Seed quality in informal seed systems

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ABSTRACT

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Seed is a crucial input for agricultural production. Approximately 80% of the smallholder farmers in Africa depend for their seed on the informal seed system, consisting of farmers involved in selection, production and dissemination of seed. The lack of overhead, distribution and seed testing costs enables seed-producing farmers to offer seed for low prices, but seed quality is not always good. Seed-producing farmers multiply their seed on-farm without frequent seed renewal, referred to as seed recycling, which may lead to low seed quality. This research analysed the effect of seed recycling on physiological quality and seed health of cowpea and maize, and compared seed quality of the formal and informal seed system.

We tested the physical and physiological quality of cowpea seeds produced by the formal and informal seed system. Five out of six foundation seed samples, 79 out of 81 samples of farmers' seed, and six out of six seed company samples failed to meet standards for foundation and certified seeds of the National Agriculture Seed Council (NASC), the seed industry regulatory agency in Nigeria. No evidence was found for a negative effect of seed recycling on physiological quality of cowpea seeds. We analysed 45,500 cowpea seeds for seed-borne bacteria and fungi to compare the performance of formal and informal seed systems. All samples were heavily infected with seed-borne pathogens, including *Fusarium oxysporum* (69% of the samples) and *Macrophomina phaseolina* (76%). No evidence was found that seed recycling in the informal seed system did lead to increased levels of seed-borne pathogens. We also analysed seed quality of farmer-produced maize seed to compare it with the formal seed system. The seed company samples had significantly higher germination (99.3%) than farmer-produced seed (97.7%), but not a single sample passed the requirements for certified seed of the NASC. Twelve seed-borne pathogens were identified including *Bipolaris maydis* (found in 45% of the farmer-produced samples), *Botryodiplodia theobromae* (97%) and *Fusarium verticillioides* (100%). Seed recycling had no negative effect on the physiological quality or seed health of maize seed. We analysed formal and informal seed systems to assess the opportunities to prevent mycotoxigenic fungi infection in maize seeds. A range of control methods to avoid fungal infection and mycotoxin production is discussed in relation to three criteria for sustainable implementation in developing countries. An integrated approach is recommended, with special attention towards the local seed system. As an

overall conclusion of the work it can be stated that the informal seed system did not underperform compared to the formal seed system for cowpea, but did underperform in relation to seed company samples of maize. There was no evidence that seed recycling reduces seed quality of cowpea and maize seed samples, so frequent seed renewal will not improve seed quality of the informal seed system. We recommend a new quality assurance system for the informal seed system based on seed quality testing by farmers themselves, without interference by government or external laboratories. Farmers publish their seed testing results on the bag, while buyers can retest the seed to verify the quality. Further research is required to develop and implement this system in different countries, agro-ecologies and crops, and to develop methods that enable farmers to test seed health quality themselves.

Keywords: informal seed systems, seed recycling, seed quality, germination, seed pathology, seed health, seed-borne diseases, mycotoxigenic fungi, *Fusarium verticillioides*, mycotoxins, *Vigna unguiculata*, *Zea mays*, Nigeria.

CONTENTS

CHAPTER 1

General Introduction

BACKGROUND

Seed is a crucial input for agricultural production and the most affordable external input for smallholder farmers (MacGuire, 2005). Improving the availability of high-quality seed of well-adapted varieties is important to boost agricultural productivity, leading to higher farmers' income, reduced poverty and improved food security (Abdoulaye et al., 2009); (Morris & Heisey, 2003); (Evenson & Gollin, 2003). Seed companies fail to provide small quantities of high-quality seed to remote areas for affordable prices. The majority of smallholder farmers in developing countries depend for their seed on seed-producing farmers or on their own seed saved from their food harvest, the informal Seed System (SS). In such a system seed quality remains unknown (van Gastel et al., 2002), or the perception of the quality is merely based on the reputation of the producer.

Farmers are not always well aware of the agronomic value of high-quality seed. It has to be demonstrated to them repeatedly. However, farmers planting low-quality seed risk poor field emergence and low plant vigour as a result of poor physiological quality (Matthews et al., 2012). Infection with seed-borne pathogens can result in reduced germination, stunted growth, higher disease pressure and introduction of new diseases (Maddox, 1998). Furthermore, seeds infected with mycotoxigenic fungi can initiate mycotoxin contamination, with adverse health effects for the population when affected produce is consumed (Wild & Gong, 2010).
The research described in this thesis analysed seed quality of the informal SS, and compared it with seed quality in the formal SS. The research assessed the opportunities of SS to control mycotoxigenic fungi infection, and analysed the effect of seed recycling on seed quality. The general introduction to this thesis provides the theoretical background of SS, seed quality, seed recycling and mycotoxins. The introduction concludes with the research design and an outline of the thesis.

Seed systems

Farmers can buy seed from formal and informal SS. The formal SS is defined as all formal institutions and private companies involved in breeding, varietal registration, seed multiplication, quality control and seed dissemination. The formal SS is characterized by specialisation and the use of standardized methodologies to meet

international standards. Examples are public breeding programmes, government agencies, NGOs and seed companies. The informal SS or farmer SS only consists of seed-producing farmers involved in selection, production and dissemination of seed. Farmer-saved seed is used for own production, often in combination with sales or exchange within the local community (Louwaars, 2007). Instead of farmer SS, the term informal SS was used for this thesis to emphasize the non-regulated character of the system and to avoid confusion with farmers participating in the formal SS, for example as out-growers for seed companies.

