 hp at 2,200 rpm, manufactured in 1997, and at the time of the beginning of the tests has worked for 9,010 h) and MF 297 (engine power of 120 hp at 2,200 rpm, manufactured in 2005, and at the time of the beginning of the tests has worked for 5,715 h). The studied tractors presented, in general, vibration levels higher than those established by International Standard Organization (ISO) 2631 for 8 h daily work, ranging between 5 and 10 Hz.

Key words: Frequency, ergonomics, personal protective equipment.

INTRODUCTION

Nowadays, stimulated by the increase in the rigor of work safety standards, there is a tendency to improve the conditions of ergonomics and safety of the operator. However, most agricultural tractors present comfort and safety problems, causing among others, a decrease in the operational capacity and the occurrence of accidents in agricultural activities.

Kyuhyun et al. (2017) stated that even with the advancement in technology, agricultural tractors have a level of comfort lower than what is recommended when compared to passenger cars, paving way for researchers aiming to improve the ergonomics of these machines. Vibration problems make the agricultural tractor operator more susceptible to accidents. According to Araújo et al. (2002), environmental risks are classified as physical, chemical and biological hazards.

Among the ergonomic factors that harm agricultural tractor operators, vibration stands out among the main ones. These vibrations, in general, are of low frequency, but can generate problems of vision, irritability, lumbar deformations and digestive problems (Servadio et al., 2007).

Cutini et al. (2016) made it clear in their research that the vibrations to which tractors operators are exposed to,

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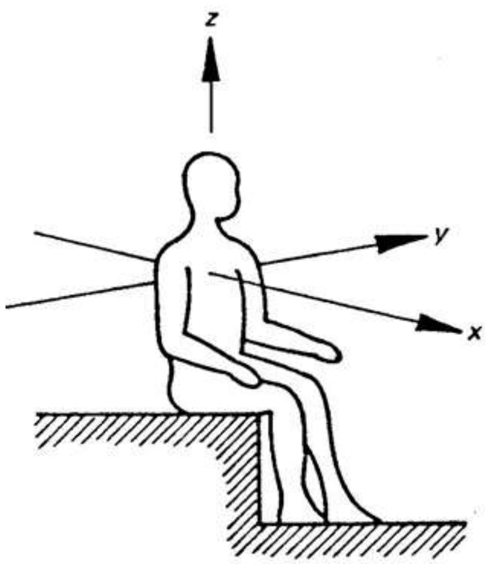

Figure 1. Directions of the coordinate system in human bodies (Adapted from ISO, 1978).

if it continues this way may result to a number of harmful effects on their health. In this same line of thought, Stojic' et al. (2017) commented that for the tractors, the vibration spectrum to be studied is the low frequency one, with emphasis on the 5 and 10 Hz range, which is mainly caused by traffic on unpaved roads.

In the rural environment it is common to use personal protective equipment (PPE) in the application of phytosanitary products. However, little attention is paid towards preventing the effects of vibration at the operator's operating station. Cunha et al. (2012) stated that, even with the technological advancement in the production of agricultural machinery, these are not ergonomically adequate for an eight-hour workday.

The vibrations consist of a complex mixture of several waves, with different frequencies and directions. However, several researches only studied vertical vibration, making it difficult to analyze this parameter in depth.

According to ISO 2631 (ISO, 1978), three points should be taken into account for the evaluation of this interaction: preservation of work efficiency, preservation of health and safety, and preservation of comfort. To do so, ideal levels were set for each of the concerns. They are, respectively, the reduced efficiency level (NER), the exposure limit (LE) and the reduced comfort level (NCR).

NER is understood as the values that, when exceeded, will affect the efficiency of the work performed, more specifically time dependent, generating fatigue. LE values are the reduced efficiency level values multiplied by 2, characterizing a maximum level of safe exposure due to the multiplication of NER criteria values.

As for the NCR, this is also derived from the NER, which is divided by 3.15, characterizing the level in which the loss of the comfort state occurs. For such analyses, there are established patterns of curves in function of time of exposure to vibration, plotted in graphs of frequency (Hz) × acceleration (m s^{-2}) (ISO, 1978), for each of the different levels or limits evaluated, as well as for each of the actuation directions (x, y, z), as exemplified in Figure 1. Thus, further research to understand how the three-dimensional vibration of agricultural machinery is transmitted to the human body is needed (Tiemessen et al., 2007; Antonucci et al., 2012).

Therefore, the present study has as its objective to evaluate the vibration levels emitted by agricultural tractors with different potencies, comparing the results to the current norms in Brazil.

MATERIALS AND METHODS

This study was developed at the Faculty of Animal Science and Food Engineering (FZEA) at the University of São Paulo (USP), in Pirassununga County, in an area owned by USP Administrative Office at Fernando Costa Campus, in Pirassununga. The geographical location of the campus is 21° 59' south latitude and 47° 26' west longitude and average altitude of 635 m.

The test was carried out with four tractors of different strengths: Massey Ferguson MF 4292 (110 hp engine power at 2,200 rpm, manufactured in 2011, with 2,560 worked hours at the moment tests began), MF 4275 (75 hp at 2,200 rpm, manufactured in 2011, with 3,036 worked hours, at the moment tests began), MF 680 (engine power of 173 hp at 2,200 rpm, manufactured in 1997, with 9,010 worked hours, at the moment tests began) and MF 297 (engine power of 120 hp at 2,200 rpm, manufactured in 2005, with 5,715 worked hours, at the moment tests began), all of which were located at USP Administrative Office at Fernando Costa Campus, in Pirassununga.

Regarding the evaluation of vibration, the primary magnitude used was the acceleration, expressed in m s^{-2}, based on ISO 2631 (ISO, 1978). A human vibration analyzer, model HD-2030HA-WB, brand Delta Ohm, serial number 12062930149, was used, it was under the responsibility of Laboratory of Agricultural Machinery and Precision Agriculture (LAMAP), which enabled the study to evaluate the vibrations transmitted by the whole body with filter band pass. The data acquisition device was set to read full body accelerations, with storage at every second.

In conjunction with the vibration meter, an accelerometer in the form of a seat, model 356B41, brand PCB Piezotronics, serial number LW145553, of tri-axial analysis, also under LAMAP's responsibility, was used. The vibration sensor was placed between the tractor seat and the operator.

The analyses were taken in three different terrains: asphalt, land and field. For ground analysis, roads commonly used daily by tractors were used for locomotion to the fields, and were characterized by their compaction. Regarding asphalt analysis, the tracks and streets between the shed where the machines were located and the work area (field), was considered.

Finally, the field condition was understood by the mobilized soil characteristic used for crops in general, where the machine worked directly. The total time of acquisition was 20 min, with tractors operating under similar conditions, and five replications were performed in each treatment (tractor × terrain).

The signals were transformed to the frequency domain using the FFT function (amplitude and phase), being digitally filtered in 1/3 octave bands to obtain the effective acceleration. The maximum values obtained in each band were multiplied by weighting factors, thus generating the weighted accelerations. The overall weighted effective acceleration analysis was performed in the range of 1 to 80 Hz. The range between 5 and 10 Hz was highlighted for studies of whole body vibration due to its range of important vibration frequencies and its influence on the spine.

The evaluations were made with the tractor motor in nominal rotation. The tractor seat was the original factory for all tractors, and before the beginning of the test, it was adjusted for the weight and size of the operator.

The results were compared to the values recommended by ISO 2631 (ISO, 1978) for exposure to LE, NCR and NER, both considering the daily 8 h work.

RESULTS AND DISCUSSION

The vibration values for the 1/3 eighth-octave band measured for the three directions (x, y and z axis) with tractors on the asphalt are shown in Figures 2, 3 and 4, respectively. LE, NCR, and NER values account for a daily work period of 8 h.

For the asphalt terrain on both axes, there is a downward trend in acceleration values in all tractors, observing that in frequencies between 6 and 10 Hz the values are generally below the reduced efficiency level. However, the results indicate that, for the MF 4275 tractor, the values were above the allowed limit, which will possibly affect the efficiency of work, more specifically the time dependent generating fatigue (ISO, 1978).

Although for higher and lower frequencies, the tractors exhibit similar behaviors, it is frequently observed that NER and NCR values are exceeded, with the usual extrapolation of LE. Considering the greater influence of the z-axis vibrations on spinal disorders, the constant overrunning of limits emphasizes not only a reduction in comfort due to NCR overruns, but also a decrease in the quality of the work due to the passage of NER, as well as the possibility of the development of occupational diseases with the overcoming of LE.

The results indicated that, in general, the seat structures were able to absorb part of the impact and in some situations create a good working condition for the machine operator. The vibration values for the 1/3 eighth-octave band measured for the three directions (axes x, y and z) with the tractors on the ground are shown in Figures 5, 6 and 7, respectively. LE, NCR, and NER values account for a daily work period of 8 h.

With the change of terrain, the tractors presented similar behavior with respect to the vibration patterns, showing a tendency of decrease in the values of acceleration. For the x and y axes within the critical range (between 5 and 10 Hz), there was little extrapolation of LE. However, the few times the acceleration values were below the limits of NER, they were not included within the NCR.

As for the z-axis analysis, the acceleration values exceeded the limits of the LE, and consequently, extrapolated the limits of NCR and NER in the frequencies of 5 to 10 Hz, being evident of the risks to which the operators of such machines are exposed, since the frequent overshoot of LE, as previously mentioned, highlights the possibility of the development of diseases related to the vertebral column.

In the terrain, compared to the asphalt terrain, it is possible to affirm that there was deterioration in the values of vibration in the studied range. Such an event can be explained by the fact that, even with the presence of compaction, the so-called terrain presents a greater irregularity than the asphalt.

Santos Filho et al. (2003) found similar results, in which the instantaneous vibrations at the base of the seat were eight times higher than the vibrations at the seat. This claim is within the parameters that were used by Da Silva et al. (2011) in a similar study of the ergonomic evaluation of a sugarcane harvesting machine.

The vibration values for the 1/3 eighth-octave band measured in the three directions (axes x, y and z) with the tractors in the field are shown in Figures 8, 9 and 10, respectively. Exposure limit (LE), reduced comfort level (NCR), and reduced efficiency level (NER) values were considered in a daily work period of 8 h.

In the so-called field, in the x and y axes, the descending curves up to the range of 5 to 10 Hz are again remarkable, with a subsequent increase in values. Still for these axes, the constant extrapolation of NCR and NER to the study range is observed, and visually exceeding the limits of the LE.

In the z-axis, the values are above the LE for frequencies below 10 Hz. If based on the vibration parameters suggested by Gialamas et al. (2016), all the tractors studied need improvement (decrease) in the vibration values to which the operators of these machines are exposed. The vibration levels were typically below the limits established for 8 h of work at frequencies of 5 to 10 Hz for the three directions (az-axis, ax-axis and ay-axis) in all of the studied tractors.

The presence of tractors exceeding the limits of LE demonstrates the possibility of occupational diseases development due to the incident vibrations, as well as the reduction of productivity due to NER extrapolation. It is believed that these values would have a much greater variation if the measures had been carried out during the course of a continuous agricultural operation, as demonstrated in several experiments (Vilibor et al., 2014; Antonucci et al., 2012; Cunha et al., 2012).

Conclusion

The studied tractors in general presented vibration levels higher than those established by ISO 2631 (1978) for an 8 h workday for the range of 5 to 10 Hz, in the three axes studied (x, y and z) (asphalt, terrain and field). There is a need for further studies on mitigation, as well as study of vibration levels emitted by agricultural tractors and its

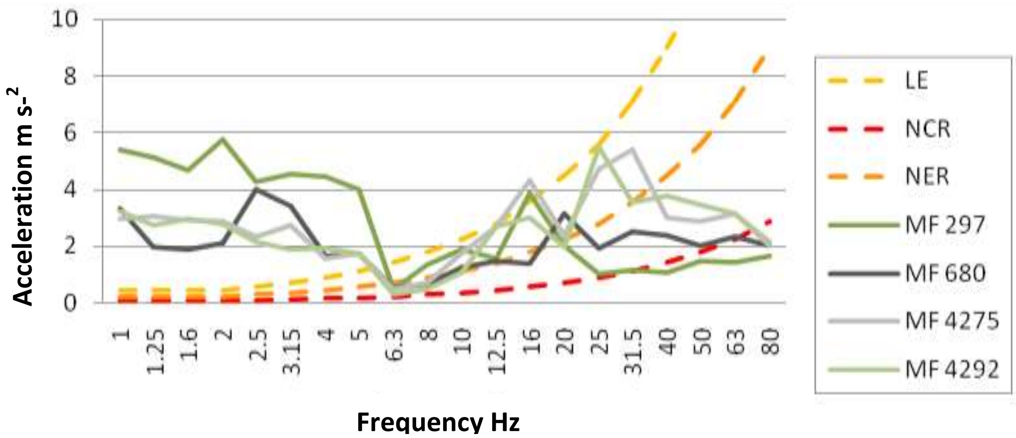

Figure 2. Acceleration (x-axis) for 1/3 octave bands of tractors operating on asphalt according to ISO 2631 (ISO, 1978) for limit of exposure (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

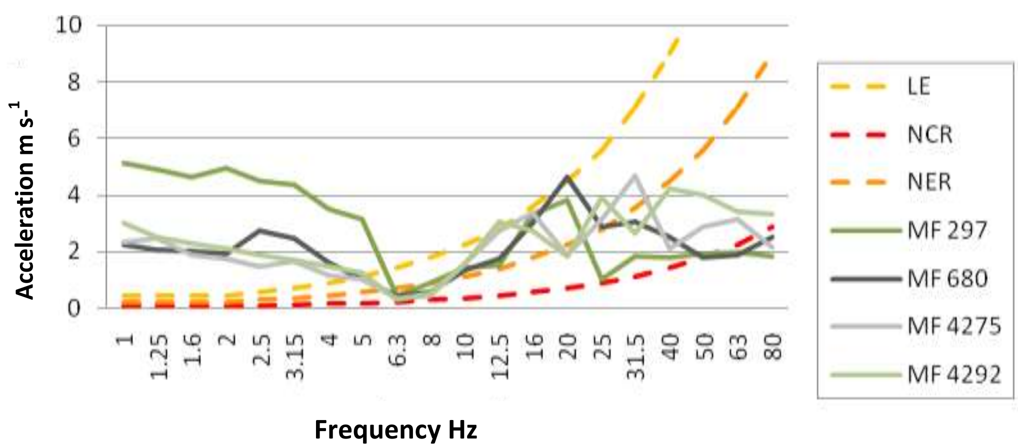

Figure 3. Acceleration (y-axis) for 1/3 octave bands of tractors operating on asphalt according to ISO 2631 (ISO, 1978) for limit of exposure (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

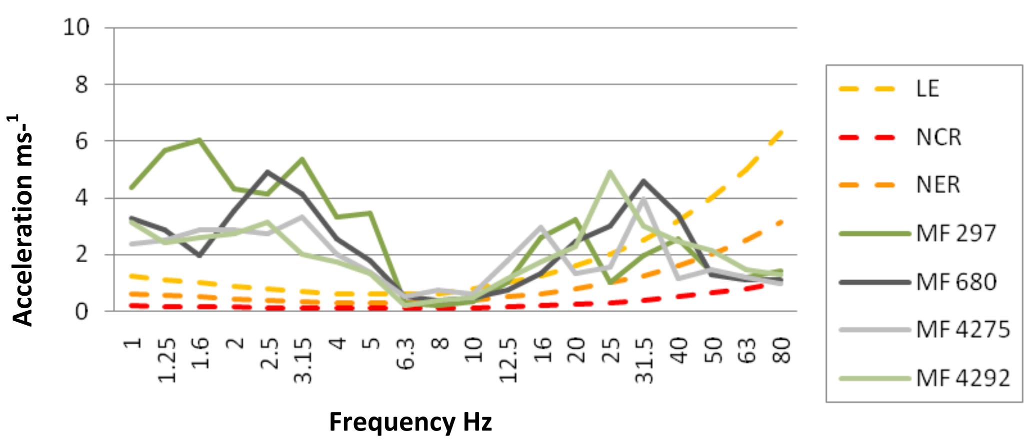

Figure 4. Acceleration (z axis) for 1/3 octave bands of tractors operating on asphalt according to ISO 2631 (ISO, 1978) for exposure limit (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

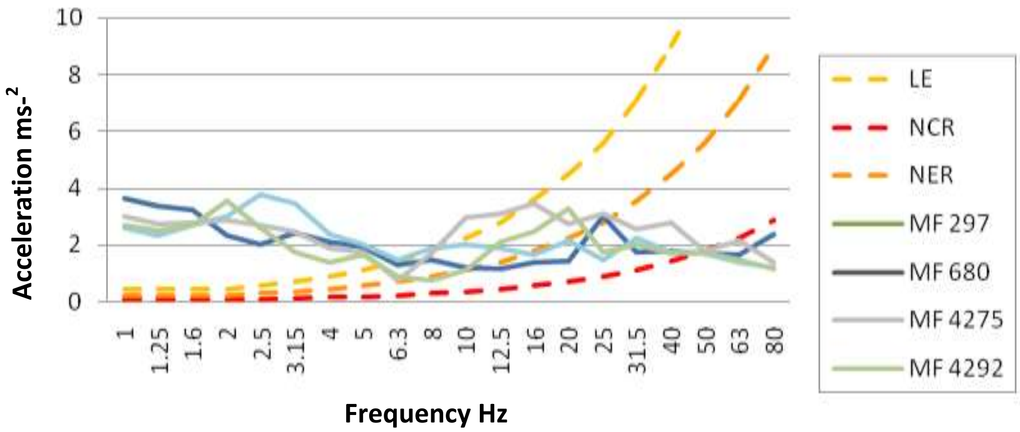

Figure 5. Acceleration (x-axis) for 1/3 octave bands of tractors operating on land according to ISO 2631 (ISO, 1978) for exposure limit (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

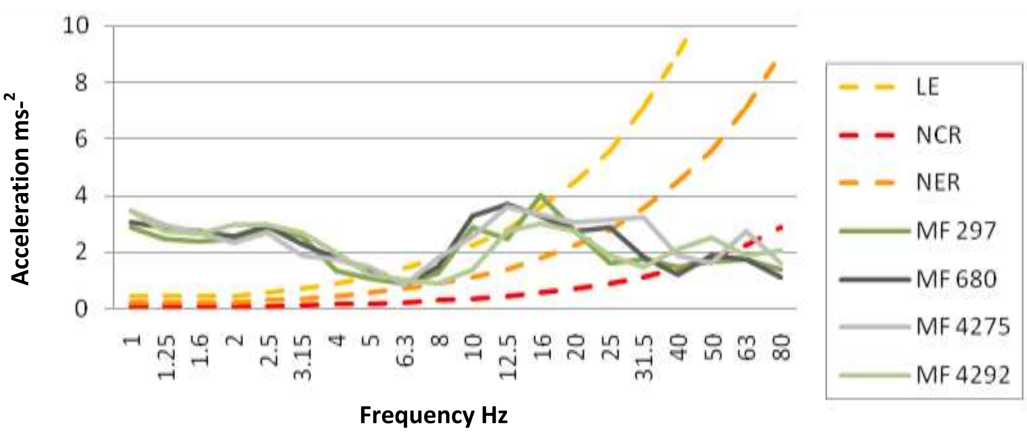

Figure 6. Acceleration (y-axis) for 1/3 octave bands of tractors operating on land according to ISO 2631 (ISO, 1978) for exposure limit (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