Table 1. Strengths (yes) and weaknesses (no) of formal and informal Seed Systems (SS).

Characteristics	Formal SS	Informal SS
Breeding	yes	only selection of landraces
Varietal registration	yes	no
Seed quality testing infrastructure	yes	no
Seed certification	yes	no
Seed prices	high	low
Seed available in remote areas	no	yes
Seed supply of hybrids	yes	no
Seed supply of minor crops	no	yes
Seed supply of all varieties	no	yes

Both SS have their strengths and weaknesses (Table 1). The formal SS has extended breeding programmes to develop new varieties, and the ability to test for seed quality. It aims at regulating the seed sector in an attempt to guarantee sufficient supply of high quality seed. Disadvantages are high overhead costs, relatively high seed prices, and insufficient supply. As a market oriented business, the private sector does not tend to offer a wide range of varieties for crops, it does not provide seed for minor crops due to limited demand, and it is not able to distribute small quantities of seed to remote areas (Almekinders & Louwaars, 2002). The informal SS fills this demand gap. Approximately 80% of the smallholder farmers in Africa depend on the informal SS for their seed. The lack of overhead, distribution and seed testing costs enables seed-producing farmers to offer seed for relatively lower prices compared to seed companies, but the flip side is the risk of producing low quality seed (Louwaars & De Boef, 2012).

Seed quality

Seed quality includes genetic quality, physical quality, physiological quality and seed health (Louwaars, 2007). The *genetic quality* of the plant determines the potential yield of the plant, and its ability to deal with biotic and abiotic stresses. Varietal registration procedures test genetic quality with criteria Distinctness, Uniformity and Stability (DUS). The criteria distinctness is only met if a variety can be clearly discriminated from existing varieties. Uniformity relates to number of off-types, which are seeds from a different variety, recognized by visual differences in colour or shape. Stability is the ability to reproduce the seed without losing essential varietal properties (Gunjaca et al., 2008). *Physical quality* is the amount of good seeds in a seed sample in relation to all sorts of visibly detectable contamination, seed damage and seed size. Seed samples may be contaminated with broken or damaged seeds, weed and other crop seeds, inert matter and off-types seeds. High physical quality can be achieved by removing all contamination through seed cleaning, in combination with adequate storage to avoid insect damage (Louwaars, 2007). It is a matter of definition whether off-types are considered as a breach of varietal purity and fall under genetic quality (Gunjaca et al., 2008), or a result of poor seed cleaning and therefore part of physical quality. This thesis categorized off-types in seed samples as a breach of physical purity in order to emphasize the responsibility of seed producers to clean their seed properly. *Physiological quality* refers to the seeds' ability to germinate, emerge from the soil and form a vigorous seedling. Especially under the stressful conditions prevailing in the fields of most smallholder farmers, high physiological quality is essential to create an optimal and uniform plant density with minimum seeding rates. Physiological quality is influenced by conditions during crop growth, harvesting and storage conditions. (Ghassemi-Golezani & Mazloomi-Oskooyi, 2008). Last but not least, there is *seed health*. Seed-borne pathogens are able to infect the seed during seed production, transmit from seed-to-seedling and infect the full-grown plant. Planting infected seeds can lead to reduced germination, increased seedling mortality, stunted growth and plant diseases, all leading to reduced yields (Solorzano & Malvick, 2011). Consequences can be severe when infected plants become an infection source for other plants, using insects as a vector to spread the disease through the canopy (Wada et al., 2002). Since various seed-borne pathogens are also soil-borne, planting infected seeds can introduce new soil-borne pathogens to hitherto uninfested soils. This threat forced governments around the world to put phytosanitary regulation in place to assure only healthy seeds are traded (Maddox, 1998).

Mycotoxins

Seed health can even threaten human health. Several seed-borne fungi can produce mycotoxins under field and storage conditions, thereby contaminating the food of millions of people. Exposure to high mycotoxin levels can be lethal, while chronic exposure to mycotoxin contaminated food can cause cancer (Wild & Gong, 2010). Most mycotoxigenic fungi belong to the genera *Aspergillus*, *Alternaria*, *Fusaria* and *Penicillium* (Tsitsigiannis et al., 2012). High income countries control the mycotoxin hazard with certification (Magan, 2006) and enforcing strict food regulation (Egmond, 2002), which is not compatible with subsistence farming in low-income countries (Wild & Gong, 2010). An important control strategy to avoid mycotoxin contamination is to prevent toxigenic fungi infection (Munkvold, 2003), which can occur by seed-to-seedling transmission from infected seeds (Bacon et al., 2001). The research in this thesis analysed how formal and informal SS can avoid mycotoxigenic fungi infection of seed based on an integrated approach.

Seed quality regulation

In contrast to the informal SS, the formal SS aims at regulating the seed sector in an attempt to guarantee sufficient supply of high quality seed. Seed certification assists the formal SS to assure seed quality. Certification schemes differ by crop and country, but often include requirements concerning the production field, foundation seed, disease control, harvest methods and seed processing. External field inspections are carried out to check for diseased and off-type plants in the field, and to check the distance with other fields to avoid outcrossing with other varieties. Seed testing is an essential aspect to evaluate the seed production process and to assure high quality standards for certified seed. Effectiveness of the system depends on the minimum seed quality requirements, and the ability to assure that all seed sales meet them. Unfortunately, certification agencies in most developing countries lack sufficient funding and qualified personnel to carry out their task properly, directly threatening seed quality of the formal SS (van Gastel et al., 2002). This thesis compared the seed quality of the informal SS with samples from the formal SS.

Seed recycling

The problems of the informal SS are not limited to seed testing and certification. Most seed-producing farmers lack access to proper foundation seed. Farmers continue with on-farm seed multiplication without seed renewal, referred to as seed recycling.

Continued seed recycling would lead to low seed quality, contributing to poor yields (Amaza et al., 2010). Several studies analysed the effects of seed recycling on hybrid and Open Pollinated Variety (OPV) seeds from various crops. Seed recycling of hybrid canola seeds in Canada resulted in lower yields (Clayton et al., 2009), but the consequences OPVs are debated. Seed recycling of several crops led to poor seed quality resulting in low yields in Nigeria (Amaza et al., 2010), while others report that OPVs allow seed recycling without yield penalty, describing this trait as an economic advantage of OPVs over hybrids (Warburton et al., 2010). Seed recycling experiments with maize hybrids and OPVs in Zimbabwe proved that OPV seed recycling of hybrids resulted in 32% yield loss against 5% for OPVs. Many farmers expecting yields below 1.5 Mg/ha were struggling with high input prices for hybrid seed and fertilizer. They were advised to purchase the cheaper OPV seed and allocate the savings to buy extra inputs like fertilizers (Pixley & Bänziger, 2002).
Seed health concerns of farmer-saved oat seed used for organic production were investigated in the Czech Republic. In comparison with certified organic seed, the farmer-saved seed did not have significantly ($P<0.05$) higher infection with Fusarium, Alternaria and Penicillium species. The 9 percent-point higher germination and 9 percent point higher field emergence of certified over farmer-produced seed were both not significant ($P<0.05$), suggesting large variability between seed lots (Konvalina et al., 2012). A report about seed recycling of farmer-saved wheat seed was carried out in Finland. Only 60% of the farmer-saved seeds exceeded 85% germination. The research could not identify a maximum number of seed-recycling generations before seed quality reduction would take place (Peltonen-Sainio et al., 2011). The effect of seed recycling on seed health might be a different story. The combination of new infections in the growing season in addition to infection from infected seeds can lead to a build-up of pathogens after each season of seed-recycling. This research analysed if more on-farm multiplications of seed without seed renewal would lead to higher levels of infection with seed-borne pathogens.

RESEARCH DESIGN

The research was designed to answer the following research questions:

1. Does the informal SS underperform compared to the formal SS in delivering high-quality seed?

2. Does continued seed recycling affect physiological quality or seed health?

3. Can SS contribute to the control of mycotoxigenic fungi infection?

Physiological quality, physical purity and seed health were assessed from seed samples produced by the formal and informal SS. The research was carried out in Nigeria, with 170 million inhabitants the most populous country of Africa. Seventy percent of the population lives below the poverty line. Agriculture provides roughly 70% of the employment opportunities in Nigeria, but contributes only 31% of Nigeria's Gross Domestic Product (GDP) (CIA, 2012). Another reason to conduct this research in Nigeria is the relatively well developed formal SS in the country compared to other countries in the region (Abdoulaye et al., 2009).

The seed samples were collected from seed-producing farmers in Borno and Kaduna state, who used the same improved varieties received from the International Institute of Tropical Agriculture (IITA) in previous years. This was done to avoid that genetic differences between local varieties would affect the testing results, and to enable comparison with foundation seed of the same varieties. Kaduna state is situated in the centre of northern Nigeria, comprising the Southern and Northern Guinea savannah agro-ecologies. Borno state is the most north-eastern state of Nigeria, containing both Guinea savannah zones and the dryer Sudan savannah zone. Borno was a focus state for the project "Promoting Sustainable Agriculture in Borno state" (PROSAB) from 2004-2008. Seed-producing farmers received foundation seed of improved varieties and were trained in seed production (Amaza et al., 2010). The year farmers received foundation seed was recorded to calculate the number of seasons farmers multiplied their seed on-farm. The number of on-farm multiplications was used to determine the effect of seed recycling on physiological quality and seed health.

One leguminous, cowpea (*Vigna unguiculata* (L.) Walp.), and one cereal, maize (*Zea mays* L.), crop were selected for this research. Both cowpea and maize are important for food security in Nigeria, but represent two extremes in SS development. Cowpea farmers depend almost solely on the informal SS, while 47% of the maize seed in Nigeria is sold by the formal SS. Cowpea farmers who are unsatisfied about the seed quality have virtually no alternative for the informal SS, while maize farmers can buy OPV or hybrid maize seed from a seed company. Maize was the most widely produced cereal in Nigeria, even more than sorghum, millet and rice. The Nigerian population consumed 29.4 kg of maize/capita/year in 2009 (FAO, 2012). Yields are constrained by poor germination and disease pressure (Daniel & Adetumbi, 2006) (Odeyemi et al., 2010). Cowpea is a widely grown legume in Nigeria, providing vital proteins to millions of people (Langyintuo et al., 2003). This popular legume can suffer significant yield losses from plant diseases, including seed-borne diseases (Bankole & Adebanjo, 1996). Moreover, cowpea suffers major post-harvest losses due to the

storage pest, in particular the bruchid beetle *Callosobruchus maculatus* F.. Bruchid damage severely reduces germination potential of the seed and facilitates infection with seed-borne pathogens (Moussa et al., 2011).

Several seed quality experiments were carried out to determine physiological quality, physical purity and seed health of the samples. Germination speed and total germination were determined with a paper towel method, while physical purity was analysed by seed cleaning. Cowpea and maize samples were planted on research farms in Kano and Kaduna to determine field emergence, but seed health required the largest effort. A total of 45,500 cowpea and 49,500 maize seeds were plated on agar to determine bacterial and fungi infection of the seed samples.