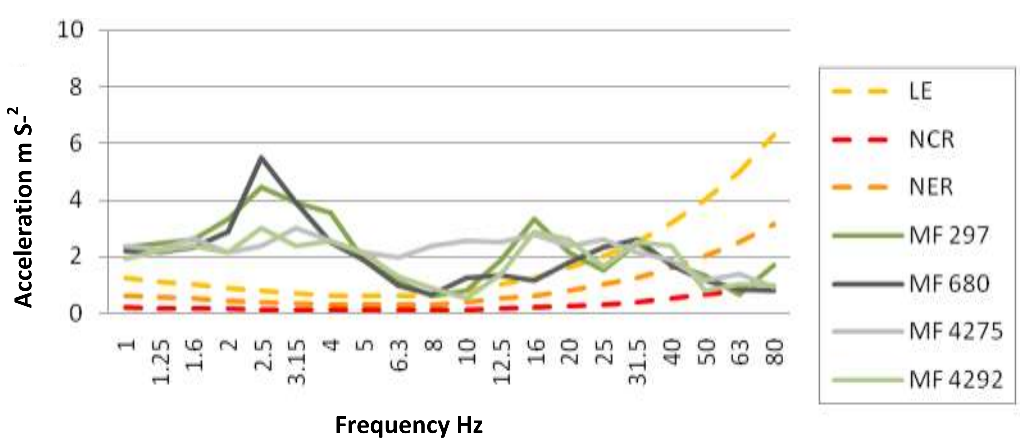

Figure 7. Acceleration (z axis) for 1/3 octave bands of tractors operating on land according to ISO 2631 (ISO, 1978) for exposure limit (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

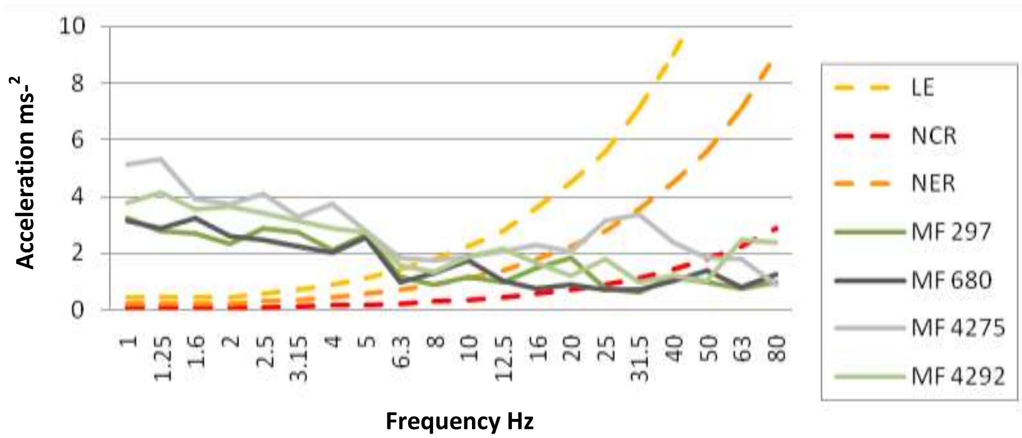

Figure 8. Acceleration (x-axis) for 1/3 octave bands of tractors operating in the field according to ISO 2631 (ISO, 1978) for exposure limit (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

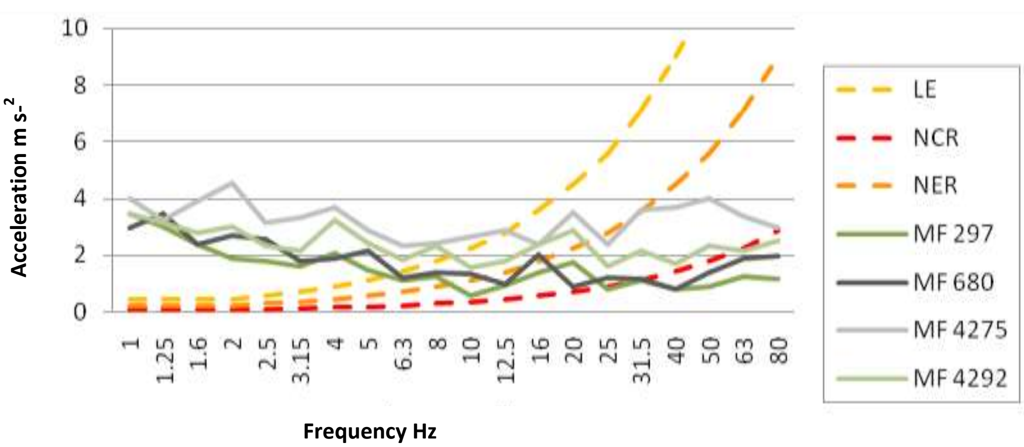

Figure 9. Acceleration (y-axis) for 1/3 octave bands of tractors operating in the field according to ISO 2631 (ISO, 1978) for exposure limit (LE), reduced comfort level (NCR) and level of reduced efficiency (NER).

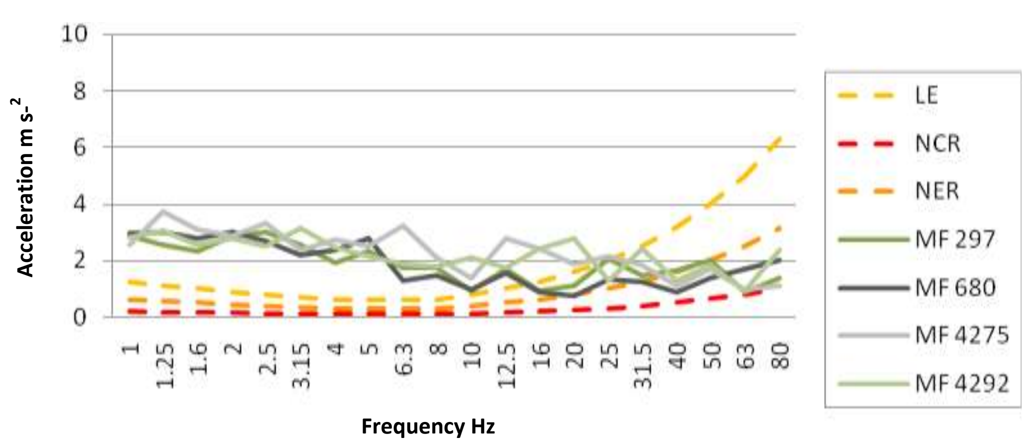

Figure 10. Acceleration (z axis) for 1/3 octave bands of tractors operating in the field according to ISO 2631 (ISO, 1978) for exposure limit (LE), reduced comfort level (NCR) and level low efficiency (NER).

origins, in order to provide better working conditions to the operators of these machines. The seat structures in general were not able to absorb the vibrations in order to create a good working condition for the machine operator.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Substrates and indolebutyric acid (IBA) concentrations in air-layering rooting of Tamarind tree

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Considering that tamarind (*Tamarindus indica* L.), an exotic fruit in Brazil, is appreciated *in natura* consumption and has been highlighted by its nutritional and pharmaceutical characteristics, its application in medicines available in the market due its long period of freshness has become an obstacle to the commercial cultivation of this fruit; therefore, the method of propagation by air-layering can solve this problem. Thus, the objective of this work was to evaluate the influence of substrates and the use of indolebutyric acid (IBA) on the production of seedlings by tamarind air-layering method. For this purpose, commercial organic compound, coconut powder and sphagnum were used to make the air-layering and IBA concentrations (500, 750, 1000 and 1250 mg L^{-1}). The experimental design was a randomized complete block, in a 3 × 5 factorial scheme, containing 10 blocks, each plant being considered a block, with 4 replicates (air-layering) per block, distributed in the plants quadrants. Influence of the substrates and IBA concentrations used for the rooting of tamarind air-layering was observed, with a significant interaction of these factors obtained in the evaluated variables. Thus, it can be concluded that propagation by the method of air-layering is viable. For organic compound and coconut powder, the concentrations of 500 and 1000 mg L^{-1} IBA, respectively, may improve the results obtained by air-layering. With the use of sphagnum as substrate, the use of 500 mg L^{-1} IBA to maximize the results is indicated in this method.

Key words: Fruits, propagation materials, *Tamarindus indica* L., tropical fruits, seedling production.

INTRODUCTION

Tamarind tree (*Tamarindus indica* L.), an exotic fruit tree in Brazil, stands out among non-traditional tropical fruits trees, due to the nutritional and pharmacological properties of all parts of the plant (Khanzada et al., 2008; Havinga et al., 2010). The potential of exotic tropical fruits has contributed to the agroindustry and pharmacological market, due to the nutraceutical properties and added value of the products from these species which are not sufficiently explored.

Tamarind fruit has an acid flavor mesocarp rich in vitamin C, and is used in national and international popular cuisine, in the production of jellies, juices, ice creams and fresh market. In addition to the nutritional properties, tamarind seeds are rich in essential oils with laxative, antimicrobial, antispasmodic, anti-inflammatory and antidiabetic functions (Ali and Shah, 2010; Escalona et al., 2010; Amado et al., 2011), which are important characteristics for the pharmaceutical market.

From this information, there is a need for the creation of germplasm banks and the production of seedlings, for the maintenance and formation of commercial orchards of this fruit. In the literature, it is reported that the production of seedlings of the tamarind tree is predominantly by seeds, and the plants cultivated from this method have a long period of juvenility (Ajiboye et al., 2011; El-Siddig et al., 2006).

Faced with this difficulty, propagation methods are fundamental in the production of seedlings of this species, and have become a line of research interest. Among the methods, air-layering is notable for integrating the rooting of a portion of the branch still connected to the matrix plant and improving the conditions for rhizogenesis (Dutra et al., 2012a). This type of propagation is feasible in several non-traditional fruits such as urucum (Montovani et al., 2007), marmelo (Pio et al., 2007), umbu (Dutra et al., 2012a) and litchi, which are the most used systems for commercial production of these species.

The success of this technique can be enhanced by the use of organic substrates, aiming to guarantee structural support, water retention, nutrient supply and the possibility of gas exchange, thus allowing the development of roots and quality seedling (Dutra et al., 2012b). Traditionally, sphagnum is used as a substrate in air-layering, due to its high water retention capacity and good aeration (Bitencourt et al., 2007). However, other alternative substrates, such as coconut powder and organic compound have been used (Dutra et al., 2012a) to search for easy availability of the seedlings producer and physico-chemical properties that accelerate the rooting of air-layering.

Besides the use of substrates as a factor to improve rhizogenesis, the application of plant regulators can also help in this process, especially exogenous synthetic auxins such as indol-3-butyric acid, which provides good results in air-layering rooting used in the propagation of fruit, ornamental and tree plants (Sasso et al., 2010; Chagas et al., 2012; Dutra et al., 2012a).

In view of the aforementioned, the objective of this work was to evaluate the influence of substrates and the use of indole-3-butyric acid (IBA) on air-layering rooting of tamarind tree for commercial production of seedlings.

MATERIALS AND METHODS

The experiment was carried out from August to November in 10-year-old tamarind trees located in Ilha Solteira/SP (20°24'04" S latitude, 51°20'55" W longitude and 320 m altitude). The climate of the region, according to Köeppen's classification (1948), is Aw type, tropical with dry winter season, presenting average annual temperature of ± 24.5°C, annual rainfall of ± 1,232 mm and air relative humidity of the ± 64.8%. The climatic conditions during the period of the experiment are shown in Figure 1.

The air-layering was randomly performed in the four quadrants of the tamarind plants, using branches with 5 mm diameter. The branches were ringed with the aid of ringing scissors all around the perimeter, at a distance of 40 cm from the apical end. At the annealed site, the concentrations of indole-3-butyric acid (500, 750, 1000 and 1250 mg L^{-1}) prepared in hydroalcoholic solution were applied with a brush.

To create a microclimate around the lesion favorable for the development of roots, the air-layering was wrapped with transparent polyethylene (PVC) bag containing the substrate and tied with nylon clamps at both ends to prevent its dehydration. Also, in this sense, the air-layering were moistened weekly with 15 ml distilled water.

Approximately 300 g of the following substrates were used in the preparation of air-layering: Organic compound based on Pinus bark, coconut powder, vermiculite, peat, charcoal, NPK and micronutrients (Basaplant®), sphagnum and coconut powder. The sphagnum was immersed for 24 h for water absorption and the other substrates were moistened with deionized water during the experimental implantation for easy handling.

The control treatment was done using branches of 5 mm diameter, ringed around the perimeter, with no IBA application and involving the substrates: organic compound, sphagnum and coconut powder. The experimental design was a randomized complete block in a 3 × 5 factorial scheme (substrates × IBA concentrations), containing 10 blocks, with each plant being considered as a block, and with 4 replicates (air-layering) per block distributed in the plants quadrants. As no block effect occurred, the experiment was analyzed as a completely randomized design.

The evaluations were done 90 days after the installation of the air-layering by collecting the following variables: 1) Callus formation (%): this evaluates the number of air-layering that form callus in the ring region; 2) rooted air-layering (%): it evaluates the number of air-layering with at least one root with more than 1 cm; 3) root length (cm); 4) number of roots and root dry matter (g). The averages obtained were subjected to the Dunnet test at a 5% probability, using SAS® v. 9.4 and analysis regression and Tukey test using SISVAR 5.3® (Ferreira, 2007).

RESULTS AND DISCUSSION

In Table 1, there is interaction between the factors tested

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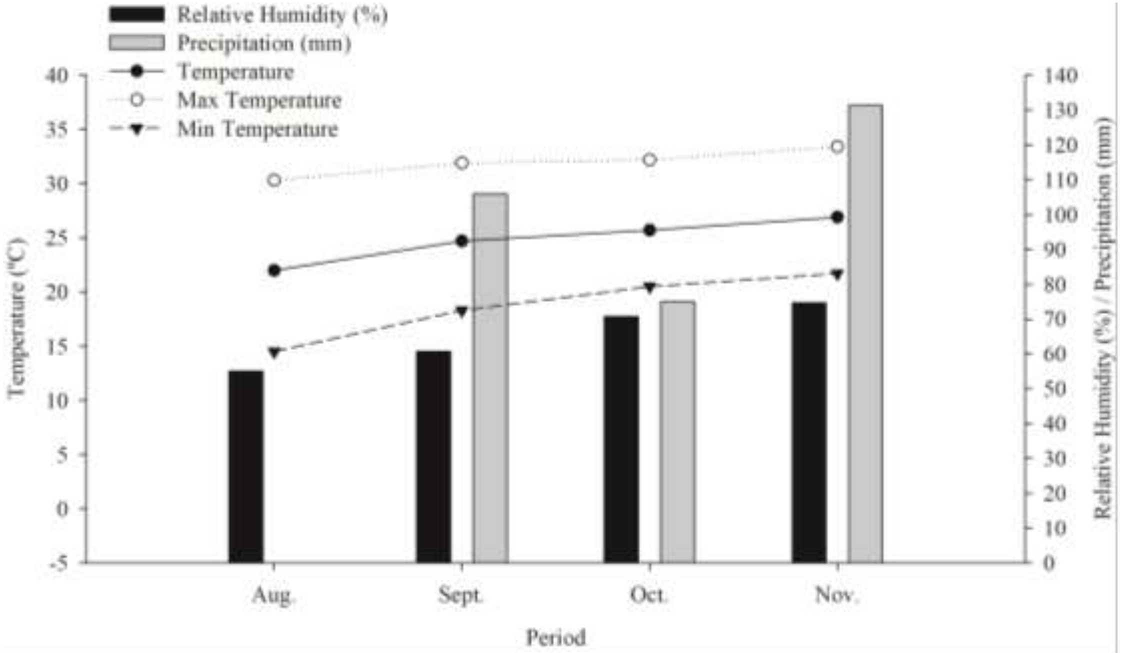

Figure 1. Meteorological data during the experiment installation period. Source: Weather Station – Area of Hydraulics and Irrigation, UNESP.

Table 1. Analysis of variance for air-layering rooted (AR), number of roots (NR), root length (RL) and root dry matter (RDM) of tamarind air-layering.

Treatments	AR (%)	NR	RL (cm)	RDM (g)
	p-value			
Substrates	0.0305**	0.6813^{ns}	0.0567^{ns}	<0.01*
IBA	0.3623^{ns}	0.4092^{ns}	0.7945^{ns}	<0.01*
Substrates x IBA	<0.01*	<0.01*	0.0327*	<0.01*
Substrates	Overall average			
Organic compound	30.00	1.05	2.06	0.87
Sphagnum	65.00	1.45	4.67	0.43
Coconut powder	40.00	1.15	2.38	0.26
IBA (mg L^{-1})	Overall average			
Control	25.00	0.67	2.17	0.25
500	58.33	1.83	4.03	0.56
750	50.00	1.08	2.89	0.75
1000	50.00	1.42	2.84	0.48
1250	41.67	1.08	3.24	0.28
Overall Average	45.00	1.22	3.04	0.51

*Significant (p≤0.01), **significant (p≤0.05) and ^{ns}non-significant (p≤0.05) at test F.

and the overall averages for the evaluated variables, with significant statistical interaction between the substrates and IBA concentrations. However, in some Brazilian fruits species such as umbu (*Spondias tuberosa* Arr. Cam.), this type of result was not observed (Dutra et al., 2012a).

For comparison of treatments with the control treatments, Dunnet test was performed; therefore, in Table 2, it is observed that for root dry matter, results were significantly different from the control treatment in the IBA concentrations: 500, 750 and 1000 mg L^{-1} with organic compound. The air-layering performed with sphagnum and IBA presented for root dry matter at concentrations of

Table 2. Dunnet test for air-layering rooting (AR), number of roots (NR), root length (RL) and root dry matter (RDM) of tamarind air-layering.

AIB (mg L^{-1})	AR (%)	NR	RL (cm)	RDM (g)
	Organic compound			
Control	0.00	0.00	0.00	0.00
500	75.00^{ns}	5.28^{ns}	3.00^{ns}	1.87^{*}
750	50.00^{ns}	4.00^{ns}	2.00^{ns}	1.95^{*}
1000	25.00^{ns}	1.00^{ns}	0.25^{ns}	0.29^{*}
1250	0.00^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}
	Sphagnum			
Control	75.00	6.50	2.00	0.74
500	100.00^{ns}	6.83^{ns}	2.50^{ns}	0.43^{*}
750	50.00^{ns}	2.75^{ns}	0.75^{ns}	0.19^{*}
1000	25.00^{ns}	$2,05^{ns}$	0.25^{ns}	0.07^{*}
1250	75.00^{ns}	5.23^{ns}	1.75^{ns}	1.02^{*}
	Coconut powder			
Control	0.00	0.00	0.00	0.00
500	0.00^{ns}	0.0^{ns}	0.00^{ns}	0.00^{ns}
750	50.00^{ns}	0.50^{ns}	1.93^{ns}	0.13^{*}
1000	100.00^{*}	3.75^{*}	5.48^{ns}	0.47^{*}
1250	50.00^{ns}	1.50^{ns}	4.50^{ns}	0.43^{*}

*Significant and ^{ns}non-significant by control for Dunnet test ($p \leq 0.05$).

500, 750, 1000 and 1250 mg L^{-1} had significant difference compared to the control. In coconut powder substrate, the concentration of 1000 mg L^{-1} of IBA provided significant difference in air-layering for root and number of roots; however, the root dry matter differed from the control treatment only at 750, 1000 and 1250 mg L^{-1} concentrations.

The sphagnum, as observed in this work, is the most indicated substrate for the production of seedlings, obtaining satisfactory results both as a control treatment and with the use of IBA. The same result was reported for ume (*Prunus mume* Sieb & Zucc) (Chagas et al., 2012), marmelo (*Chaenomeles sinensis* L.) (Pio et al., 2007) and jabuticaba (*Plinia cauliflora*) to provide a higher percentage of rooted air-layering. Lins et al. (2015) stated that in litchi, this substrate also presented the best results for this variable, without using plant regulators. For interaction between the factors, the analysis of variance in the regression for IBA doses was performed. For all variables analyzed, the regression deviation was not significant, showing that the model found is adequate.

As reported by Hartmann et al. (2011), for the initiation of adventitious roots, the divisions of the first root-initiating cells are of great importance for the presence of auxins, whether endogenous or exogenous. The use of auxins such as IBA is known to promote the development of adventitious roots, uniformity and success of rooting (Hartmann et al., 2011), and the tested concentrations influenced the percentage of air-layering rooting, number, length and dry matter of the root system (Figures 2 and 3).

Thus, it is observed that for the organic compound, the percentage of rooting (Figure 2a) and the number of roots (Figure 2b) of the air-layers showed descending linear regression with respect to the increase of IBA concentrations of 500 mg L^{-1} which provided the highest means for these variables.

In the quadratic equation of the sphagnum treatments, the minimum point observed for rooted air-layering percentage and number of roots was 925 mg L^{-1}, providing 30.75% (Figure 2a) and 915.39 mg L^{-1} with 0.32 roots (Figure 2b), respectively. The maximum point observed in the quadratic equations in treatments with coconut powder was 975 mg L^{-1} IBA with 85.25% of rooted air-layering (Figure 2a) and 1013.64 mg L^{-1} IBA with 2.46 roots formed (Figure 2b).

In Figure 3a, it is noted that the air-layering made of organic compounds with increasing concentrations of IBA linearly decreased the root length, and 500 mg L^{-1} of this regulator provided the greatest value for this variable. However, the dry matter of the roots adapted to quadratic regression, with the concentrations of 500 and 750 mg L^{-1} IBA (Figure 3b) being the most suitable for the addition of mass in the roots of tamarind air-layering.

For both length and dry matter of the roots in this experiment, the data were adjusted to quadratic regression, with the minimum points observed as 913.79 mg L^{-1} IBA, 1.86 cm long (Figure 3a) and 760 mg L^{-1} IBA with 0.23 g dry matter of the roots (Figure 3b).

The use of coconut powder as substrate with increasing concentrations of IBA linearly increased root

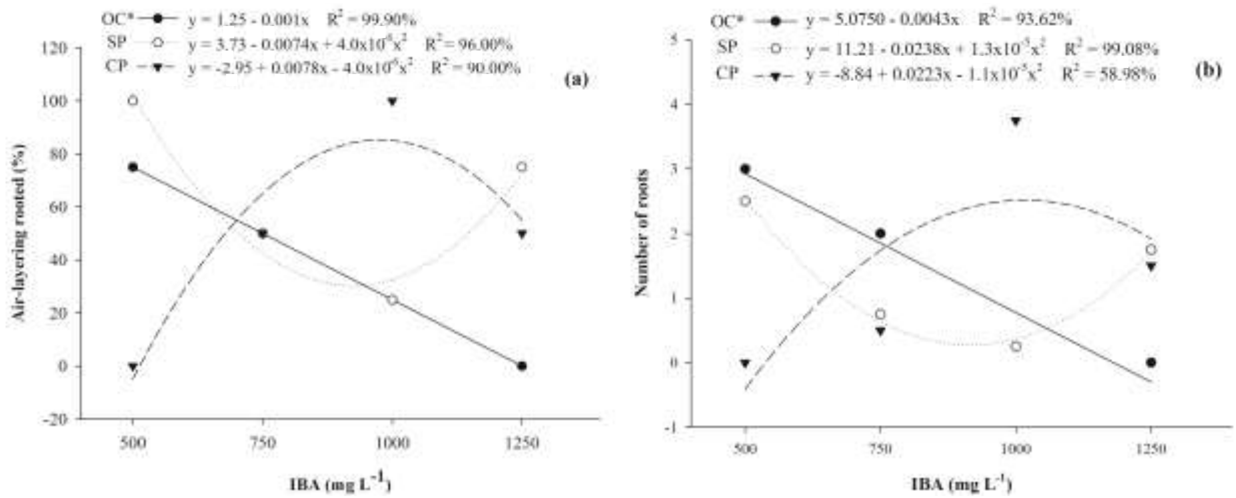

Figure 2. Air-layering rooted (%) and number of roots in tamarind air-layering. *(OC = organic compound; SP = sphagnum; CP = coconut powder).

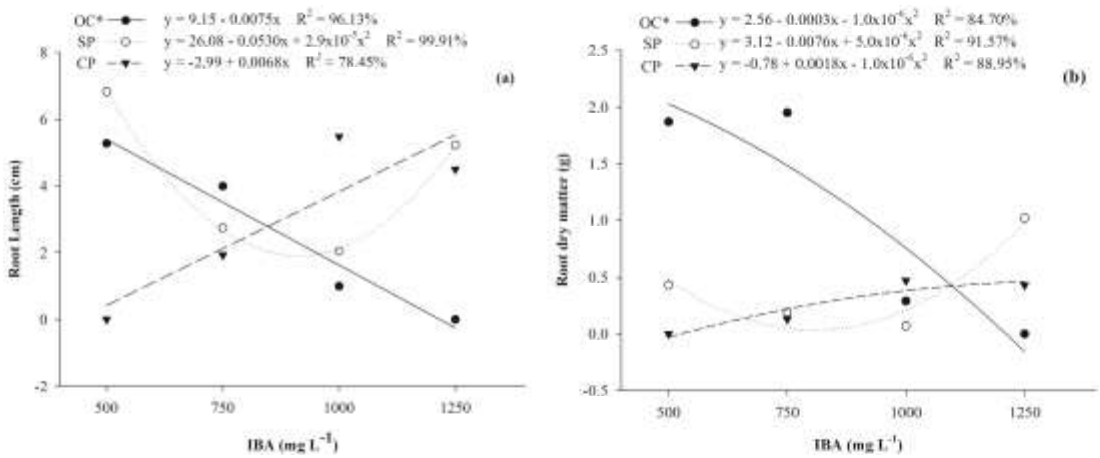

Figure 3. Root length (%) and root dry matter in tamarind air-layering. *OC = organic compound; SP = sphagnum; CP = coconut powder.

length and quadratic root dry matter. The highest root length in this substrate was observed at the concentration of 1000 mg L^{-1} IBA (Figure 3a) and the maximum point of 900 mg L^{-1} IBA provided 0.03 g of root dry matter (Figure 3b).

Smarsi et al. (2008) reported that organic substrates also had favorable results in the development of the root system in litchi air-layering, which was not expected since sphagnum is the most recommended for this type of propagation in this species. As observed in this experiment, in each substrate, the results obtained with the application of IBA provided different means (Table 3).

The organic compound favored the root development because it has nutritional supplements (NPK and micronutrients) as a source of nutrients for the roots growth and accumulation of dry matter. In this work, this substrate obtained higher values for the evaluated variables when low concentration of IBA (500 mg L^{-1}) was used (Figure 4a).

The use of coconut powder as a substrate for seedling production has been growing in the fruit growing sector due to its high porosity, high potential for moisture retention and biodegradability, even though it does not have a high nutritional content such as organic matter (Lins et al., 2015). The same author did not observe good results for this variable when coconut powder was compared with sphagnum in the litchi air-layering, demonstrating that this substrate was also not effective in this method. However, the concentration of 1000 mg L^{-1} of IBA provided good results in the tamarind air-layering when coconut powder was used (Figure 4b).

Sphagnum favored the root development at the 500 mg L^{-1} IBA (Figure 4c), corroborating with the results of Lins et al. (2015) who observed that sphagnum provided

Table 3. Tukey test for air-layering rooting, number of roots, root length and root dry matter of tamarind air-layering.

IBA (mg L^{-1})	Organic compound	Coconut powder	Sphagnum
		Air-layering rooted (%)	
500	$75.00^{ab1/a2}$	$0.00^{b/b}$	$100.00^{a/a}$
750	$50.00^{a/a}$	$50.00^{a/ab}$	$50.00^{a/a}$
1000	$25.00^{a/a}$	$100.00^{a/a}$	$25.00^{a/a}$
1250	$0.00^{a/a}$	$50.00^{a/ab}$	$75.00^{a/a}$
	Number of roots		
500	$3.00^{a/a}$	$0.00^{b/b}$	$2.50^{ab/a}$
750	$2.00^{a/a}$	$0.50^{a/b}$	$0.75^{a/a}$
1000	$0.25^{b/a}$	$3.75^{a/a}$	$0.25^{b/a}$
1250	$0.00^{a/a}$	$1.50^{a/b}$	$1.75^{a/a}$
	Root length (cm)		
500	$5.28^{ab/a}$	$0.00^{b/a}$	$6.83^{a/a}$
750	$4.00^{a/a}$	$1.93^{a/a}$	$2.75^{a/a}$
1000	$1.00^{a/a}$	$5.48^{a/a}$	$2.05^{a/a}$
1250	$0.00^{a/a}$	$4.50^{a/a}$	$5.23^{a/a}$
	Root dry matter (g)		
500	$1.87^{a/b}$	$0.00^{c/d}$	$0.43^{b/b}$
750	$1.95^{a/a}$	$0.13^{c/c}$	$0.19^{b/c}$
1000	$0.29^{b/c}$	$0.47^{a/a}$	$0.07^{c/d}$
1250	$0.00^{c/d}$	$0.43^{b/b}$	$1.02^{a/a}$

[1]Averages followed by the same letter for columns and [2]Averages followed by the same letter in the rows do not differ by Tukey test ($p \leq 0.05$).

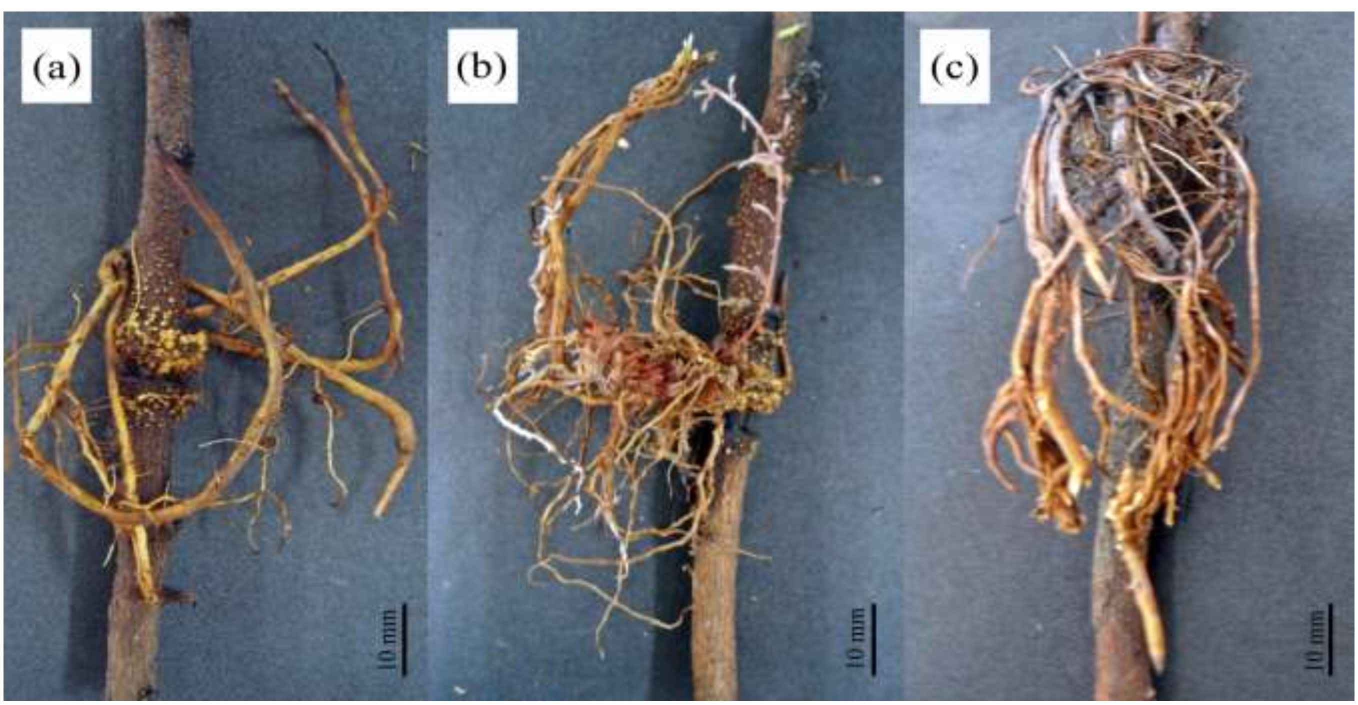

Figure 4. Formation of adventitious roots in tamarind air-layering in organic compound (IBA 500 mg L^{-1}) (a), coconut powder (IBA 1000 mg L^{-1}) (b) and sphagnum (IBA 500 mg L^{-1}) (c).

greater number and length of roots in litchi air-layering. Dutra et al. (2012a) affirmed that this favoring is important, since the quality of the root system directly influences the survival of the seedlings in the field, especially in less favorable periods.

The good results obtained with the use of sphagnum in the tamarind tree, besides the physical characteristics of the substrate and the use of IBA to promote rooting, are also correlated to the photosynthetic products such as carbohydrates produced by the plant. The availability and use of these substances by the branches favors rooting, since instead of being redirected to other areas such as for flowering and fruiting, these compounds are used by the cells to develop the roots (Maurya et al., 2013). It is worth mentioning that the tamarind plants were in excellent cultivation conditions, well-nourished and with availability of water in the soil, which are influential characteristics in the good rooting of air-layering. This difference in rooting in the substrates and IBA concentrations tested has a great applicability for the producer, since the latter may have options to perform the air-layering according to the availability of the substrate in the region. Also, the efficiency of the air-layering can be correlated with the period that the propagation was done. Maurya et al. (2013) reported that the increase in temperature, relative humidity and the beginning of precipitation during the period of air-layering can provide plant exposure to a continuous supply of water, avoiding dehydration of the branches and positively influencing rhizogenesis.

In addition to influence of availability of water on the rooting of air-layering, Hartmann et al. (2011) reported that increase in temperature favors cell division, thus helping in root formation, which indicates that the season may have been favorable for the propagation of tamarind tree by this method due to the increase in temperature and beginning of the rainy season in the region (Figure 1).

Conclusions

Propagation using the method of air-layering is viable. For organic compound and coconut powder, the concentrations of 500 and 1000 mg L^{-1} of IBA, respectively may improve the results obtained by air-layering. With the use of sphagnum as substrate, 500 mg L^{-1} of IBA is indicated to maximize the results in this method.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth, yield and fruit quality of 'Chimarrita' peach trees grafted on different rootstocks

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Rootstocks influence the performance and characteristics of fruit trees. However, there is still insufficient information on the relation of rootstocks in mainly cultivars of peach trees grown in Brazil. Therefore, this study aims at evaluating the performance of eight rootstocks: Aldrighi, Capdeboscq, Flordaguard, Nemaguard, Okinawa, Umezeiro, Tsukuba and Seleção Viamão grafted by 'Chimarrita' peach tree, planted in 2006 and evaluated in the 2013/2014, 2014/2015 and 2015/2016 in Capão do Leão, RS, Brazil. The following dependent variables were evaluated on the field: floral phenology, affinity with rootstocks, trunk diameter, crown volume, number of fruits, fruit mass and productivity. Fruit mass, diameter, color, pulp firmness, total soluble solid content, titratable acidity, total phenolic content, total carotenoid content, L-ascorbic acid content and antioxidant activity were evaluated. The Umezeiro rootstock provided the lowest values for crown volume, trunk diameter, number of fruits and productivity. The blossoming period of peach trees can be early or delayed depending on rootstock and harvest seasons. Field compatibility constant of Chimarrita cultivar was higher with the rootstocks of Capdeboscq, Okinawa, Tsukuba and Aldrighi. Fruits of plants grafted on Capdeboscq and Okinawa rootstocks showed reddish epidermis. The Okinawa rootstock enabled 'Chimarrita' peaches to have higher soluble solid concentrations and L-ascorbic acid. The highest concentrations of total carotenoids were verified in the pulp of fruits obtained from the 'Chimarrita' cultivar grafted on Aldrighi, Okinawa, Tsukuba and Umezeiro. The antioxidant activity of the peach pulp was higher on the Capdeboscq rootstock.

Key words: *Prunus persica* (L.) Batsch, vegetate-productive behavior, phytochemical.

INTRODUCTION

In order to improve peach tree cultivation, its management must be improved with regards to soil preparation, correction and maintenance fertilization, irrigation, phytosanitary control and phytotechnical

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management, besides combinations of crown and rootstock cultivars. There is a clear consensus about the need for new studies on rootstocks which aim at increasing production and improving peach quality in Brazil. The main characteristics that rootstocks need to have are control of vigor, fruit size, fruit quality (Neves et al., 2017), productivity and the ability to resist adverse soil and climate conditions.

In Brazil, most peach orchards use seed-bearing rootstocks, traditionally from the canning industry. However, these materials are genetically variable, leading to a lack of uniformity in the plants and hindering orchard management (Barreto et al., 2017). Both Aldrighi and Capdeboscq are the most commonly used rootstocks in the south of Brazil since it is easy to get their stones from industries to grow seedlings (Rocha et al., 2007). However, other rootstocks such as Flordaguard, Nemaguard, Okinawa, Umezeiro, Tsukuba and Seleção Viamão have been used as alternatives in terms of fruit adaptation, productivity and quality (Comiotto et al., 2012, 2013; Galarça et al., 2013).

Studies on crown and rootstock combination in peach trees have shown problems with regards to affinity between these materials. Lack of affinity affects plant growth and development, decreases orchard productivity over time and causes the death of adult plants, besides early death (Pereira et al., 2015). Incompatibility has been an important fact in fruit farming; although there is demand for new rootstocks and crowns, the big challenge is to find adapted genetic material which can multiply easily and seedling production which assures longevity and productivity (Comiotto et al., 2013).

In general, several studies have shown that fruit plants may be influenced by rootstocks because they can affect plant vigor, growth and development besides productivity (Picolotto et al., 2009; Galarça et al., 2013) and fruit quality parameters, such as soluble solids, antioxidants, total phenols, ascorbic acid and flavonoids in the fruit pulp (Remorini et al., 2008; Orazen et al., 2011; Forcada et al., 2013). Therefore, studies on different rootstocks are needed to determine alternatives to replace the most commonly used ones in South Brazil, so that compatible and productive combinations can be established, fruit quality can be achieved and economic benefits can be assured to producers. This is the context of this study, which aims at evaluating eight rootstocks grafted in the 'Chimarrita' cultivar.

MATERIALS AND METHODS

Location and climatic conditions

The experiment was conducted in a teaching orchard at the Palma Agricultural Center (Centro Agropecuário da Palma; CAP), Federal University of Pelotas (Universidade Federal de Pelotas) in Capão do Leão municipality, Rio Grande do Sul (RS) with Latitude 31°52'00" S and Longitude 52°21'24" W, Brazil. According to the classification of Köppen, the climate of the region is type Cfa, namely humid and temperate with hot summers. The region has an average annual rainfall and temperature of 1500 mm and 17.9°C, respectively. The experiment was conducted during the 2013/2014, 2014/2015 and 2015/2016 seasons.

Treatments and experimental design

The peach orchard used consisted of the *P. persica* (L.) Batsch Chimarrita cultivar grafted in Aldrighi, Capdeboscq, Flordaguard, Nemaguard, Okinawa, Umezeiro, Tsukuba and Seleção Viamão rootstocks. The orchard was planted in 2006 with a "V" type plant-organization system with 5 m of space between rows and 1.5 m between plants, amounting to a density of 1,333 plants ha^{-1}. The experimental design involved randomized blocks, with each plot being composed of five plants. The plants at the ends of each plot were disregarded, amounting to nine useful plants.

Traits evaluated

The variables evaluated in the field were: trunk diameter (mm), measured at 20 cm above the soil surface in two transverse positions with the aid of a digital caliper; canopy volume (m^3) was calculated using the equation:

$$V = [(L/2) \times (E/2) \times A \times \pi]/3,$$

where V is the volume of the canopy, L is the distance between the main branches, E is the average thickness of the main branches, and A is the height of the canopy.

Field compatibility coefficient (FCC) was calculated using the equation:

$$\text{Affinity coefficient} = [(C/A)+(C+A)/2B]+10$$

where A is the trunk diameter above the grafted point, B is the trunk diameter at the grafted point and C is the diameter of the trunk under the grafted point, considering that parameters A and C were measured 10 cm above and 10 cm below the grafted point (Gokbayrak et al., 2007).

Flowering was evaluated by subperiods including early flowering when 10% of the flowers were open, full bloom when 50 to 70% of the flowers were open, late flowering when the petals were falling, and early sprouting when 10% of the fruit were formed. Fruits were monitored until harvest time when the number of fruit per plant (fruit $plant^{-1}$) and productivity (t ha^{-1}) were evaluated to determine production amounts.