OUTLINE OF THE THESIS

The thesis starts with this introduction, followed by three original research papers, a perspective, and concludes with a general discussion. Chapter 2 compares the physiological quality of cowpea seed samples from seed-producing farmers (informal SS), with seed company and foundation seed samples (formal SS). The effect of seed recycling on cowpea germination and field emergence is analysed with a multiple regression model. Chapter 3 compares seed health of cowpea samples from the formal and informal SS. The effect of seed recycling on seed-borne pathogen infection is visualized by plotting the percentage of infected seeds against on-farm multiplications. Chapter 4 analyses physiological quality and seed health of maize seed samples from the informal SS, and compares it with seed samples from the formal SS. The effect of seed-recycling on seed-borne pathogen infection and the number of off-types was analysed too. Chapter 5 assesses how maize SS can contribute to the control of mycotoxigenic fungi. A schematic overview of the formal and informal SS is presented to visualize fungi infection and mycotoxin contamination risks in the maize value chain. The perspective recommends an integrated approach to control mycotoxigenic fungi infection. The general discussion (Chapter 6) presents a brief answer to the scientific research questions of this thesis. Literature and the current results were used to identify five major bottlenecks for seed quality in the informal SS. The general discussion concludes with recommendations for a new quality assurance system based on capacity building in the informal SS.

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CHAPTER 2

Are investments in an informal seed system for cowpea a worthwhile endeavour?*

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Abstract

High seed quality is a critical component for realising yield potential. For smallholder cowpea farmers in northern Nigeria the informal seed system is a major supplier of genetically high-quality seed, but the physiological quality of farmers' produced seed remains unknown. The project "Promoting Sustainable Agriculture in Borno State" (PROSAB) trained and supported farmers in seed production in Borno State, Nigeria. We analysed the quality of farmers' produced cowpea seed based on standard quality testing criteria, and evaluated its field emergence as a proxy for non-genetic seed quality. We carried out a survey among seed producing farmers about their production and storage practices, and tested seed quality of samples from these farmers, from seed companies and compared these to foundation seed. Field emergence of farmers' produced seed was not significantly different from that of foundation seed (P=0.47) or seed company samples (P=0.12). Cowpea seed quality, however, was inadequate in both the formal and informal seed systems. Five out of six foundation seed samples, 79 out of 81 samples of farmers' seed, and six out of six seed company samples failed to meet standards for foundation and certified seeds of the National Agriculture Seed Council (NASC), the seed industry regulatory agency in Nigeria. Multiple regression analyses predicting field emergence showed that projects like PROSAB can improve seed quality. Especially proper storage and reducing seed damage can increase field emergence significantly. Our findings suggest that it is worth to invest in improving the informal seed system of cowpea.

Keywords: Cowpea; Vigna unguiculata; Seed systems; Seed quality; Northern-Nigeria.

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INTRODUCTION

Seed is a crucial input for agricultural production, and the most affordable external input for smallholder farmers. The genotype of the planting material affects the plant's ability to cope with harsh weather conditions, diseases and pests, and determines the potential yield of a crop. Seed is the only way for farmers to benefit from investments in crop improvement. High physical quality of seed is essential to establish a sufficient plant stand, directly affecting the yield (McGuire, 2005). High-quality seed should be free from diseases to avoid seedling mortality or introduction of diseases (Haque et al., 2007).

Farmers' access to seed is organized in seed systems (SS), which involve all actors in breeding, seed production, quality control and dissemination. The formal SS consists of public institutions and private companies specializing in their own role in the seed value chain. They apply defined methodologies to meet national and international standards, and in many countries are supported by national legislation and oversight. The formal SS usually controls seed multiplication to assure sufficient quantities of breeder, foundation and certified seed of guaranteed quality. The informal SS, also called the farmers' SS, is operated solely by farmers involved in local seed selection, production and diffusion. Production and dissemination takes place at farmer and community level (Louwaars, 2007). In developing countries, 60-100% of the farmers depend fully on the informal SS for their planting material, despite all investments in the development of a formal SS. Smallholder farmers in general request relatively small quantities of seed, live in remote areas, and have very limited budget for seed purchases. As a market-oriented business, the private sector does not tend to offer a wide range of varieties for crops, it does not provide seed for minor crops due to limited demand, and it is not able to distribute small quantities of seed to remote areas (Almekinders & Louwaars, 2002).

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important legume in West and Central Africa, providing vital proteins for human consumption and fodder for livestock (Uzogara & Ofuya, 1992). The grains are utilized in a wide variety of local dishes and have great potential to fortify food. Alene and Manyong (2006) suggested that adaptation of improved varieties can further enhance cowpeas' impact on rural life. Nigerian farmers planting improved cowpea varieties were more food secure and had higher income compared with farmers growing local varieties. However, the availability of seed is still a bottleneck for adoption of these new varieties. In Sub Saharan Africa, due to issues described above, farmers cultivating cowpea depend

largely on the informal SS as the source of their cowpea seed (DeVries & Toenniessen, 2001). Almekinders et al. (1994) suggested that a combined approach of strengthening the informal SS along with creating and enhancing linkages with the formal SS may act as a vehicle for addressing the issue of availability of improved germplasm. A recent study of cereal SS in Syria showed that improving seed delivery systems can only be successful when actors understand the functioning of the whole SS and know farmers' motivations to choose for certain varieties and seed sources (Bishaw et al., 2011).