After harvesting, fresh weights (grams) of the peaches were evaluated by digitally weighing 20 fruit per repetition, totaling 60 fruits. The firmness of the pulp (Newtons), was measured at two opposite points in the equatorial region of peeled fruit using a manual penetrometer (model 53205, TR TURONI-Italy) with an 8 mm tip; color of the epidermis was examined using a colorimeter type Minolta CR-300®with a D65 light source that measured "L" (luminosity), "a*", "b*", and hue or chromatic tonality represented by "hue angle"; soluble solids (ºBrix) were measured using an Atago digital refractometer®; and titratable acidity (% citric acid) was quantified in 10 mL of juice diluted in 90 mL of distilled water and titrated with 0.1 mol L NaOH solution to pH 8.1 with the aid of Quimus pHmeter®.

The following analyses were carried out in order to determine the main phytochemical groups or compounds of the peach pulp: total phenolic compounds (mg gallic acid per 100 g of sample) were determined according to the adapted method of Singleton and Rossi (1999) using the reaction with the Folin-Ciocalteau reagent; total carotenoids (mg β-carotene in 100 g of sample) were

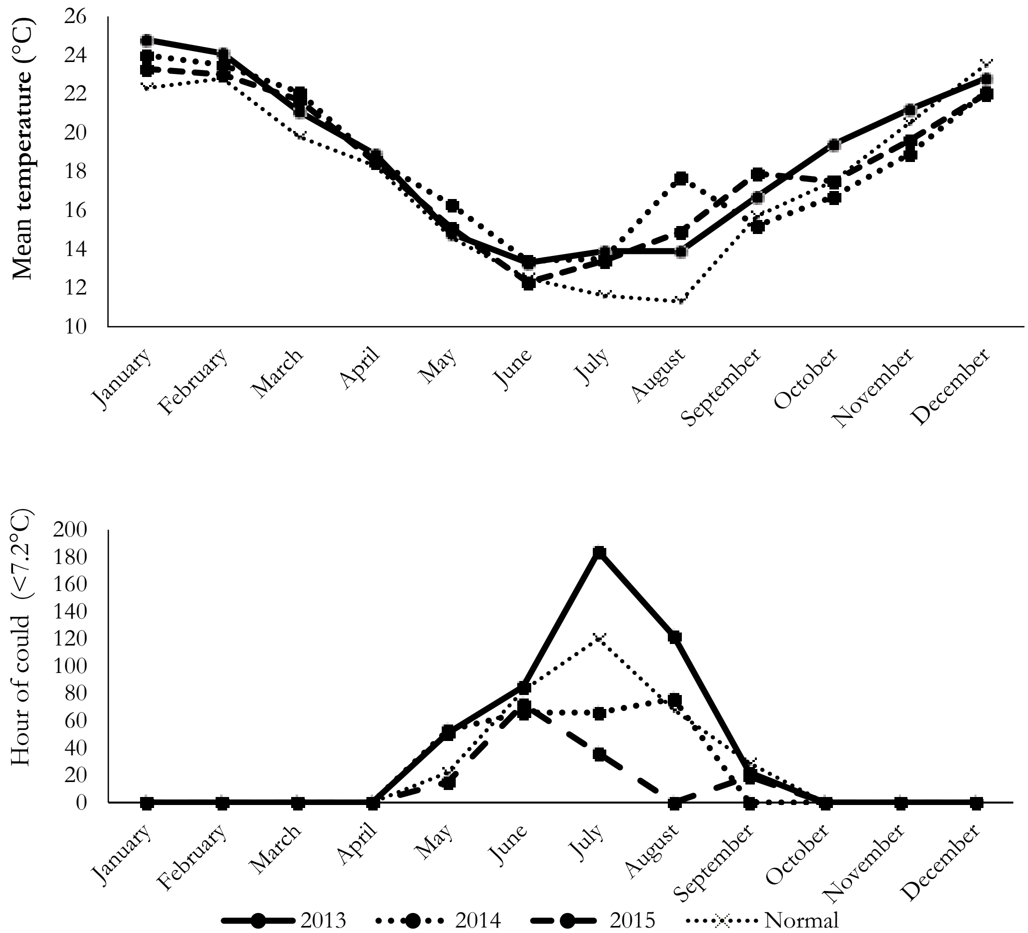

Figure 1. Mean temperature and hour of cold in 2013, 2014 and 2015 and normal ones in Capão do Leão, Rio Grande do Sul, Brazil, Terras Baixas Experimental Station. Embrapa Clima Temperado.

determined using the modified method of AOAC (2005) and an extraction solution (hexane-acetone-alcohol-toluene); and antioxidant activity (mg trolox per 100 g fresh weight) was determined using the radical DPPH method adapted from Brand-Williams et al. (1995); and L-ascorbic acid determined by high-performance liquid chromatography (HPLC), with HPLC Shimadzu system equipped with an automatic injector and a UV-VIS detector (254 nm), in agreement with AOAC (2005) method, and the results are expressed as mg L-ascorbic acid/100 g fresh sample.

Statistical analysis

The data were evaluated for normality (Shapiro-Wilk test) and then subjected to a variance analysis (F-Test), with the means compared using Tukey's range test to identify significant differences ($p \leq 0.05$).

RESULTS AND DISCUSSION

In the 2013/2014 and 2014/2015 seasons, the development and blooming of 'Chimarrita' peach trees occurred in weather conditions similar to the traditional ones in the region (South Rio Grande do Sul, Brazil) where the experiment was carried out (Figure 1). In these crops, blooming was uniform and concentrated, that is, at the end of July. However, in the 2015/2016 season, it was strongly affected by the El Niño phenomenon and the behavior of the 'Chimarrita' cultivar was characterized by non-uniform blooming on all rootstocks.

Peach trees grafted on Aldrighi, Capdeboscq, Flordaguard, Nemaguard, Tsukuba and Seleção Viamão began blooming (BB) on July 25th, 2013. It occurred five days before Okinawa and Umezeiro started blooming (Table 1). Full blooming (FB) of peach trees occurred on August 15th, 2013, except in the cases of Okinawa and Tsukuba, whose FB occurred seven days earlier (August 8th, 2013). Blooming ended (EB) on August 29th, 2013, except in the cases of Capdeboscq and Nemaguard, whose EB occurred eight days earlier.

The peach trees grafted on Capdeboscq, Flordaguard and Tsukuba began blooming on July 28th, 2014, eight days before the beginning of full blooming of the other

Table 1. Beginning (B), full bloom (FB), end of blooming (EB) and fruit growth of the 'Chimarrita' cultivar grafted on different rootstocks in Capão do Leão, Rio Grande do Sul, Brazil in 2013/2014 and 2014/2015 seasons.

Rootstock	2013			2014		
	B	FB	EB	B	FB	EB
Aldrighi	25/July	15/August	29/August	05/August	12/August	26/August
Capdeboscq	25/July	15/August	21/August	28/July	12/August	19/August
Flordaguard	25/July	15/August	29/August	28/July	19/August	26/August
Nemaguard	25/July	15/August	21/August	05/August	19/August	26/August
Okinawa	30/July	08/August	29/August	05/August	12/August	26/August
Tsukuba	25/July	08/August	29/August	28/July	12/August	19/August
Umezeiro	30/July	15/August	29/August	05/August	12/August	19/August
Seleção Viamão	25/July	15/August	29/August	05/August	12/August	26/August

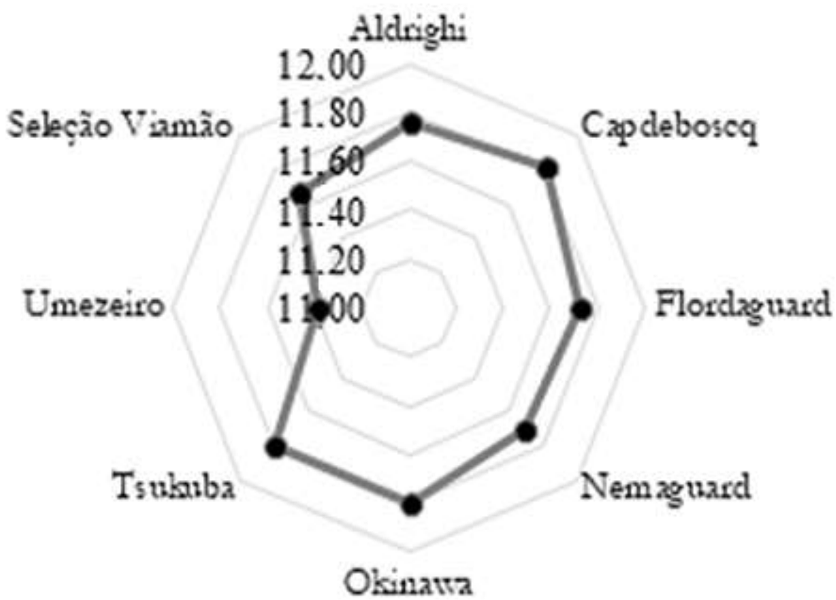

Figure 2. Field compatibility constant of the 'Chimarrita' peach tree cultivar grafted on different rootstocks in the municipality of Capão do Leão, Rio Grande do Sul, Brazil, during the 2013/2014, 2014/2015 and 2015/2016 seasons.

rootstocks. Full blooming of peach trees occurred on August 12th, 2014, except in the case of Flordaguard and Nemaguard, whose FB occurred on August 19th, 2014. Even though differences in blooming were found among the rootstocks, fruit maturation was similar, regardless of the rootstock, that is, maturation peaks occurred in December 4th, 2013 and November 30th, 2014.

In the region where the experiment was carried out, blooming of the 'Chimarrita' cultivar historically occurs in the second week in August (Raseira et al., 2014). Blooming of peach trees occurred irregularly, that is, one of the first blooming periods even occurred two months before the normal period. First blooming began on 16th June, 2015 on Flordaguard, Nemaguard, Okinawa and Umezeiro and on 20th June, 2015 on the other rootstocks. After the first blooming, there was floral abortion on all rootstocks due to temperature oscillation. As a result, the plants had their phenological phases modified. The second blooming occurred at the end of July 2015. None of the rootstocks contributed to production losses in the conditions of the El Niño phenomenon.

Analysis of field compatibility constant of the 'Chimarrita' cultivar– the measure of the affinity between the rootstock and the crown cultivar, showed that the values were close to 12 in the case of Capdeboscq (11.82), Okinawa (11.80), Tsukuba (11.80) and Aldrighi (11.76) rootstocks (Figure 2). According to Gokbayrak et al. (2007), when the value is close to 12, the affinity between the crown cultivar and the rootstock on the grafted point is high.

Regarding field compatibility constant, the Umezeiro

Table 2. Trunk diameter (TD), crown volume (CV), number of fruits per plant (NF), estimated productivity (EP) and fruit mass (FM) of 'Chimarrita' peach trees on different rootstocks in the municipality of Capão do Leão, Rio Grande do Sul, Brazil, for the 2013/2014, 2014/2015 and 2015/2016 seasons.

Rootstocks	TD (mm)	CV (m³)	NF (n°)	EP (t.ha^{-1})	FM (gramas)
			2013/2014		
Aldrighi	82.04^{a}	3.98^{a}	116^{b}	17.17^{b}	110.33^{bc}
Capdeboscq	79.52^{ab}	3.59^{a}	115^{b}	18.69^{ab}	120.40^{ab}
Flordaguard	85.56^{a}	2.38^{ab}	109^{b}	19.64^{ab}	130.30^{a}
Nemaguard	80.78^{a}	3.06^{a}	152^{a}	17.70^{ab}	97.90^{c}d
Okinawa	76.84^{ab}	2.10^{ab}	116^{b}	14.02^{c}	90.67d
Tsukuba	83.73^{a}	2.17^{ab}	136^{a}	21.49^{a}	104.13^{cd}
Umezeiro	70.56^{b}	1.50^{b}	79^{c}	10.37d	98.63^{cd}
Seleção Viamão	84.37^{a}	2.37^{ab}	135^{a}	18.92^{ab}	105.87^{cd}
CV (%)	4.91	16.66	5.28	4.98	4.90
			2014/2015		
Aldrighi	82.55^{ns}	3.71^{a}	97^{b}	13.45^{ab}	103.05^{ns}
Capdeboscq	85.40	3.82^{a}	144^{ab}	15.91^{ab}	82.52
Flordaguard	93.16	3.51^{a}	111^{ab}	13.42^{ab}	87.82
Nemaguard	84.24	3.44^{a}	107^{ab}	12.64^{ab}	88.27
Okinawa	83.61	3.06^{a}	116^{ab}	14.63^{ab}	95.20
Tsukuba	85.47	3.26^{a}	157^{a}	20.20^{a}	83.47
Umezeiro	79.33	1.69^{b}	83^{c}	9.66^{b}	98.05
Seleção Viamão	86.46	3.00^{a}	118^{ab}	12.52^{ab}	86.37
CV (%)	7.71	15.6	17.5	24.4	19.90
			2015/2016		
Aldrighi	98.06^{a}	2.20^{a}	-	-	-
Capdeboscq	94.34^{ab}	2.40^{a}			
Flordaguard	105.2^{a}	2.13^{a}			
Nemaguard	98.38^{a}	2.27^{a}			
Okinawa	94.38^{ab}	2.23^{a}			
Tsukuba	91.53^{ab}	2.16^{a}			
Umezeiro	80.70^{b}	1.82^{b}			
Seleção Viamão	96.61^{a}	2.13^{a}			
CV (%)	6.91	15.3			

Means followed by the same small letter do not differ by the Tukey's test at 5% error probability. VC (%) = variation coefficient. ns = not significant. (-) Peach trees did not yield.

rootstock had values which were far less than 12 (11.39), a fact that shows incompatibility on the grafted point and excessive development below the point of union. Besides, not only its trunk diameter (2013/2014 and 2015/2016) but also its crown volume (in all three crops) was observed to be smaller (Table 2). This rootstock influenced the crown volume, vigor and diameter of 'Chimarrita' peach trees. It may have occurred as a result of the incompatibility of the rootstock and the crown cultivar, which decreases the transport of water and nutrients and reduces the leaf area and the crown volume (Tombesi et al., 2011). Incompatibility between the Umezeiro rootstock and the 'Chimarrita' cultivar, besides smaller trunk diameter, was also observed in other regions in Rio Grande do Sul state by Comiotto et al. (2013), Galarça et al. (2013) and Pereira et al. (2015).

Regarding plant growth, the trunk diameter of 'Chimarrita' peach trees was observed to vary among crops and rootstocks, but in 2014/2015 season, there was no difference among rootstocks. In 2013/2014 and 2015/2016 seasons, the largest trunk diameters were observed in Flordaguard, Nemaguard, Aldrighi and Seleção Viamão rootstocks. They did not differ statistically from Okinawa, Tsukuba and Capdeboscq.

The Umezeiro rootstock made the 'Chimarrita' cultivar to yield a small number of fruits and offered low productivity in 2013/2014 and 2014/2015 seasons. In the same orchard, Comiotto et al. (2012) observed that the

Table 3. Epidermis color, pulp firmness, soluble solids, total phenols, ascorbic acid, total carotenoids and antioxidant activity of 'Chimarrita' peach trees on different rootstocks in the municipality of Capão do Leão, Rio Grande do Sul, Brazil, for the 2014/2015 season.

Rootstock	Epidermis color (°Hue)	Pulp firmness (Newtons)	Soluble solids (°Brix)	Total phenols¹
Aldrighi	75.87ᵃ	25.13 ns	11.73bc	52.66ab
Capdeboscq	68.76b	22.69	12.23bc	58.73ab
Flordaguard	75.00a	23.66	11.06c	50.87b
Nemaguard	79.64a	21.19	11.20c	50.56b
Okinawa	65.94b	18.43	14.20a	63.74a
Tsukuba	79.94a	18.03	11.06c	60.70ab
Umezeiro	74.98a	23.25	12.23bc	47.57b
Seleção Viamão	75.10a	24.03	13.23bc	48.58b
CV (%)	7.44	16.79	4.90	7.89

	Ascorbic acid²	Total carotenoids³	Antioxidant activity ⁴
Aldrighi	0.57d	29.60a	188.86b
Capdeboscq	0.95ab	14.34b	235.61a
Flordaguard	0.73c	15.38b	157.79c
Nemaguard	0.69cd	14.80b	146.98c
Okinawa	1.02a	29.63a	202.68b
Tsukuba	0.69cd	20.04ab	147.25c
Umezeiro	0.75c	18.50ab	200.35b
Seleção Viamão	0.81bc	13.40c	138.39c
CV (%)	6.68	17.79	5.77

¹mg galic acid equivalent 100 g^{-1} fresh weight; ²mg L-ascorbic acid 100 g^{-1} fresh weight; ³mg β-carotene equivalent 100 g^{-1} fresh weight; ⁴mg trolox equivalent 100 g^{-1} fresh weight.

Umezeiro rootstock with the 'Chimarrita' cultivar had low productivity in 2009. The fact that this rootstock leads to low productivity of peach trees may be due to the incompatibility between the grafted point and the crown cultivar.

Productivity of 'Chimarrita' peach trees was higher on Tsukuba, Flordaguard, Seleção Viamão, Capdeboscq and Nemaguard rootstocks in 2013/2014 season. Their productivity was higher on Tsukuba rootstock, even though it did not differ from the other rootstocks, except in the case of Umezeiro, in the 2014/2015 season. Both crops had lower productivity of 'Chimarrita' peach trees on the Umezeiro rootstock.

Peach mass was larger on plants grown on Flordaguard rootstock in comparison with the other rootstocks in 2013/2014 season. However, there was no difference in this variable in 2014/2015 season. Galarça et al. (2013) observed that seven rootstocks did not change fruit mass of 'Maciel' and 'Chimarrita' cultivars in three crops.

In 'Chimarrita' peach trees, rootstocks did not change pulp firmness (Table 3). However, the color of 'Chimarrita' peach epidermis depended on the rootstocks. Fruits of Tsukuba, Nemaguard, Aldrighi, Seleção Viamão, Flordaguard and Umezeiro rootstocks had cream and greenish epidermis, thus, °hue values were high. Fruits of plants grafted on Capdeboscq (75.87 °hue) and Okinawa (65.94 °hue) rootstocks were cream and reddish. Red epicarp is desirable in fruits which are consumed *in natura*, such as the ones of the 'Chimarrita' cultivar.

The 'Okinawa' rootstock enabled 'Chimarrita' peaches to have higher soluble solid concentrations than the others. It is relevant, since consumers prefer sweetish fruits, an advantage of 'Chimarrita' cultivar grafted on Okinawa rootstock, which also provided high values of total soluble solids in the cases of Talismã and Tropical cultivars (Montes et al., 2008).

Variation in concentrations of the main groups of phytochemical compounds (total phenols, L-ascorbic acid, total carotenoids and antioxidant activity) depended on the rootstocks on which the 'Chimarrita' cultivar was grafted. Peaches with the highest content of phenolic compounds were yielded by plants grafted on the Okinawa rootstock, whereas the lowest one was found in plants grafted on Flordaguard, Nemaguard, Seleção Viamão and Umezeiro rootstocks. Gil et al. (2002) reported that phenolic compounds are the main sources of antioxidants in peaches and attributed the amount of these compounds to both the crown cultivar and the rootstock.

Regarding the content of L-ascorbic acid, there were also differences among the rootstocks. Its highest concentrations were found in peaches yielded by plants grafted on the Okinawa rootstock, whereas the lowest ones were found on Aldrighi, Nemaguard and Tsukuba rootstocks. The highest concentrations of total carotenoids were found in the pulp of fruits yielded by the 'Chimarrita' cultivar grafted on Aldrighi and Okinawa rootstocks, whereas the lowest ones referred to the Seleção Viamão rootstock. The antioxidant activity of the peach pulp was higher on the Capdeboscq rootstock than on the others.

Variation in fruit phytochemicals among rootstocks may be due to the capacity of plants to absorb nutrients which are available in the soil and efficiency in converting the assimilated compounds to fruits. Phytochemicals are affected by combinations between rootstocks and crown cultivars. However, the phytochemicals under analysis do not have similar behavior. Besides the rootstocks, other factors such as harvest conditions, position of fruits in the plant, genotype (Forcada et al., 2013), weather conditions, off season period and maturation stage (Daza et al., 2008), can affect fruit quality.

Conclusion

The Umezeiro rootstock reduces the crown volume, trunk diameters, number of fruits and productivity of the 'Chimarrita' cultivar in south Brazil. Fruits of plants grafted on Capdeboscq and Okinawa rootstocks showed reddish epidermis. The Okinawa rootstock enabled peaches to have higher soluble solid concentrations and L-ascorbic acid. The highest concentrations of total carotenoids were verified in the pulp of fruits yielded by the 'Chimarrita' cultivar grafted on Aldrighi, Okinawa, Tsukuba and Umezeiro. The antioxidant activity of the peach pulp was higher on the Capdeboscq rootstock.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physical properties of soil and productivity of maize intercropped with different cover plants

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The system of intercropping maize with other plant species has been a common practice for several years, but its use has been increasingly mainly to improve the quality of soil physical properties of no-tillage system. The objective of this study was to investigate the effect of intercropping using different cover plants on the physical properties of soil and the productivity of maize. Field experiments were conducted on the property of Mr. Arno Paulo Deimling, located in Linha São João, in the municipality of Quatro Pontes-PR. The experiment was carried out using a randomized complete block design, with four treatments and five repetitions. The treatments were winter maize intercropped with *Urochloa brizantha*, *Urochloa ruziziensis* or black oats (*Avena strigosa*), and maize sown in monoculture. In winter period, the production of maize used for silage and the production of dry matter from cover crops were assessed. The physical properties of soil samples were also investigated. Cover crops did not influence the productivity of winter maize used for silage, but it promoted improvements in soil macroporosity. The cover crops promoted improvements in the macroporosity of the soil and reduction at soil penetration resistance, particularly in the area planted with *U. brisantha*, demonstrating its potential in improving water infiltration and soil aeration.

Key words: Direct seeding system, green manures, macroporosity, soil penetration resistance, conservationist system.

INTRODUCTION

Increasing agricultural production without adversely affecting the environment is a big challenge for technology. The use of management practices and soil conservation is one of the means of maintaining or improving production systems. Among the management practices employed is the no-tillage system, the use of green manure and intercropping systems (Board and Modali, 2005).

Intercropping between grain and forage plants is possible due to the time and space differential in the

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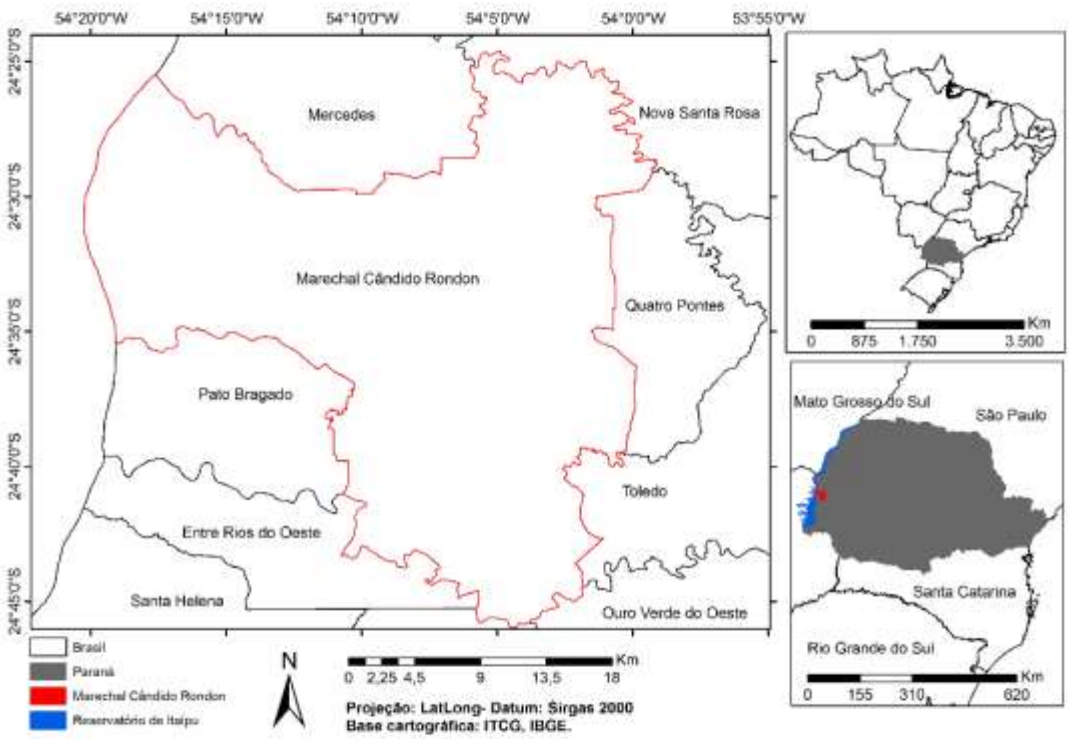

Figure 1. Location of Marechal Cândido Rondon/Paraná/Brazil.

accumulation of biomass between the species (Kluthcouski and Yokoyama, 2003). In the management system with intercropping plants, there is a higher production of dry mass of the aerial part, and of the root system. The roots release exudates and mucilage; involving the soil particles promoting their aggregation, reflecting on the increase of bulk density, macroporosity, aeration and infiltration of water in the soil. The objective of this system is to maintain the physical, chemical and biological properties of the soil, which reflects in the good development of the intercropped commercial culture, as well as in successor crops (Tirloni et al., 2012).

In the western region of Paraná, farmers carry out diverse agricultural activities, including pig farming, dairy farming, beef cattle, and cultivation of grains, mainly soybean and maize. It is a common practice to produce maize silage, which is used to feed dairy cattle. With silage production and even with grain production, there is insufficient plant cover for a no-tillage system (Mendonça et al., 2014) due to the transportation of plant material during the silage process. This leaves the soil completely unprotected, making it more susceptible to erosion, compaction and degradation. To help reverse the physical degradation of the soil, as well as avoid the loss of crop productivity, numerous soil management practices are recommended, such as no-tillage, green manuring, intercropping and crop rotation (Andreola et al., 2000).

According to Castoldi et al. (2011), when maize is associated with cover crops such as oats (*Avena* species) and Brachiaria, this provides a sustainable production system. In this system, management and conservation ensure that natural resources are used in a more appropriate way.

In addition, this system is considered to be economically viable. The maize crop is one of the most favorable to the practice of the intercropping, due to plant height and height of spike insertion. Therefore, maize harvesting, either as dry grain or as silage, can be carried out without interfering with the development of forage plants (Alvarenga et al., 2006).

The objective of this experiment was to evaluate the effect of intercropping maize with different cover plants on soil porosity, soil penetration resistance, as well as maize yield.

MATERIALS AND METHODS

Description and location of the experimental site

The experiment was conducted on a commercial property belonging to Mr. Arno Paulo Deimling, located in the São João Line, municipality of Quatro Pontes-PR, coordinates 24°34'8.32 "S 54°0'2.46" O (Figure 1), during the season 2014 to 2015. The soil was classified as an Oxisol with clayey texture (Santos et al., 2013). Before the planting started, samples of soils were collected at a depth of 0 to 0.20 m, for the purpose of chemical characterization. The analyses were performed according to the methodology described by Donagema et al. (2011), and soil granulometry according to the recommendations described by Santos et al. (2013).

Soil sampling and analyses of chemical properties

Before the experiment started, soil samples were collected at a depth of 0 to 0.20 m, and analysed for chemical properties and particle size characteristics. Chemical and physical analyses were performed according to the methodology proposed by Raij et al. (2001). Clay content was 532.5 g kg^{-1}, silt was 422.62 g kg^{-1} and sand was 44.88 g kg^{-1}. The results of chemical analysis of soil samples are as follows:

pH ($CaCl_2$) 4.83; organic matter 28.71 g dm^{-3}; P ($Mehlich^{-1}$) 45.77 mg dm^{-3}; Ca^{2+} 5.84 $cmol_c$ dm^{-3}; Mg^{2+} 2.02 $cmol_c$ dm^{-3}; K^+ 0.46 $cmol_c$ dm^{-3}; Al^{3+} 0.00 $cmol_c$ dm^{-3}; and base saturation (V%) 59.09%.

Experimental design and treatments

The experiment was conducted using a randomized complete block design, with four treatments and five replicates. The treatments used were maize intercropped with *Brachiaria brizantha* (*Urochloa brizantha*), *Brachiaria ruziziensis* (*Urochloa ruziziensis*) and black oats (*Avena strigosa*) and maize sown alone (control).

Soil sampling and analyses of porosity, bulk density and soil penetration resistance

The evaluation of the total porosity, macroporosity, microporosity, soil bulk density and soil resistance to penetration were made after desiccation of the cover plants. Physical analysis of soil samples was carried out using the methodology proposed by Santos et al. (2013).

Soil samples were collected with their structure preserved using metallic rings of known volume, at depths of 0 to 0.10 m and 0.10 to 0.20 m. After collection, samples were saturated for 24 h in a tray containing water at a depth of up to two-thirds of the ring height.

After the saturation period, samples were drained at the potential equivalent to -0.006 MPa using a tension table method. The macropores volume was estimated as the difference between the water content of the saturated soil and the water content of the soil after applying the potential of -0.006 MPa.

The micropores volume was calculated as the water content retained at the potential of -0.006 MPa. The total porosity was calculated as the sum of macroporosity and microporosity. Soil bulk density was determined using the volumetric ring method, in which undisturbed soil samples were oven-dried at 105°C for 24 h (Santos et al., 2013).

In order to evaluate soil resistance to penetration, an impact penetrometer model (Stolf, 1991) was used. For determination of the gravimetric moisture content of soil, samples were collected at the time of analysis at depths of 0 to 0.10 m and 0.10 to 0.20 m.

Management and cropping systems

In February 2014, maize (Pioneer 30F53 variety) was mechanically sown in a no-tillage system at a density of 50,000 plants ha^{-1}, with 0.70 m spacing between rows. Basic fertilizing was performed using 310 kg ha^{-1} of a 10-15-15 formulation (N, P_2O_5 and K_2O, respectively).

Thirteen days after sowing, the forage species used for intercropping was manually sown in inter-row spaces at rates of 12 kg ha^{-1} for *B. brizantha* (*U. brizantha*), 12 kg ha^{-1} for *B. ruziziensis* (*U. ruziziensis*) and 80 kg ha^{-1} for black oats (*A. strigosa*). During the course of the experiment, rainfall data were collected on a monthly basis.

Harvesting of maize for silage was performed when the maize was in the R5 stage of growth (farinaceous grains). The plants of the three central rows were cut manually. The cutting height was 0.20 m above ground level. Plants were then crushed, collected in sacks and taken to the laboratory to be weighed on a digital scale with a precision of 5 g. The results were used to calculate silage production (kg ha^{-1}).

After cutting the maize for silage, the area was fenced and intercropping plants remained in the area until August. After that, production of dry matter within each treatment was determined. This assessment was performed using an inventory square. The area was then desiccated using 2.75 L ha^{-1} of glyphosate.

Statistical analysis

The data were subjected to analysis of variance with a significance level of 5% for the F-test. When significant, the averages were compared using the Tukey's test at 5% probability, using the statistical software SISVAR® (Ferreira, 2011).

RESULTS AND DISCUSSION

Production of dry mass of cover plants

At the beginning of maize crop growth (February, 2014), a water deficit occurred with a monthly precipitation of 33 mm. This was anticipated to affect the development of maize silage and consequently its productivity (Figure 2).

Table 1 shows the average productivity results for the dry mass of maize silage and cover crops. It was verified that there was no effect of the intercropping in the production of dry mass of the winter maize silage, even with water deficit. It is observed that the average production of silage was of 2,497 kg ha^{-1}. This result is considered to be low for the region.

Reduced production occurs due to a water deficit occurring at the beginning of crop development. According to results obtained by Pinho et al. (2002), the average production of corn silage is 12.400 to 20.000 kg ha^{-1}, and for Pinho et al. (2007) values it varied from 8.000 to 23.000 kg ha^{-1}. These yields are well above those reported in this experiment.

Dry mass production data for the intercropped plants showed significant differences between treatments (Table 1). The highest dry mass production was with black oats (3027.31 kg ha^{-1}), which was significantly higher than with *U. brizantha* (2287.84 kg ha^{-1}; $p<0.05$) or controls (spontaneous plants) which produced the lowest yield (892 kg ha^{-1}).

Therefore, black oats contributed 29.5% dry matter to the soil. Increased amount of cover straw in the no-tillage system will result in greater soil protection from rainfall impact, lower incidence of solar rays under the soil, and consequently increase in available water for plants

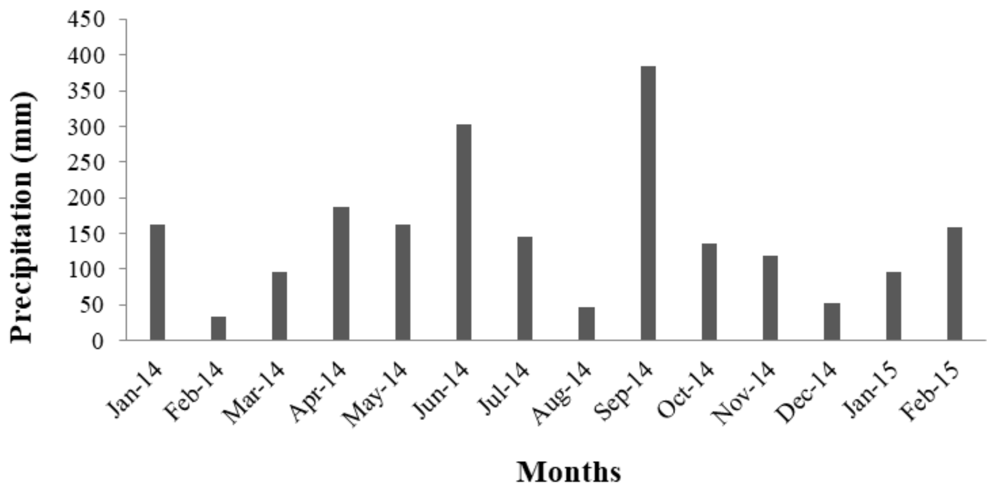

Figure 2. Monthly accumulated precipitation during the experimental period. SM: Sowing maize. Source: Agrícola Horizonte Ltda., Quatro Pontes-PR, Brazil.

Table 1. Mean results for dry mass of silage from intercropped maize and cover crops used in intercropping.

Treatment	Dry mass of silage (kg ha^{-1})	Dry mass of cover plants (kg ha^{-1})
Maize + Brachiaria ruziziensis	2526.77^{a}	2723.88^{ab}
Maize + oats	2609.06^{a}	3027.31^{a}
Maize + Brachiaria brizantha	2360.80^{a}	2287.84^{b}
Maize not intercropped (spontaneous plants)	2493.00^{a}	891.76^{c}

Means followed by the same lowercase vertical letters do not differ significantly from each other according to the Tukey's test (5%).

Table 2. Mean results for macroporosity, microporosity, total porosity and soil density in soil samples collected at depths of 0-0.10 and 0.10-0.20 m, after intercropping using different winter cover crops.

Treatment	Macroporosity (m³ m^{-3})		Microporosity (m³ m^{-3})	
	0-0.10 m	0.10-0.20 m	0-0.10 m	0.10-0.20 m
Maize + *Brachiaria ruziziensis*	0.06^{Aab}	0.06^{Aab}	0.44^{Aa}	0.41^{Aa}
Maize + oats	0.05^{Ab}	0.06^{Ab}	0.47^{Aa}	0.48^{Aa}
Maize + *Brachiaria brizantha*	0.07^{Aa}	0.07^{Aa}	0.49^{Aa}	0.49^{Aa}
Maize not intercropped	0.04^{Ab}	0.04^{Ab}	0.48^{Aa}	0.47^{Aa}
	Total porosity total (m³ m^{-3})		Bulk density (mg m^{-3})	
Maize + *Brachiaria ruziziensis*	0.50 ^{ns}	0.47 ^{ns}	1.31 ^{ns}	1.35^{ns}
Maize + oats	0.52	0.54	1.29	1.27
Maize + *Brachiaria brizantha*	0.56	0.56	1.30	1.28
Maize not intercropped	0.52	0.51	1.28	1.29

Means followed by the same capital letters in horizontal and low in the vertical do not differ statistically from each other by the Tukey test (5%).

(Bescansa et al., 2006).

Porosity and bulk density of soil

Table 2 shows that there was effect of cover plants for macroporosity, microporosity, and soil bulk density. In the area planted with *B. brizantha* (*U. brizantha*), soil samples collected at a depth of 0 to 0.10 m presented the highest values for macroporosity (0.07 m³ m^{-3}).

These values differed significantly from the area planted

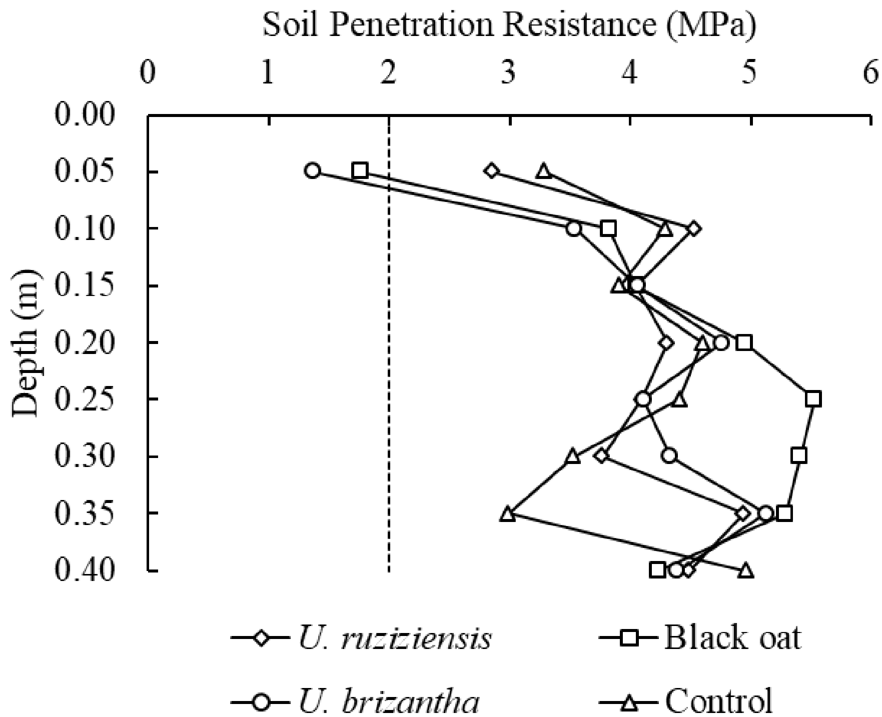

Figure 3. Soil penetration resistance after cultivation of maize intercropped with different winter cover crops.

with oats (0.05 m³ m⁻³) and the control area (0.04 m³ m⁻³), but were not significantly different to the area planted with *B. ruziziensis* (*U. ruziziensis*; 0.06 m³ m⁻³).

A similar result was observed in samples collected at a depth of 0.10 to 0.20 m, demonstrating the potential for *Brachiaria* species to improve this physical property of soils. Brachiaria plants have a fasciculate root system with extensive root production. This large root volume decompresses the soil, releases exudates and increases soil microbiota, resulting in an increase in soil aggregation and consequently changes in the macroporosity (Salton and Tomazi, 2014).

Macroporosity values for the control samples (0.04 and 0.04 m³ m-³) are low, indicating physical impediments to root development (Table 2). Macroporosity values should be 7 to 10% (0.07 to 0.1 m³ m-³) of the total soil volume to allow gas and liquid exchange between the soil and the atmosphere (Drewry et al., 2003). Values below 10% hinder the aeration process and water conduction in the soil (Beutler et al., 2001; Secco et al., 2005). This can lead to a reduction in productivity in adverse climatic conditions.