The current evidence on farmers' seed production indicate that seed production and storage methods need to be improved to increase seed quality. Nigeria's National Agricultural Seed Council (NASC) published certification standards for cowpea seed. Samples should consist for minimal 98% of cowpea seed, maximal 10 off-type seeds per kg sample, and should have a minimum germination rate of 85%. One of the most important traits is the seeds' ability to create a uniform field stand of the desired plants (van Gastel et al., 2002), which is mostly referred to as seed vigour. Especially under the suboptimal environmental conditions of most smallholder farmers, vigorous seeds are required to achieve high field emergence and an uniform crop stand (Ghassemi-Golezani & Mazloomi-Oskooyi, 2008). Cleaning cowpea seed samples had a positive effect on field emergence, especially when small and broken seeds were removed (Asiedu et al., 2003). A specific threat for cowpea are storage pests, in particular the bruchid beetle *Callosobruchus maculatus* (Fabricius), which is locally called weevil. Bruchids cause characteristic holes in cowpea, affecting seed weight and viability whilst enabling the introduction of pathogenic fungi and bacteria into the seed. Farmers traditionally store their seeds in polyethylene bags, but storage pests forced them to look for alternatives like metal drums, double bagging and the Purdue Improved Cowpea Storage (PICS) bags (Moussa et al., 2011). Although airtight storage technologies like the PICS bags can successfully suppress bruchid damage, most farmers still use inferior storage bags (Sanon et al., 2011).

The project "Promoting Sustainable Agriculture in Borno State" (PROSAB) addressed the seed quality problem from 2004-2008. The project identified the lack of quality seeds as a major constraint for agricultural production, contributing to food insecurity in Borno State, Nigeria. PROSAB tried to strengthen the informal SS of cowpea by introducing improved varieties, in combination with initiatives and incentives to enhance local seed production. A community-based seed scheme was implemented by training farmers in seed production. Farmers participated in a workshop to be trained in all relevant aspects of seed production including plot selection, land clearing, pest

and weed control, removal of off-type and diseased plants, harvesting and storage methods. Project staff assisted farmers with selecting appropriate plots to avoid outcrossing or mixing with other varieties, or problems with witch weed or drainage problems. Furthermore, farmers received foundation seed and were registered as seed producers by the National Seed Council (NSC). NSC officers inspected the field for certification twice a season, and made sure that farmers implemented the required procedures for seed production. The project turned out to be successful in terms of an increased seed availability of improved varieties (Amaza et al., 2010). The projects' success in terms of cowpea seed quality remains unevaluated. An assessment is crucial for stakeholders and donors targeting to invest in the cowpea SS. This research analysed whether the PROSAB seed producers can match the formal SS in terms of seed quality, and identified the most successful elements of the project approach. The first objective of the study was to evaluate the quality of farmer produced seed. A comparison was made between farmer's seed, samples from seed companies and as a benchmark with samples of the foundation seed that farmers received to start up the seed production. The second objective of the study was to evaluate the effect of individual project elements on cowpea field emergence to establish the most important characters contributing to uniform emergence and optimised crop establishment.

MATERIAL AND METHODS

General approach

Seed samples were collected from seed-producing farmers, seed companies and foundation seed in Borno and Kaduna State, Nigeria. Farmers were interviewed during seed collection about factors that might influence seed quality, including inputs, storage and certification. The seed quality parameters assessed included physical purity, germination rate and field emergence. A multiple regression model was used to analyse the relation between farmer practices and field emergence. In the following sections, we will describe the plant material used, and the methodology of the field experiments and the survey in detail.

Plant material

In May 2009 and April 2010, 2-3 months prior to planting, 41 and 40 samples of between 2.5-5.0 kg each were collected, respectively, from seed producing farmers in Borno and Kaduna State (Table 1). Kaduna State is located in Northern Nigeria comprising the Southern and Northern Guinea Savanna zone. Borno State forms the

most north eastern part of Nigeria, and also includes the dryer Sudan Savanna zone. Three improved cowpea varieties were selected based on their maturity type and popularity among farmers. The late-maturing variety IT89KD288 and the medium-maturing IT89KD391 were most popular in Borno State, the former PROSAB area. The very early maturing variety IT93K452-1 was the most preferred variety in Kaduna State. From 2001-2009, farmers received foundation seed only once, and kept multiplying the seed until the year we collected the seed samples. The number of seasons that farmers multiplied their seed on farm was indicated by "multiplication". Only farmers that once received foundation seed from the selected varieties were part of the sampling frame.

Table 1. Overview of cowpea seed samples collected from farmers. Farmers received foundation seed between 2001 and 2009, and multiplied their seed for 1-9 seasons until our sampling in 2009 or 2010.

Variety	Number of multiplications by farmer	No. of samples taken	
		2009	2010
IT89KD288	1	2	0
	2	2	2
	3	2	2
	4	2	2
	5	2	1
	6	0	1
IT89KD391	1	3	3
	2	3	3
	3	3	3
	4	3	3
	5	0	1
IT93K452-1	1	3	0
	2	3	3
	3	3	3
	4	2	3
	5	3	2
	6	2	3
	7	0	2
	8	3	0
	9	0	3
Total		41	40

Comparing multiplication 1-9 within a variety showed the effect of seed recycling on seed quality. Six samples were purchased from seed company outlets in Kano, Borno and Kaduna State; two samples in 2009 and four in 2010. Foundation seed from each

variety included in the study was collected from the International Institute of Tropical Agriculture (IITA). All seed samples were stored at room temperature between collection and planting time to mimic storage conditions of farmers buying seed from their colleagues.