Results for microporosity, total porosity and soil density showed no significant differences between treatments. These results agree with those of Almeida et al. (2008) who reported that, with only one crop cycle, these physical characteristics of the soil were not altered. The extensive root systems of cover crops promoted the decomposition, due to the presence of channels that facilitate water infiltration and diffusion of gases. This leads to improvements in soil physical quality for the next crop (Foloni et al., 2006).

For clay soils, the appropriate values of total porosity vary between 0.43 and 0.52 m³ m^{-3} (43 and 52%), and are strongly influenced by the type of crop, vegetation and soil compaction (Michelon et al., 2009).

Soil penetration resistance

The determination of soil penetration resistance was performed when the soil presented gravimetric moisture of 0.23 kg kg^{-1} or 23%. The soil penetration resistance at different depths was statistically different in layers 0 to 0.05 m. Treatment with *U. brizantha* resulted in lower penetration resistance values at a depth of 0 to 0.05 m, and differed significantly from values obtained with *U. ruziziensis* (Figure 3). The results of soil penetration resistance at different depths showed that the values increased in the depth of 0.05 to 0.10 m, indicating soil compaction from this depth. Most values were above 2 MPa, therefore, above the critical value of 2.0 MPa, considered critical for a majority of crops (Santos et al., 2015), but this limit may vary from 2 to 3 MPa (Imhoff et al., 2000) as a function of the soil unit at the time of evaluation.

Conclusion

The highest production of dry mass from the cover crops investigated was reported for oats and *U. ruziziensis*, and the cover plants used in this study did not reduce the productivity of maize used for silage. These results indicate that the intercropped system is an excellent option for the producer to increase the organic matter content of the soil, increase the straw on the surface, resulting in less impact of the raindrops, and decrease in soil temperature. The cover crops promoted improvements in the macroporosity of the soil and reduction at soil penetration resistance, particularly in the area planted with *U. brisantha*; demonstrating its potential in improving water infiltration and soil aeration.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Relative chlorophyll index on doses of nitrogen fertilization for cherry tomato culture

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Tomato culture is very nitrogen demanding and periodical evaluation of the nutritional state of tomato plants can help in handling nitrogen fertilization. Determining the relative content of chlorophyll using a chlorophyll meter or soil plant analysis development (SPAD) has come up as an alternative method among the common methods. The aim of this study was to determine the relative chlorophyll index in the leaf blade of tomato plants 'BRS Zamir' in response to nitrogen doses throughout 112 days in a protected environment. A randomized delimitation was used with subdivided parcels in time with four replications. The parcels (two plants) received treatment constituted by 5 Nitrogen (N) doses: 80, 160, 240, 320 and 400 kg ha^{-1} of N, using urea as source. The doses were divided and applied on the 14th, 28th, 42nd, 56th, 70th, 84th, 98th and 112nd days after transplant. Determination of relative content of chlorophyll was done using a portable meter, with readings in the following schedule: 7:00 a.m, 9:00 a.m, 11:00 a.m, 1:00 p.m, 3:00 p.m and 5:00 p.m. The data were subjected to analysis of variance by F-test at 5% probability, and when they were significant, they were analyzed by a regression. The study verified that relative content of chlorophyll presented a positive correlation of 0.84 with content of total N, and the dose increments of N influenced the chlorophyll content in leaf blade.

Key words: *Solanum lycopersicum* L., chlorophyll meter, relative chlorophyll index (RCI).

INTRODUCTION

Cherry tomato (*Solanum licopersicum* var. *cerasiforme*) is considered to be an exotic vegetable due to its sensorial properties, taste and red color. In Brazil, it has already been found since the 90s, and it is becoming common in restaurant menus, plate ornamenting and appetizers, besides having attractive prices for producers (Machado et al., 2003; Rocha et al., 2009; Silva et al., 2011; Araújo et al., 2013). Because cherry tomato culture demands fertilization, producers face a high production cost. Thus, it is important to apply proper fertilization and the use of adequate techniques when harvesting and handling the culture, in order to minimize costs and enhance fruit

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quality (Ferreira et al., 2003; Souza et al., 2010).

Forage fertilization must happen according to the physiological development of the culture. Up to the moment of blooming, the leaf (specifically in chloroplasts) is the organ of the tomato plant which contains the highest concentration of nutrients and dry mass. Nitrogen (N) is among the nutrients that are more required by the tomato plant. This is a vital element for the plants and its application can be parceled according to the demand of N from the plant (Argenta et al., 2003; Ferreira et al., 2003; Fontes and Araújo, 2007).

Monitoring chlorophyll content in the leaf is one way of determining the need for N. These contents have high correlation with nitrogen, and can be obtained through laboratory means (destructive) or in loco (non-destructive) through portable devices, allowing for the monitoring of the nutritional state of the plants and creating a toll at decision making for nitrogen fertilization (Salla et al., 2007; Silveira et al., 2003; Carvalho et al., 2012).

The use of portable meters to estimate chlorophyll content in a sensible, precise and non-destructive way is far more beneficial than lab methods (Rezende et al., 2010; Rigon et al., 2012). The measures retrieved by these devices are known as relative chlorophyll index (RCI) or soil plant analysis development index (SPAD), and allows the identification of the nutritional state of the plant. From this index it is possible to estimate the proper quantity of nitrogen to be applied (Gil et al., 2002; Amarante et al., 2010).

The aim of this study is to determine the correlation of the RCI in the leaf blade of the cherry tomato 'BRS Zamir' in response to nitrogen doses, with chlorophyll content obtained in the laboratory.

MATERIALS AND METHODS

The experiment was conducted in a protected environment at State University of Goiás – UEG, in the Campus of Exact and Technologic Sciences, Anapolis – Goiás, Brazil. The area is located at 16°20'34"S and 48°52'51"O, 997 m above sea level (Moura et al., 2005). Cherry tomato seedlings, cultivar BRS Zamir, were transplanted on April 20, 2014 into polyethylene vases containing 7 L of water, using a commercial substratum as backup and one seedling per vase. They were transplanted with 2 to 3 pairs of well-formed leaves 28 days after sowing, with 1.00 m spacing between rows and 0.40 m between plants. Culture practices were performed according to the recommendations from Fontes and Silva (2002), in which thinning and vertical transport of the culture were performed weekly; pruning of tips happened when plants reached two meters of height and vertical tutoring with plastic straps. The experiment was performed in a randomized delimitation with sub-subdivided parcels in time with four replications. The parcels were constituted by two plants and received randomly one of the five doses of N: 80, 160, 240, 320 and 400 kg ha^{-1}, using urea as source. The sub-parcels correspond to the evaluation days: 14, 28, 42, 56, 70, 84, 98 and 112 days after transplant (DAT), and the sub-subparcels to the schedule within each evaluation day: 7:00 a.m, 9:00 a.m, 11:00 a.m, 1:00 p.m, 3:00 p.m and 5:00 p.m.

The RCI in the leaves of tomato plants was retrieved with the aid of a portable chlorophyll meter, model CFL 1030 (Falker Farm Automation®) (Falker Automação Agrícola, 2008). The readings from the RCI in field follow the methodology proposed by Fontes and Araújo (2007), in which measures of each parcel were performed on adjacent leaves to each plant bunch. The readings of the leaves were conducted on five leaflets, being two on each side of the leaf (laterals) and the central end leaflet. In the evaluation conducted on the 98th DAT the evaluated leaves in the field were taken and sent to the lab of Agriculture Engineering in the Campus of Exact and Technologic Sciences of State University of Goiás to determine chlorophyll contents and total nitrogen content. The chlorophyll content was analyzed following the procedure/method of Lichtenthaler (1987). A precision scale was used to weigh 150 mg of leaves and they were fragmented in Eppendorf flasks with 1.5 ml of pure acetone without compacting. The tubes were under agitation for 24 h, low temperature and wrapped with aluminum foil to prevent contact with light. After the stipulated period, the acetone solution was removed from the tube, and then absorbance of acetone solution was read in 661.6 nm for chlorophyll a (Ca) and 644.8 nm for chlorophyll b (Cb).

To determine the total nitrogen content adequacy to Kjeldahl method (1883) proposed by Embrapa (2006) was adopted. The collected leaves were placed in paper bags, dried in a hothouse with forced circulation of air at 60° for 24 h. After this period the material was analyzed and grinded in a Wiley type mill with a colander of 20 mesh and then stored in a place without light. Each sample was weighted to 0.1 g on a precision scale, homogenized and transferred to a Kjeldahl tube. For each sample, 2.5 g of catalytic mixture was added and 7 ml of sulfuric acid, so that they could be heated over a digestion block, at 50°C for one hour, afterwards the temperature was increased gradually up to 350 to 400°C. When the liquid became transparent, the samples were taken away from heating; 10 ml of distilled water was added after they cooled down. After that, distilling of samples was performed by engaging them to Erlenmeyer distillery, with working conditions of 20 ml of boric acid solution at 4%, with 4 drops of mix indicator solution. The Kjeldahl tube was adapted to the distillery and added to the sodium hydroxide solution at 50% until a dark color solution was obtained. When distillation was completed, the samples were titled with pattern sulfuric acid solution (0.05 mol L^{-1}) up to the turn of the indicator.

The following data analyses were performed: a) Pearson's correlation between the values from leaf analysis on the 98th DAT of nitrogen content and RCI, to measure the amount of linear correlation between two quantitative variables, reflecting the intensity of a linear relation between the data groups; b) Nitrogen sufficiency index (NSI) which is the relation of the values from RCI present in doses of 80, 160, 240 and 320 kg ha^{-1} of N by the values found by reference doses, which in this study is 400 kg ha^{-1} of N; c) Variance analysis for the variable RCI, considering random delimitation with sub-subdivided parcels in time regression analysis for the split of significant interactions for the quantitative factors, was performed.

RESULTS AND DISCUSSION

The summary of analysis of variance for RCI is presented in Table 1. It is possible to observe the significant interaction ($p<0.05$) between the doses of N (80, 160, 240, 320 and 400 kg ha^{-1}) and the evaluation times (7:00 a.m, 9:00 a.m, 11:00 a.m, 1:00 p.m, 3:00 p.m and 5:00 p.m.) and respective days. Regarding doses of N deployed through evaluation days, it was observed that on the 42nd, 56th, 70th, 84th, 98th and 122th DAT, respectively significant differences ($p<0.05$) in RCI were

Table 1. Variance analysis of Relative Chlorophyll Index (RCI) from cherry tomato.

Variance source	Liberty degree	Average square
Doses of N	4	1990.38*
Residue (a)	15	195.81
Evaluation Days	7	8975.9*
Doses of N throughout evaluation days	28	136.23*
Residue (b)	105	28.79
Evaluation times	5	111.2*
Doses of N throughout evaluation times	20	5.32^{ns}
Residue (c)	75	4.73
Evaluation Schedule throughout the days	35	60.26*
Doses of N throughout evaluation times and days	140	5.28^{ns}
Residue (d)	524	4.73
Total	958	-

*Significance at 5% probability; CV: (a) 26,47%; (b) 10,15%; (c) 4,13% and (d) 4,12%.

presented, because the readings on RCI gathered through the portable chlorophyll meter increased due to the amount of N furnished to the plants in the different days of evaluation. Similar outcomes were found by Ferreira (2006), who evaluated the RCI and chlorophyll content in the leaf blade of the tomato plant concerning the doses of nitrogen and organic fertilization in two harvest times. These results corroborate with the one gathered by other authors for different cultures, when evaluating the RCI in the leaves, which showed an increase in the index with the increment in the dose of N applied in cotton (Neves et al., 2005), common beanstalk (Silveira et al., 2003) and cabbage (Moreira and Vidigal, 2011).

According to what was proposed by Gomes (2000), the variance coefficient indicates the accuracy of an experiment, and they are considered low variance when they are inferior to 10%; average variance from 10 to 20%; high variance from 20 to 30%; and very high variance when they are superior to 30%. For Silva et al. (2011), the variation coefficient is a quality indicator of an experiment. With this, the outcomes showed a high variance for the RCI in the doses of N, which possibly indicates that the doses used up might present significant differences. For evaluation days in relation to the doses of N applied, the RCI presented average variance, indicating that evaluation days interfere with SPAD values according to the doses of N applied.

RCI in the evaluated times in relation to doses of N and in relation to evaluation days presented low variance (<5%), which possibly explains the lack of significance from RCI in the evaluated times. According to Figure 1, RCI for each dose of N applied decreased up to a certain point, followed by a decrease throughout evaluation days, which characterized a quadratic behavior. Ferreira (2006) found a quadratic behavior from RCI in response to doses of N in two harvest moments of tomato, with index evaluation on the 34th, 54th, 83rd DAT during spring/summer of 1998/1999 and 37th, 52nd, 81st DAT during fall/spring of 1999/1999.

In the study by Xu et al. (1997) with tomato plant in a green house, there was a decrease in RCI throughout the cycle. For Fontes and Araujo (2007) and Porto et al. (2011), this occurred because N is a nutrient that takes part in the synthesis and structure of molecules of chlorophyll, and increase of doses of N up to a certain extent results in the increment of chlorophyll content and green color intensity in the leaves of the plant. For doses of 80, 160, 240, 320 and 400 kg ha^{-1} of N the critical levels of RCI were on 74th, 66th, 27th, 21st and 26th days of evaluation, respectively. The dose of N which presented the highest RCI in the critic level was of 240 kg ha^{-1} of N with RCI of 63, 61, 51, and 47 units, respectively (Figure 1).

Fayad et al. (2002) believed that the maximum absorption of nutrients is coincident to the initial blooming period of the tomato plant, and justify such affirmatives in their study of absorption of nutrients by the tomato plant in field conditions and protected environment, where they have found that absorption of N was crescent up to the ^{46th} day followed by decrease of the former.

Schuelter et al. (2003) studied the behavior of RCI of three cultivars of tomato plant fertilized according to doses of N recommended by Guimarães (1998), and found a critical level of RCI for hybrid F1 around the 75th DAT, and have justified these outcomes, affirming that chlorophyll tends to degrade from a certain period due to plant physiology, with RCI as an indicator of photosynthesis activity. Possibly, chlorophyll degradation happens because as soon as fruits start to develop, there is increase in metabolic activity in the plant, leading to the absorbing of higher quantities of nutrients in the reproduction stage. Taiz and Zeiger (2009) and Fayad et al. (2002) affirm that when fruits start to develop, there is

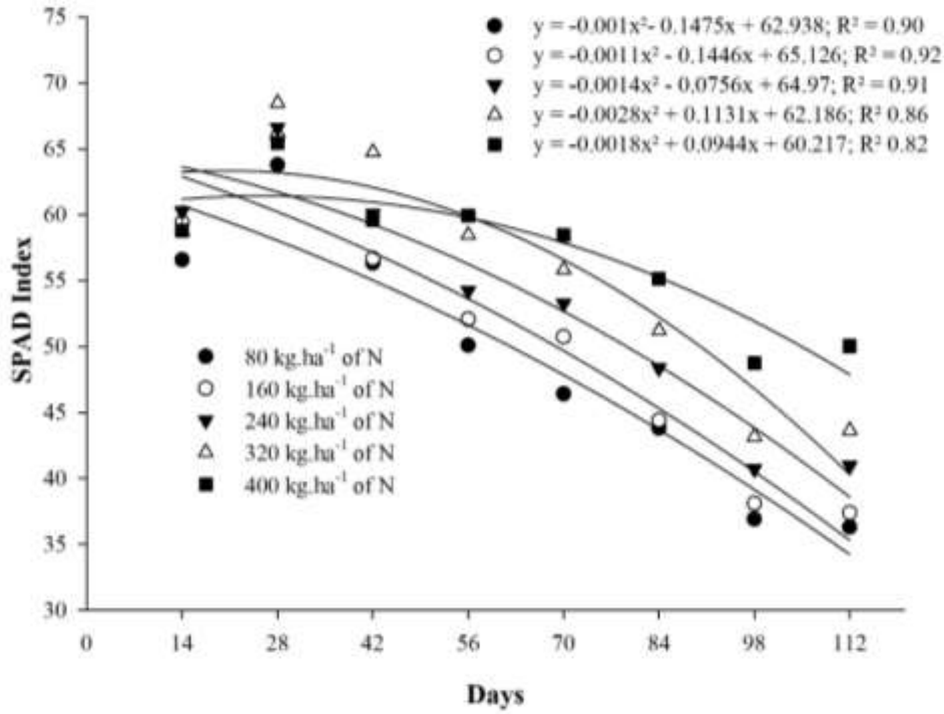

Figure 1. Behavior from Relative Chlorophyll Index (RCI) according to nitrogen (N) dose throughout evaluation days (14, 28, 42, 56, 70, 84, 98 and 112 DAT) of the cherry tomato.

a mobilizing force of nutrients which assimilates due to increment in metabolic activity, which can be associated to hormonal activities, division and cellular growth. From that point some nutrients such as nitrogen, phosphorus and potassium start to gradually build up in greater quantity in the fruit.

Total chlorophyll rate in the leaves of the cherry tomato plant presented a significant difference ($p<0.05$). Figure 2 portrays a quadratic behavior according to the doses of N applied. Chlorophyll content increased up to a critical level of 250 kg ha^{-1} of N, and after that it decreased with the increase of doses of N. For Fontes and Araujo (2007), N takes part in the synthesis and structure of the chlorophyll molecules, whereas doses of N increase up to a certain limit; there is increment in chlorophyll content and intensity of the green color in the leaves of the plant.

Porto et al. (2011) found similar results in the study with nitrogen fertilization for zucchini, where chlorophyll increased due to doses of N up to 40 kg ha^{-1}, decreasing afterwards, presenting a quadratic behavior. Costa et al. (2008) verified increase in chlorophyll content in the leaf, with the increase of doses of N. Specifically about tomato culture, Ferreira (2006) found a quadratic behavior in chlorophyll content in relation to N doses, where it presented a similar behavior to the one in this study; that is, from this point on, the increase of doses led to decrease in the values of chlorophyll content.

Coelho et al. (2012) found a linear increase in chlorophyll content regarding the increase of N doses and justified this behavior because the portable chlorophyll meter detected indirectly, increase of N that is incorporated to chlorophyll molecules, not detecting the free form which are non-incorporated, where N builds up when there is luxury consumption. However, in this study, a quadratic behavior for chlorophyll content concerning the increase in N doses has been verified.

The total content of N in the dry mass of the cherry tomato leaves presented a significant difference ($p<0.05$). N content in the tomato plant leaf increased with the increment of N dose of kg.ha^{-1}, presenting a linear behavior (Figure 3), where the dose of 400 kg.ha^{-1} of N presented higher total value of N with 32 mg g^{-1} of dry mass of the leaf. Possibly, the content of N in the dry matter of the leaf increases with the increment of N dose, because the lab analysis does not extract only N involved in the chlorophyll molecule of the leaf, but also what comes from luxury consumption. Luz et al. (2014) observed that the higher the nutrient availability, the higher the absorption of N by potato plants, thus increasing its transportation to the leaves, linearly.