Table 2. Descriptive summary of regression variables.

Variables	Description
Measured variables	
Off types	Seeds visibly different from expected variety (colour/shape)
Broken seeds	All seeds that are broken, or missing an embryo
Bruchid damage	All seeds damaged by cowpea bruchid beetles
Other damage	All damaged seeds except broken seeds and seeds with bruchid damage
Germ2	Germination rate after 2 days
Total germination	Germination rate after 7 days
Femergence	Field emergence after 14 days
Survey variables	
Year	Year of seed sample collection (2009 /2010)
State	State where sample was collected (Borno / Kaduna)
Multiplications	Number of on-farm seed multiplications
Field inspection	Field inspection by extension agents for certification
Selection	Farmers selected good cowpea pods before or during harvest to provide seed
Storage	Method and location to store cowpea seed from harvest until sale or planting
Drum	Metal, tightly closed drum, used to store cowpea seed or grains
Polybag	A single-layer polypropylene bag
Double bag	One inner high density polyethylene bag surrounded by an outer polypropylene bag
PICS bag	Purdue Improved Cowpea Storage (PICS) bag; two inner high density polyethylene bags surrounded by an outer polypropylene bag
Store	Storage location only used for storage activities
Room	Storage location also used for non-storage activities

Experiments

Physical purity was measured by sorting 1 kg of each seed sample. The composition of the seed lot was divided into categories of pure seed, other seeds and inert matter as described by the International Seed Testing Association (ISTA) standards for pure seed (Manino et al., 2010). Instead of two separate analyses for physical purity and other species count as described by ISTA, all analyses were done on one sample of 1 kg. The procedure for seed damage, off-types and hundred-seed weight deviated from

the ISTA standards to analyse the effects on field emergence in detail. Off-types were removed by visually observed differences in shape and colour. Damaged seeds were divided into broken seeds, bruchid damage, and other damage. Off-type seeds with seed damage could belong to both categories, but were always categorized as off-type. Inert matter and other crop and weed seeds were also measured separately. Hundred-seed weight was measured from the total sample prior to sorting, but inert matter, broken seeds and other crop seeds were replaced with intact seeds from the sample. The definitions of the various categories used in the sorting process are shown in Table 2. The results of farmers, seed companies and foundation seed samples were compared and tested for significance with the two-sided t-test in MS Excel®.

Germination rate was determined based on unsorted seeds with the exclusion of materials farmers would not plant: broken seeds, very small seeds and other crop and weed seeds. Broken seeds are considered to be inert matter by the NASC, and very small seeds are not suitable for use in germination tests. Germination rate was determined with the paper towel method on 400 seeds as described by ISTA. Fifty seeds were rolled in one paper towel and put vertically in a cup. The cups holding eight paper towels were filled with 1 cm water and placed into an incubator at 27 °C. The paper towels were unfolded every 24 hours to count and remove the germinated seeds, up to 7 days after initiation of testing. Although ISTA germination standards for cowpea only require observation on 5^{th} and 8^{th} day after test initiation, all non-germinated seeds appeared to be disintegrated after 7 days, making observation at the 8^{th} day unnecessary. Germinated seeds were counted daily instead of only twice to allow analysis of germination speed as a parameter for seed vigour.

Field emergence was tested in two seasons with 41 samples in 2009, and 35 samples in 2010. Although 40 samples were collected in 2010, some samples had to be omitted due to insufficient seed delivery of five farmers in 2010. Field was measured under rain fed conditions at Minjibir farm (12808.9970 N, 8839.7330 E) in Kano State, Nigeria. The field was planted in July, the time that most farmers planted cowpea. The farm lies in the Sudan Savanna agro-ecological zone (Boukar et al., 2011). The field was harrowed and ridged with inter ridge space of 0.75 m. The field was divided in three replicates, containing 50 plots of 5.0 m × 4.5 m. Three seeds were planted per hole on an intra-ridge space of 20 cm. The number of planted seeds was estimated by multiplying number of seeds per hole and planting holes. Field emergence was determined by counting the emerged seedlings after 14 days, divided by the number of planted seeds times 100%. To improve plant stand, the number of seeds per hole was increased from three to four in 2010.

Survey

The 40 farmers contributed 81 seed samples. Thirty-two farmers delivered one sample in 2009 and one in 2010, while four of them could even provide seed of a second variety. One farmer delivered three samples, and eight farmers only 1 sample. Only the 14 farmers who lived in Borno State participated in the seed production workshop and benefited from the support of the PROSAB project. The other 26 seed producers from Kaduna State lived outside the project area. The survey consisted of 22 multiple choice questions divided into three categories: farmer's personal information, background of the seed, and the seed production and storage process. Farmer's personal information included the farmer's name and village, including the State and agro-ecological zone the village was situated. To determine the number of on-farm multiplications, farmers were asked the source and year they received "fresh" seed from PROSAB, IITA or another source. Seed production questions included field clearing, fertilizer and agrochemical application. Farmers were asked whether they removed off-type and diseased plants prior to harvest to maintain seed quality or whether they carried out "selection at harvest" by selecting the best pods during harvest to obtain seed. Most farmers received extension agents for "field inspection" as part of a certification program. Farmers also indicated which storage method they used, as well as placement of storage bags in a separate store solely meant for storage, or in a room in the house that was also used for non-storage activities.