Similar outcomes were found by Coelho et al. (2012), who found a linear behavior in N content in relation to N doses applied to potato culture on the 21st day after emergency, and justified that N concentration in the leaf is due to quantity of N applied. Ferreira and Fontes (2011) studied nitrogen indexes in tomato plant leaves

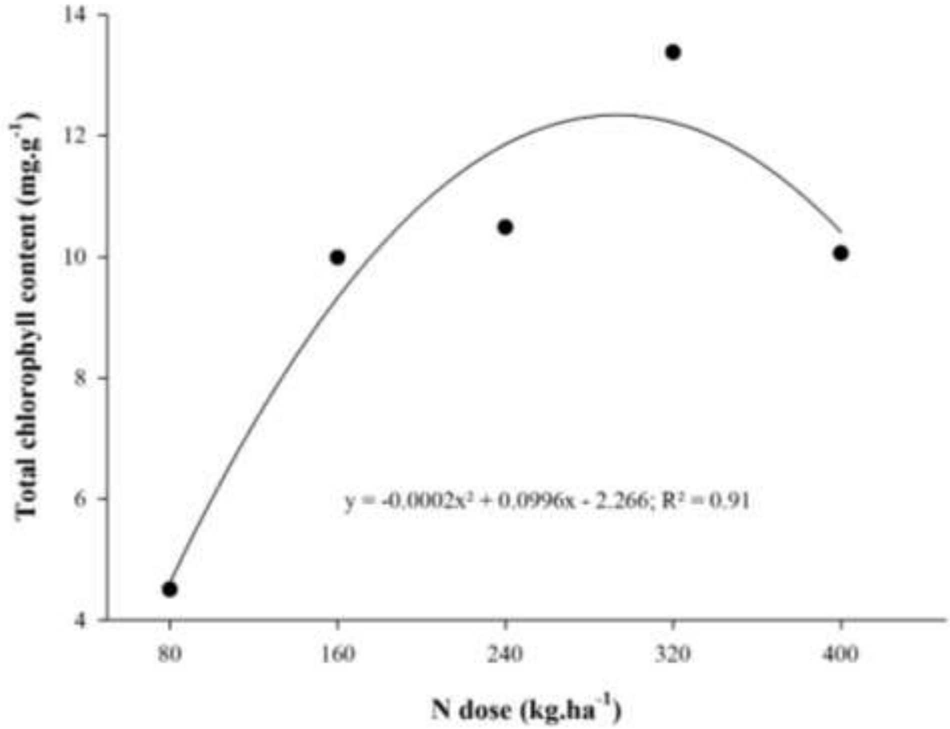

Figure 2. Total chlorophyll content in the fresh matter of cherry tomato leaf on the 98th DAT.

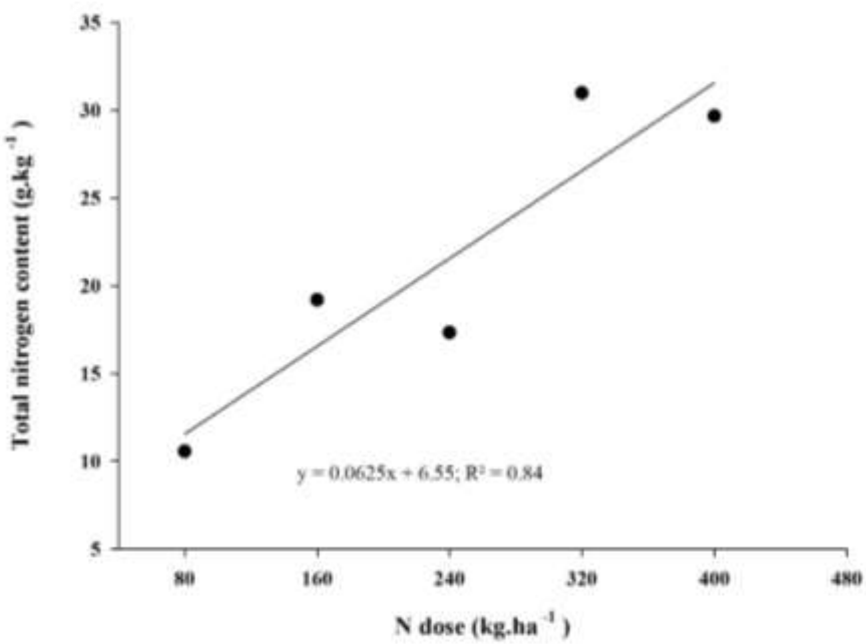

Figure 3. Total nitrogen content in the fresh matter of the leaf of cherry tomato on the 98^{th} DAT

concerning nitrogen and organic fertilization, and observed a quadratic behavior in N content concerning N doses applied to zucchini culture, and justified such results by the fact that N is a component of chlorophyll

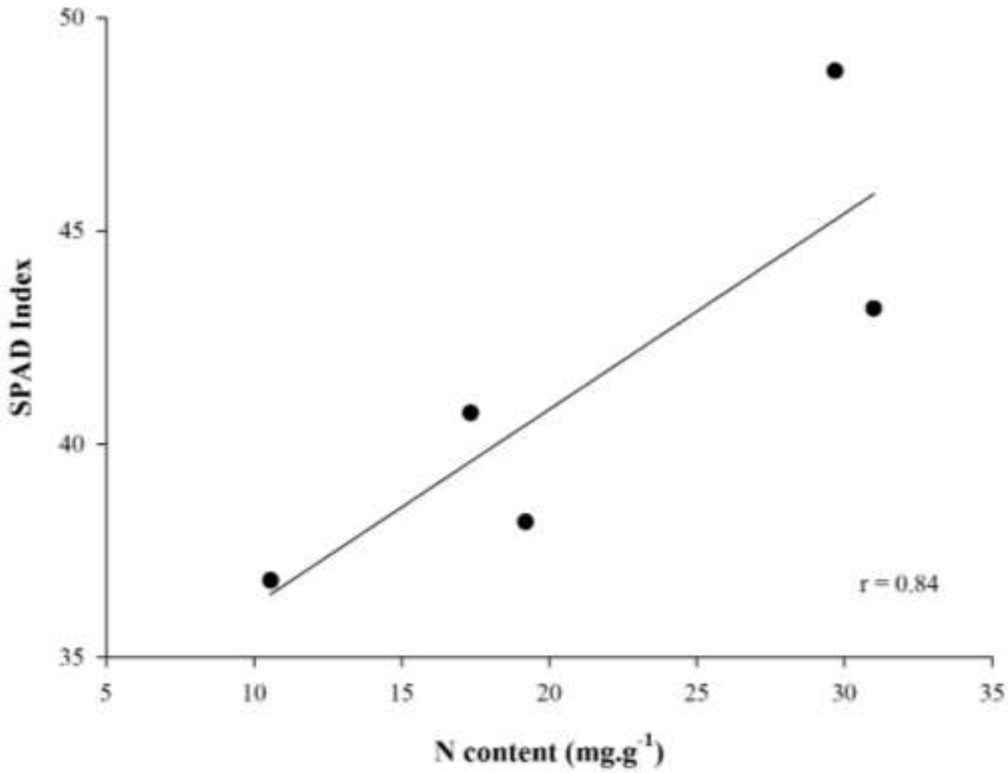

Figure 4. Correlation between N content in the dry matter and Relative Chlorophyll Index (RCI) of cherry tomato leaves on the 98th DAT

molecule, being a limiter of photosynthetic activity.

The readings for total N content and RCI (Figure 4) determined by the portable chlorophyll meter presented positive correlation of r=0.84, which indicates that the readings retrieved with the portable chlorophyll meter can be used to estimate N concentrations determined by the standard method. Pôrto et al. (2014) highlight that the portable chlorophyll meter presents indexes that correlate to N concentration in the plant, serving as an aid tool to determine indirectly N deficiency. Satisfactory outcomes were obtained about the nitrogen state of the tomato through studies from Ferreira (2006) and that of Guimarães et al. (1999).

The INS was retrieved with a portable chlorophyll meter through the relation of the values of RCI found in the leaves referent to doses 80, 160, 240 and 320 kg ha^{-1}of N by values found in the N dose of 400 kg ha^{-1}, considered as dose of reference. According to Braun et al. (2013) and Varvel et al. (1997) when the plant presents INS at 90 or 95% of the reading taken from the plants regarded as references, this indicates that the plant is deficient of N. It could be observed in Figure 5, that the dose of 80 kg ha^{-1} cherry tomato culture presented a need for N application from the 42nd DAT. For dose 160 kg.ha^{-1}, INS was stable at 95% in the period between the 42nd and the 56th DAT and after this period the index started to decrease coming up to 50% on the 112th DAT. The dose 240 kg ha^{-1} of N presented INS below 95% around the 70th DAT and the dose 320 kg ha^{-1}of N presented high INS up to the 70th DAT. It has been observed that all doses of N presented decrease in INS, staying below 95% up the 112th DAT. It demonstrates that, based on this criterion, none of the doses presented INS above 95% during the whole evaluation period.

Godoy et al. (2003) studied the use of Relative Chlorophyll Index (RCI) in handling nitrogen fertilization for bell pepper plants and concluded that the INS can help and indicate the moment of the application of the nitrogenous fertilizer, enhancing efficiency of N usage. Coelho et al. (2010) varied N doses in intermissions of 0, 100, 200 and 400 kg.ha^{-1} of N, and found out INS lower than 95% in the doses of 0 and 100 100 kg.ha^{-1} of N on the 21st and 14th days after emergency respectively, and for the dose 200 kg.ha^{-1} of N the INS was lower than 95%.

Conclusion

The correlation between the readings of total N content in the cherry tomato leaves and RCI retrieved through portable equipment was 84%. RCI can be an indicator of the need for nitrogen fertilization for cherry tomato, for it has a positive correlation with N content in the leaves

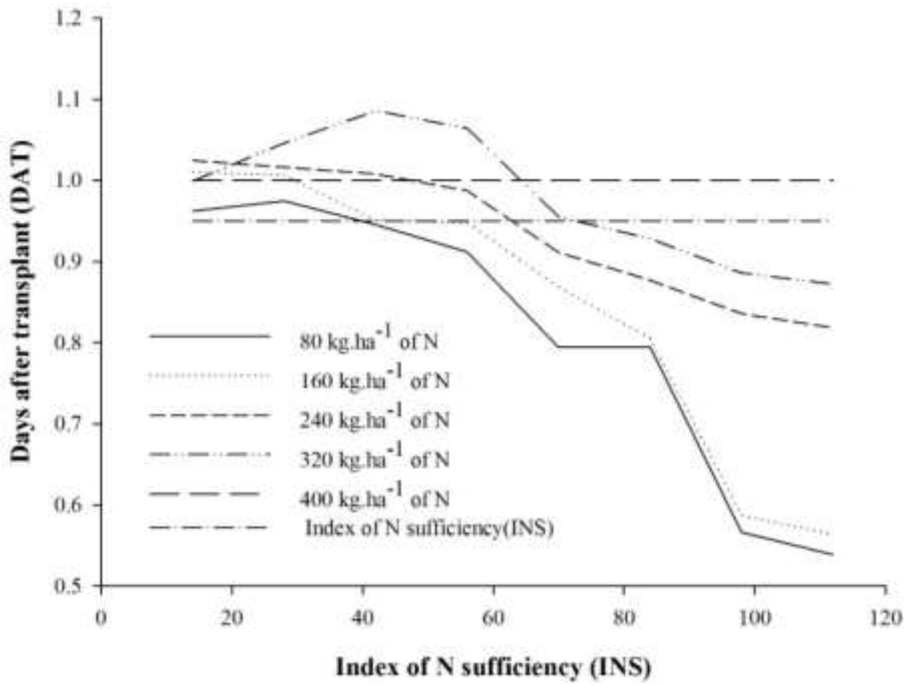

Figure 5. Index of N sufficiency calculated by the quotient between the Relative Chlorophyll Index (RCI) of one parcel and one of another that received a higher N dose (reference), during the cycle of cherry tomato

found in laboratory. The critical value found for cherry tomato in the readings from RCI and chlorophyll content through lab analysis remained close, indicating a good relation between both methods. Chlorophyll content in cherry tomato can be monitored by the portable chlorophyll reader without the need for analysis which destroys the leaves, yielding more economy and speed in the analysis. Increase in N doses influenced chlorophyll content in the leaf blade.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

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Enhancing surface liming rate on a no-till cropping system in an oxisol of Southern Brazilian

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The aim of the experiment is to evaluate the physical and chemical effects caused on the properties of red latosol containing high levels of organic matter in the aftermath of 40 months after surface liming and the effects on yield components of no-till maize. The experiment was conducted in an area of 1000 m^2 split into 40 plots of 25 m^2, and the experimental design adopted was randomized complete blocks with five treatments and four replications. The treatments consisted of five rates of lime: 0, 2.5, 5, 12.5 and 30 $ton.ha^{-1}$ (1 SMP pH 5.5). After 40 months of experiment installation, soil samples were taken in a total of four sub samples per plot, that were properly homogenized at different depths (0 to 5, 5 to 10 and 10 to 20 cm) and evaluated on chemical parameters (pH, H+Al, Ca, Mg, Al saturation and bases); on physical parameters (resistance to penetration and soil water infiltration) and on maize crop parameters (yield, height of insertion of first cob and plant's height). It was observed that equal and above 1 SMP to pH 5.5 ameliorated soil pH in the layers up to 20 cm after 40 months of application, interfering in the levels of Al+H, Ca, Mg, saturation of bases and saturation of aluminum. However, significant effects on soil pH were observed in a depth up to 10 cm. The application of different surface liming rates did not interfere on penetration resistance or soil water infiltration capacity. The effects on soil chemical properties with the rate of 1 SMP to pH 5.5 resulted in higher maize grain yield.

Key words: Chemical correction, soil physics, maize, acidity.

INTRODUCTION

The efficiency of surface liming in no-till cropping systems, particularly aiming subsoil acidity amelioration, and liming recommendation criteria for this purpose were widely investigated (Caires et al 2000; Rheinheimer et al., 2000; Amaral et al., 2004; Kaminski et al., 2005; Nolla and Anghinoni, 2006; Bortoluzzi et al., 2014). However, when soil pH is not properly ameliorated at the moment of implementation of a no-till cropping system, the rates

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of surface liming on elevated acidity soils with elevated levels of organic matter may not be enough for amelioration in an efficient way.

In a consolidated no-till cropping system Fidalski et al. (2015) observed that liming incorporated into about 20 cm deep of the limestone with plow and harrow, had not exhibited any advantage in relation with surface liming (zero cm incorporation). Yagi et al. (2014) observed that the soil limestone incorporation reduced the carbon frameworks of macro-aggregates in the layer of 0 to 0.10 m and concluded that surface liming is essential for the maintenance or even enhancement of carbon stocks in the soil. Surface liming in no-till cropping systems for a long period boosts an increment of soil organic carbon and total nitrogen (Briedis et al., 2012).

Surface liming is an effective practice, and it is important to maximize grain yield of no-till crops, also the acidity amelioration of deeper soil layers is more pronounced with the use of higher limestone rates (Rodrighero et al., 2015). Veronese et al. (2012) verified that the higher the rate, the greater the progress of the alkaline front in the soil, and the higher the grain yield even with deep liming. Moreira et al. (2017) suggested that applications of limestone in soil up to a rate necessary to achieve 70% of base saturation are not related with micronutrient deficiency in no-till soybeans. Caires et al. (2006) suggested that liming application aims to elevate the bases saturation to 70% with surface liming on a single application or within three consecutive years. The likelihood correspondence between the saturation of bases and the pH of reference crops, for soils in south Brazil (Rio Grande do Sul and Santa Catarina states), utilized by the Slightly Modified Shoemaker-McLean-Pratt Single Buffer Method for limestone prescriptions (SMP method), would be: pH = 5.5 correspond to V (Base saturation) = 65%; pH = 6.0 correspond to V = 75% and pH = 6.5 correspond to V = 85% (CQFS-RS/SC, 2016).

Therefore, it turns out to be still necessary to the assessment of efficient rates for surface liming in soils with elevated potential acidity and high organic matter content, looking for alternative strategies for soil amelioration at depth without tillage, and so at deeper soil layers. Thus, the experiment aimed to evaluate the physical and chemical effects of surface liming for a long time (40 months) on the properties of a brown Oxisol containing high levels of organic matter, and the effects on yield components of no-till maize.

MATERIALS AND METHODS

The experiment was carried out in an experimental field at the University of Caxias do Sul on the municipality campus of Vacaria, Rio Grande do Sul state, Brazil, with geographic coordinates of latitude 28º31´S, longitude 50º 54´W and elevation of 965 m above sea level. The soil was a brown Oxisol (Embrapa, 2013), with native pasture presenting the following chemical features (Tedesco et al., 1995): pH 4.5; SMP (Slightly Modified Shoemaker-McLean- Pratt Single Buffer Method for limestone prescriptions) index 4.5; organic matter 60 $g.kg^{-1}$; calcium 1.4 $comol_c.dm^{-3}$; magnesium 1.0 $comol_c.dm^{-3}$; aluminum 6.4 $comol_c.dm^{-3}$; H+Al 24.4 $comol_c.dm^{-3}$; effective cation exchange capacity 9 $comol_c.dm^{-3}$; cation exchange capacity at pH 7.0, 27 $comol_c.dm^{-3}$; aluminum saturation 71.1%; bases saturation 9.6%; phosphorus 1 $mg.dm^{-3}$; sulfur 5.1 $mg.dm^{-3}$; and potassium 74 $mg.dm^{-3}$.

The treatments were applied in a single time at December 20, 2012 to study the residual effect of liming, in twenty plots of 25 m^2 (5x5 m) arranged in completely randomized blocks, with four replications (blocks) and five rates of calcitic lime applied on soil surface. Since for the region being tested there is a recommendation from the Soil Chemistry and Fertility Commission for the states of Santa Catarina and Rio Grande do Sul, Brazil (CQFS-RS / SC, 2016), which limits the surface application of limestone to 5.0 ton ha-1 if soil analysis has recommended higher doses. If the doses are below 5.0 ton ha^{-1}, the dose may be applied in its entirety.

Therefore, the doses were: 0 ton.ha-1 (control); 2.5 $ton.ha^{-1}$ (50% of the maximum rate recommended for superficial liming); 5 $ton.ha^{-1}$ (maximum rate recommended for surface liming); 12.5 $ton.ha^{-1}$ (necessary rate to enhance the pH of this soil to 5.5 based in a soil analysis by SMP method), and 30 $ton.ha^{-1}$ (6 times the maximum rate for surface liming). The rates adjusted to the actual effective neutralizing power of the limestone utilized (68 to 70%) in this area, was kept native grassland without animal occupation for 3 seasons (2012 to 2015) and finally in the cropping season of 2015/2016, was cultivated maize of the variety Pioneer 30F53. At the first fortnight of May 2016, 40 months after surface lime application, the residual effect of lime mobility over the physical and chemical parameters of the soil as well as yield components of maize was evaluated (Plant height, insertion of the cob corn, maize yield).

It was executed six evaluations in each plot for soil penetration resistance with the use of an electronic measurer Penetrolog PLG1020, from Falker® at a depth of 0 to 40 cm, resulting in 24 evaluations per treatment. The evaluations of the rate of water infiltration in the soil were executed through the Soil Quality Test Kit method (USDA-ARS, 1998) using four replications per plot, totalizing 16 replications per treatment. For the evaluation of chemical features of the soil, four samples were collected per plot and were homogenized, at the soil depths of 0 to 5 cm, 5 to 10 cm and 10 to 20 cm. In each plot 20 measurements were made for the variables plant height, considering from the soil surface to the base of the maize tassel and the height of first feasible cob. Additionally, it harvested the cobs manually in an area of 2x3 m into each plot. The cobs were threshed with a maize mechanical thresher of the brand Lavrale®, where the grains were weighted, and the values were adjusted to a humidity of 13%.

The results of the evaluations were subjected to analysis of variance (ANOVA) using WinStat (1.0 version). In case of significance, the effects of the rates were compared through analysis of regression and depth effects were compared by the Tukey averages test at 5% probability. The criteria are to fit regression equations were the magnitude of the coefficient of determination (r^2) is significant at 5% probability.

RESULTS AND DISCUSSION

The physical parameters evaluated, penetration resistance (PR) and soil water infiltration was not influenced by the enhancement of surface liming rate. The soil water infiltration had a mean of 325 $mm.h^{-1}$, which is considered as fast by the Soil Quality Kit Test Guide (USDA-ARS, 1998). Notwithstanding, despite the method to be more sensitive than the traditional ones, it

Table 1. pH H_2O, H + Al, calcium, magnesium, saturation of bases and saturation of aluminum in different soil depths and doses of soil surface liming on no-till cropping system.