Analysis

The relation between field emergence and germination on day 1-7 was analysed with the Pearson r correlation coefficient, calculated with MS Excel®. Two multiple linear regression models were tested with stepwise regression in Genstat 13th edition, using only the data of farmer produced seed samples. The two models explained the variance in germination on day 2 (germ2) and field emergence, respectively. The independent variables were storage, broken seeds, bruchid damage, other damage, multiplication, year, variety, inspection, 100 seed weight and State (Table 2). The quality of the models was assessed by the $R^2_{adj.}$ and the Akaike Information Criterion (AIC). The AIC compares the relative goodness of fit of the models as a trade-off between complexity and accuracy. To determine the importance of a single variable, the explained variance was calculated for each independent variable. The explained variance was calculated by dividing the sum of squares of the independent variable by the total sum of squares of the model.

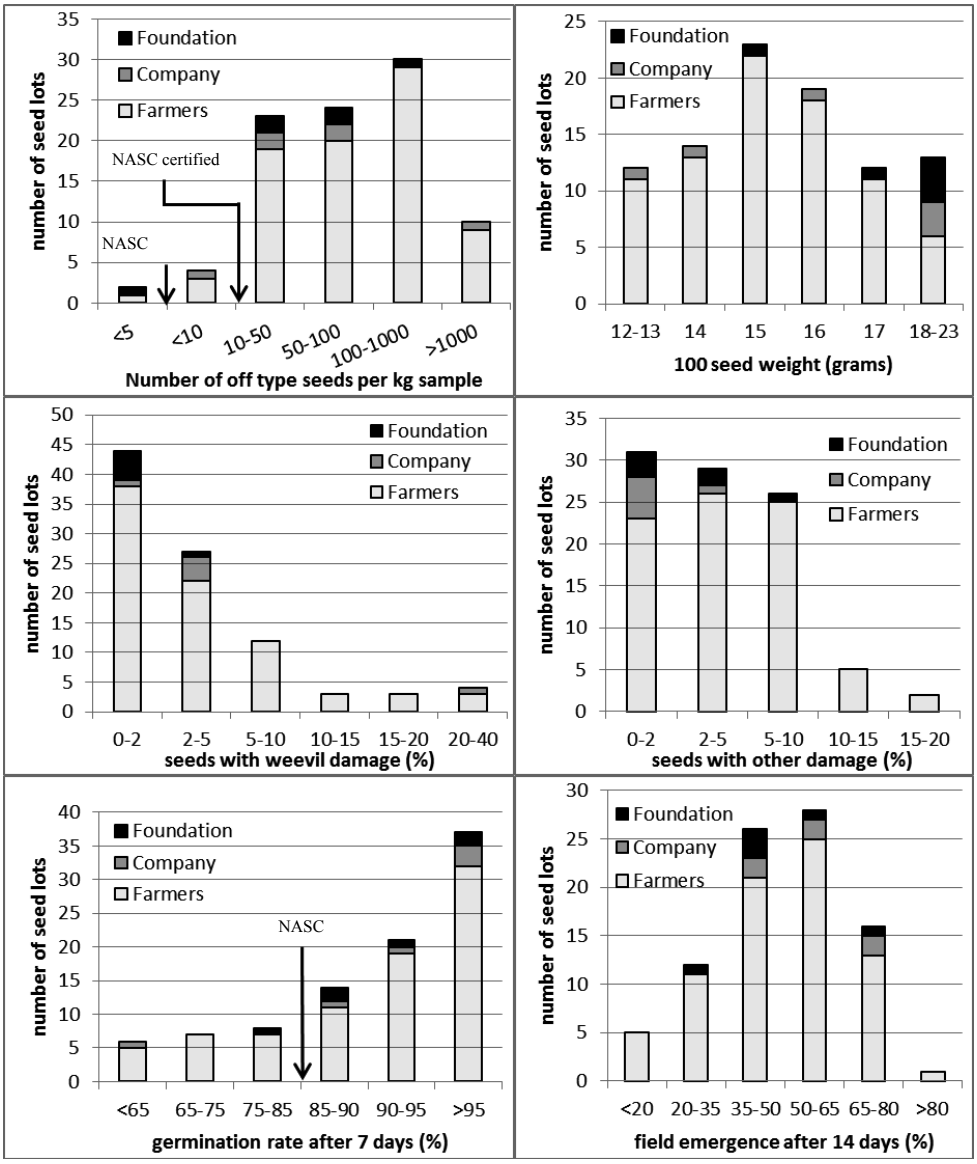

Figure 1. Distribution of farmer produced seed lots, seed company samples and foundation seed for off type seeds, 100 seed weight, bruchid damage, other damage, germination rate and field emergence. Arrows show standards set by the National Agricultural Seed Council (NASC) of Nigeria for the maximum number of off type seeds and minimum germination rate as indicated for certified and foundation seed.