Depth (cm)	Limestone dose (ton.ha^{-1})				
	0	2.5	5.0	12.5	30
			pH H_2O		
0.0 - 5	4.70^{B}	5.45^{A}	5.83^{A}	6.80^{A}	$7. 47^{A}$
5.0 - 10.0	4.97^{AB}	5.10^{B}	5.27^{B}	5.42^{B}	5.82^{B}
10.0 - 20.0	5.07^{A}	4.97^{B}	5.00^{B}	5.10C	5.20^{C}
Coefficient of variation (CV%)			3.09		
H+ Al (comol$_c$.dm.$^{-3}$)					
0.0 - 5	15.49^{A}	7.42^{B}	5.12^{C}	2.07^{C}	1.02^{C}
5.0 - 10.0	12.85^{A}	11.40^{A}	8.12^{B}	7.27^{B}	4.27^{B}
10.0 - 20.0	13.85^{A}	13.85^{A}	15.92^{A}	11.65^{A}	9.55^{A}
CV(%)			19.19		
Ca (comol$_c$.dm.$^{-3}$)					
0.0 - 5	3.80^{A}	7.75^{A}	9.30^{A}	14.95^{A}	17.02^{A}
5.0 - 10.0	2.77^{AB}	3.32^{B}	4.00^{B}	4.50^{B}	7.22^{B}
10.0 - 20.0	1.15^{B}	1.30^{C}	1.45^{C}	1.65^{C}	2.95^{C}
CV(%)			21.18		
Mg (comol$_c$.dm.$^{-3}$)					
0.0 - 5	1.82^{A}	2.67^{A}	2.75^{A}	2.50^{A}	1.65^{A}
5.0 -10.0	1.28^{B}	1.35^{B}	1.25^{B}	1.33^{B}	1.70^{A}
10.0 - 20.0	0.48^{C}	0.55^{C}	0.58C	0.60^{C}	1.00^{B}
CV(%)			22.22		
Saturation of bases (comol$_c$.dm.$^{-3}$)					
0.0 - 5	28.07^{A}	59.60^{A}	70.27^{A}	89.37^{A}	94.80^{A}
5.0 - 10.0	25.40^{AB}	30.37^{B}	40.30^{B}	45.32^{B}	68.00^{B}
10.0 - 20.0	11.62^{B}	12.35^{C}	12.00^{C}	17.25^{C}	30.85^{C}
CV(%)			19.30		
Saturation of aluminium (comol$_c$.dm.$^{-3}$)					
0.0 - 5	24.37^{B}	4.35^{C}	2.25^{C}	0.00^{C}	0.00^{A}
5.0 - 10.0	38.42^{B}	31.17^{B}	24.02^{B}	16.92^{B}	2.15^{B}
10.0 - 20.0	57.27^{A}	57.27^{A}	57.37^{A}	58.35^{A}	28.42^{B}
CV(%)			32.40		

Analyses of variance significant (F≥0.01). Averages followed by the same capital letter in the column do not differ from each other (Tukey at 5% probability).

is efficient in detecting great differences in the rate of water infiltration (Santi et al., 2012).

PR differed only among the depths evaluated and presented in the depths of 0 to 40 cm a mean of 1099 kPa. Values above 2000 kPa are proven to be a critical value for root elongation according to Reinolds et al. (2002) and were achieved at depths beginning from 20 cm, reaching a maximum of 2717 kPA at depths of 30 to 40 cm. Similarly, Bortoluzzi et al. (2014) found values of PR above 2 Mpa only at depth of 20 cm, while the greatest value for PR (2882 kPa) was found in the layer of 30 to 40 cm. However, the authors stated that greater values of PR seem not to affect root distribution of the treatments.

The chemical parameters evaluated exhibited significant interaction among rates of surface liming and the depths evaluated is based on analysis of variance (p ≤0, 05). The depth effects at each dose are shown in Table 1 and the effects of doses at each depth are presented in Figures 1 to 4.

In the soil tested, the parameters of each experimental plot (different limestone doses) presented natural variability with increasing depth for pH H_2O, Ca, Mg, Base Saturation and Aluminum Saturation (Table 1). With

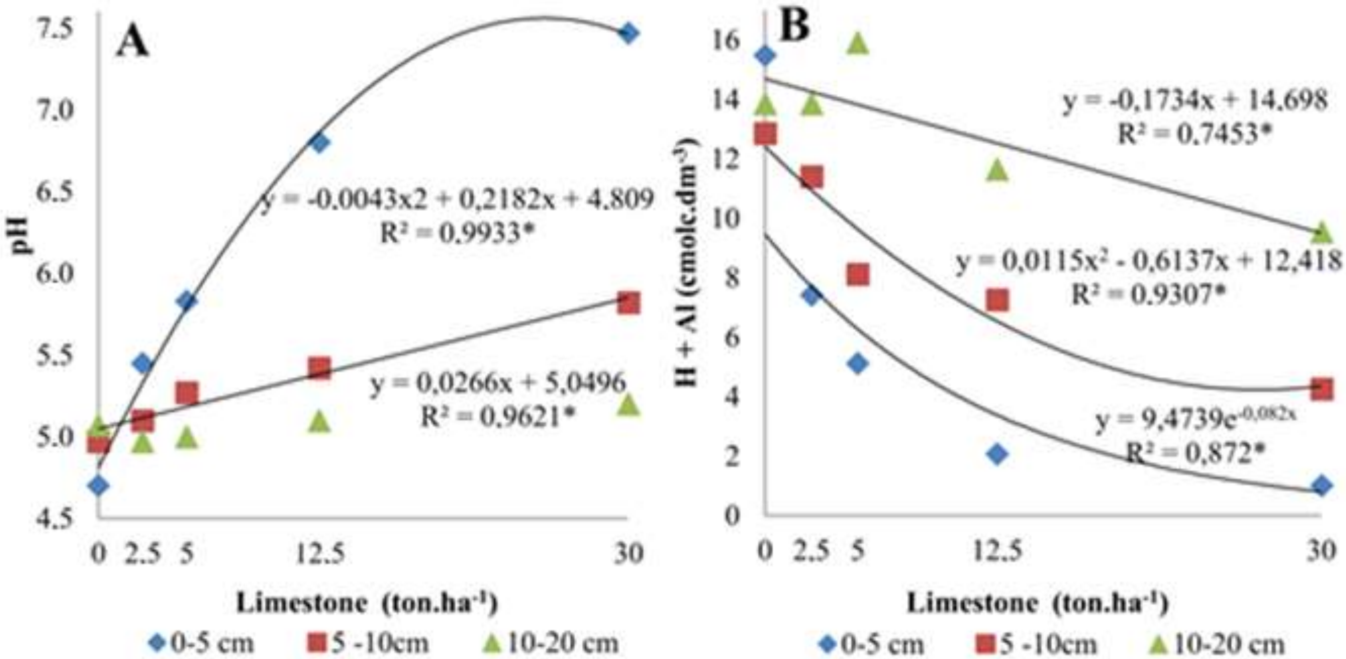

Figure 1. pH H_2O (A) and H+Al (B) as function of different rates of soil surface liming on no-till cropping system in at the soil depths of 0-5, 5-10 and 10-20 cm. *Coefficient of determination significant at 5% of probability and ANOVA (F≥0.01).

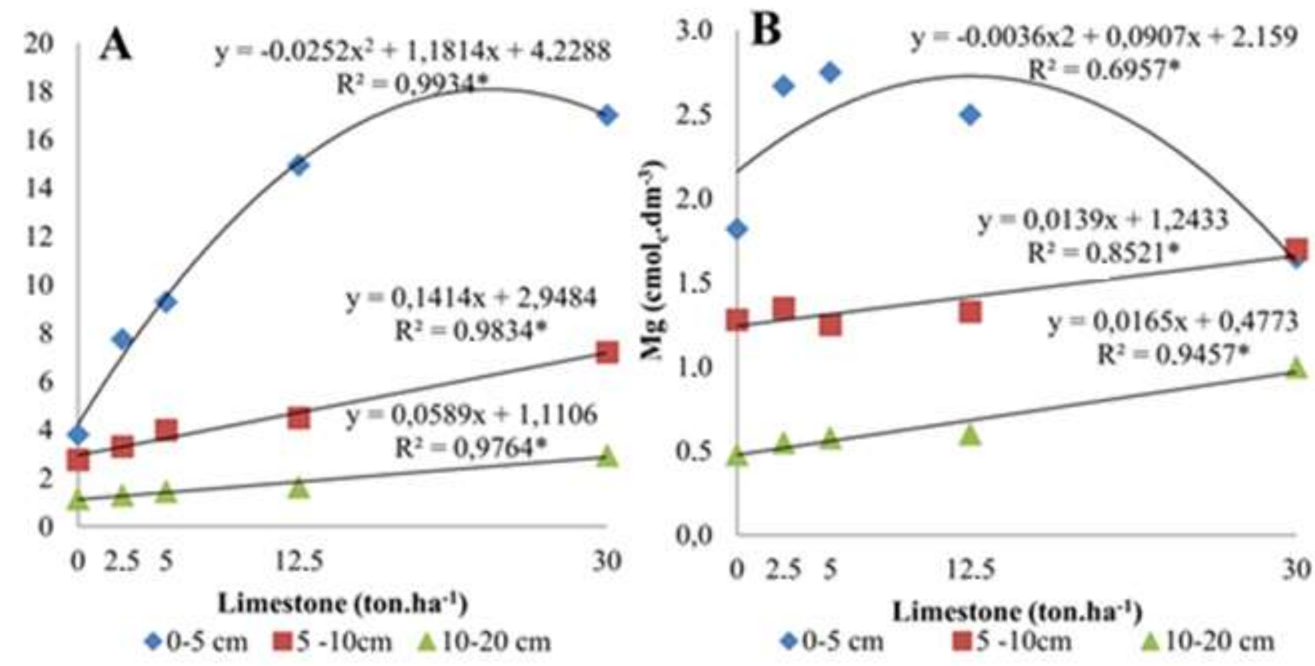

Figure 2. Calcium (A) and magnesium (B) as function of different rates of soil surface liming on no-till cropping system in at the soil depths of 0-5, 5-10 and 10-20 cm. *Coefficient of determination significant at 5% of probability and ANOVA (F≥0.01).

the application of limestone, this variation reduces to the pH of the soil and intensifies with the increment of the applied dose for the other evaluated parameters. The chemical gradient in the different depths with the application of surface limestone has already been observed in several studies (Rheinheimer et al., 2000;

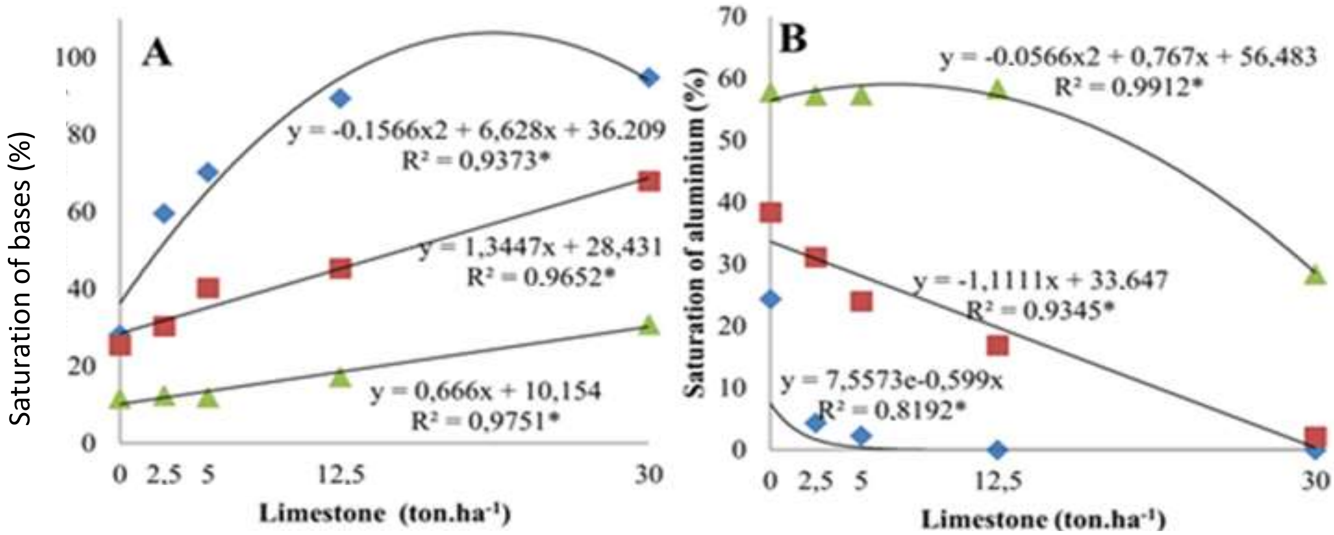

Figure 3. Saturation of bases (A), saturation of aluminium (B) as function of different rates of soil surface liming on no-till cropping system in at the soil depths of 0-5, 5-10 and 10-20 cm. *Coefficient of determination significant at 5% of probability and ANOVA (F≥0.01).

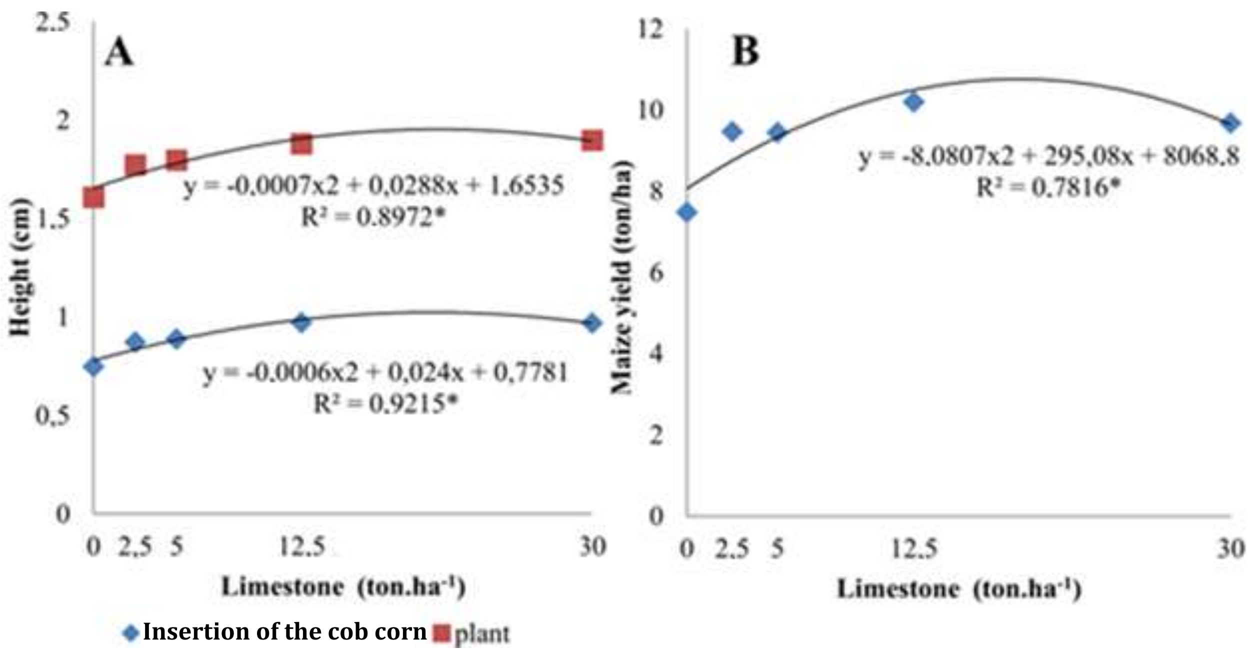

Figure 4. Plant height and height (A) and maize yield (B) as function of different rates of soil surface liming on no-till cropping system. * Coefficient of determination significant at 5% of probability and ANOVA (F≥0. 01).

Kaminski et al., 2005) and can be attributed to its low mobility when applied in this condition.

Surface liming stimulated the soil pH to increase, which intensified with the increment of the lime rates at depths of 0 to 5 and 5 to 10 cm (Figure 1A). However, the effect in the layer of 0 to 5 cm was quadratic, while the layer of 5 to 10 cm was linear, indicating a stabilization of the pH in the superficial layer at higher rates. In the layer of 10 to 20 cm the surface lime rates applied did not result in a significant effect.

The levels of H^{+}+Al^{3+} exhibited reduction in all depths evaluated after the application with increased lime rate, being the layer of 0 to 5 cm and 5 to 10 cm the effect was quadratic, that is, with a tendency to stabilize at higher rates and at depth of 10 to 20 cm the effect was linear. These results demonstrate that the potential acidity was reduced until the depth of 20 cm (Figure 1B)

Despite surface liming did not alter the pH of the layer 10-20 cm (Figure 1A), it indeed altered the potential acidity in this layer with greater intensity at the higher rates applied. Soratto and Crusciol (2008) found similar results where liming promoted a decrease of the soil potential acidity (H+Al) in the layer up to 20 cm deep after surface liming inversely proportional to the rate applied.

The level of Ca^{2+} exhibited an increase accordingly to the increment of limestone rate applied, at all depths evaluated demonstrating that Ca^{2+} applied as limestone can reach depths higher than 10 cm after 40 months of surface liming (Figure 2A). The decrease of Ca^{2+} at depths greater than 20 cm was reported in other studies (Caires et al., 2000; Soratto and Crusciol, 2008; PaulettI et al., 2014), demonstrating that an improvement in the root zone environment was proportional to the rate applied at soil surface.

Magnesium presented a reduction of the levels in the layer of 0 to 5 cm at the highest lime rate tested (Figure 2B) and the increase of the levels in the layers of 5 to 10 and 10 to 20 cm according to the rate applied. This result can be the consequence of an interference with the high Ca^{2+} content of the superficial layer at excessive rates of surface liming, reason been that according to Caires et al. (1998), with the presence of limestone the magnesium leaching is more pronounced. The same authors observed that after 12 months of liming an increment in the level of magnesium in the layer of 5 to 10 cm, similarly with this experiment.

With increasing surface liming rates, it was observed that an increment in the saturation of bases and a reduction in the saturation of Al at all depths were evaluated (Figure 3A and B), reflecting the effects in H+Al and the levels of Ca^{2+} and Mg^{2+}. Therefore, evident improvement in the root zone environment up to a depth of 20 cm can be reached with the application of rates equivalent to 1 SMP to pH 5.5 in a soil with elevated levels of organic matter and potential acidity. It is important to point out that this effect on soil chemistry is less efficient than deep liming at the implementation of a no-till cropping system (Bortoluzzi et al., 2014) but an alternative to tillage in a consolidated no-till cropping system.

Maize yield components presented significant variations to the rates of surface liming applied. Plant height and height of first cob (Figure 4A) had a quadratic increase according to the increment of lime rate. Maize plants reached maximum height development with lime rate of 12.5 ton/ha and rate of 1 SMP to reach pH 5.5 in the layer of 0 to 20 cm. Caires et al. (2002) also reported an increment in maize plant height with the increment at the rates of limestone, in a latosol in Paraná state.

Maize yield also presented a significant quadratic increase with the increment of the rates of surface liming (Figure 4B), demonstrating a beneficial effect to soil for maize development. The increment of plant height and maize yield found in this experiment with the application of the rate obtained for 1 SMP to pH 5.5 are the consequence of the improvement of the soil features, being an indicative of the rate for soil amelioration through surface liming, in soil with elevated potential acidity. These results corroborate with the findings of Caires et al. (2000), where the rate of limestone for a maximum economic efficiency is indicated by the elevation of bases saturation method to 65%, for a soil sample collected at a depth of 0 to 20 cm, corresponded in this experiment to the rate of 15 ton/ha^{-1}. Therefore, considering the correlation between these methods, the results indicate that this criterion is adequate for the recommendation of surface liming rates in a no-till cropping system, even with rates higher than 5 ton/ha^{-1}.

Conclusion

The limestone application at surface, according to the total amount based on the soil analysis recommendation, is not a commonly used practice in the southern region of Brazil, since the fertilization and liming recommendation manuals establish 5.0 ton.ha-1 as the annual maximum limit for the superficial application, or its incorporation, if the recommended doses are higher than this value, when it is necessary the correction of the acidity in the depth of 10 to 20 cm. However, for the soils of the evaluated region, with high potential acidity and high levels of organic matter, the superficial application of the total limestone dose required to raise the pH H_2O to 5.5 (according to the recommendation based on soil analysis by the SMP method), is an effective alternative for improvements in soil chemical characteristics in the long term and increase in maize crop productivity without changes in soil physics, resulting in the maintenance of soil organic matter and in the economics of soil management activities associated with liming

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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