RESULTS AND DISCUSSION

Seed quality

Seed samples from farmers, seed companies and foundation seed were compared for the number of off-type seeds, the percentage seeds with bruchid damage and otherwise damaged seeds, 100 seed weight, germination rate and field emergence (Figure 1). Foundation seed had on average the least number of off-types (62.2 per kg), followed by seed companies with a mean of 212 per kg and farmers with on average 484 off-types per kg. For comparison, 1 kg of seed with the average 100 seed weight of 15.6 g contained approximately 6400 seeds. On average, farmer-produced seed had significantly (t-test, P=0.0003) more off-types than foundation seed, but the number of off-types in seed lots of seed companies was not significantly (t-test, P=0.2019) different from that of farmers' seed. The NASC guidelines for seed certification allow a maximum number of off-types per kg seed sample of five and ten seeds for foundation and certified seeds, respectively. Figure 1 shows that only one out of six foundation seed samples met this requirement. The same conclusion is valid for seed companies where five out of six samples exceeded 10 off-type seeds per kg sample. The majority of foundation and seed company samples, four samples each, had between 10-100 off-types per kg seed. One foundation sample had 195 off-types, 39 times the NASC limit, while a company sample exceeded the NASC limit more than 100 times with a total of 1048 off-types per kg seed sample. Only four out of 81 farmers' samples met the NASC guidelines, and 19 samples fell in the next category of 10-50 off-types. Thirty-eight samples had more than 100 off-type seeds. From the ten samples with more than 1000 off-type seeds, nine samples belonged to variety IT89KD288. Seven of these samples had more than 3000 off-type seeds, meaning that approximately 60% of the seeds were off-type.

Seed size of farmers' seed lots were almost normally distributed with 40 out of 81 samples with a hundred-seed weight of 15-16 grams. Foundation seed samples had four samples in the category of heaviest seeds (18-23 g), against three seed company samples. The average 100 seed weight of foundation seed was not significantly (P=0.2438) different from that of seed company samples. Bruchid damage showed a binomial distribution, with 71 out 93 samples having less than 5% seeds with bruchid damage. A total of four samples, one foundation and three farmer samples, had no bruchid damage at all, while 32 samples had bruchid damage between 0.1-1%. Apparently, bruchid infestation does not directly have to lead to widespread damage.

Foundation seed samples had less bruchid damage than seed company samples. One seed company sample even had 31.7% seeds with bruchid damage.

Damaged seeds that were not broken or affected by bruchids were put into the category "other damage", including heavily damaged seeds as well as seeds with light damage to the seed skin. Other damage ranged from 0-20%, with 60 out of 93 samples having less than 5% other damage. Five out of six seed company samples had less than 2% other damage. Foundation seed samples had more other damage than seed company samples, with even one sample in the category 5-10%. Only farmer seed samples had more than 10% other damage. A comparison with bruchid damage showed that the range of other damage was smaller, but that more samples had 5-15% other damage. Foundation samples had less bruchid damage compared with seed company samples, while company samples outperformed foundation samples in other damage.

The germination rate ranged from 59-100%, with an average of 89.4%. The germination rate of farmers seed was not significantly (t-test, P=0.4684) different from foundation seed, neither from seed company (t-test, P=0.9746) samples. Germination rate had a binomial distribution with 37 samples having over 95% germination. Five out of six seed company samples met the NASC guidelines of 85% germination, but the remaining sample had a germination rate of only 60%. The only foundation seed sample that failed the NASC standard had a germination rate of 84%, only 1% less than the required percentage. Nineteen out of 81 farmer samples did not meet the NASC standard for total germination.

Field emergence was normally distributed ranging from 8.6% to 88.1%, with an average of 49.5%. Five seed samples of farmers did not have enough seeds to plant, leaving a total of 88 samples. Five farmer samples had less than 20% germination, meaning that only 1 out of 5 seeds could produce a viable seedling. Forty-six of 76 farmer samples had a field emergence between 35-65%. Only 14 out of 88 farmer seed samples had a field emergence of more than 65%. Foundation seed was not significantly (t-test, P=0.7106) different from farmer-produced seed, while seed company samples performed a little better. Four out of six seed company samples had more than 50% field emergence, against two out of six for foundation seed. Seed company samples had on average the highest field emergence of 58.2%, but that was not significantly different from that of farmers' (t-test, P=0.1192) or foundation (t-test, P=0.1838) seed samples.

Table 3. Pearson r correlation coefficient between field emergence and germination rate (germ) on day 1-7, and the germination on day 1-7 as percentage of the number of seeds germinated on day 7.

Days to germination	1	2	3	4	5	6	7
Correlation coefficient (germ) with field emergence	0.78	0.83	0.75	0.76	0.76	0.76	0.76
Average % of germinated seeds	45.5	88.5	95.8	98.8	99.9	100.0	100.0

The average field emergence of 49.5% was almost 40% lower than the average germination rate. Ellis and Auma (1980) related the gap between germination rate and field emergence to seedbed conditions and seed viability. Storage time and suboptimal storage conditions increased the time barley seeds needed to germinate, which negatively affected field emergence. Many different factors including seed production conditions, physical purity and seed health affect seed vigour. Tests such as tetrazolium, accelerated ageing and electric conductivity are well described to test seed vigour (Pekşen et al., 2004), but these tests require laboratory facilities. In contrast, germination speed can be easily observed by farmers or extension agents under very basic circumstances. Table 3 shows the correlation coefficients between field emergence and the cumulative germination on day 1-7. The correlation with germination on day 1 was 0.78, which increased to 0.83 on day 2, followed by a decrease to 0.75 on day 3. The correlation with germination rates on day 4-7 remained stable at 0.76. The highest correlation coefficient was with germination rate on day 2 when 88.5% of total germination was reached. The remaining 11.5% that germinated between day 3-7 was of little importance for field emergence.

The correlation coefficient for germ1, with only 45.5% of the seeds germinated, was higher for germ3, emphasizing the importance of seed viability over total germination. Germ2 was more important for cowpea field emergence than total germination, because it had the highest correlation coefficient with field emergence. An additional advantage for Nigerian seed producers would be that germ2 can be determined in two days instead of seven, saving time and costs. The NASC is recommended to consider reviewing the standards for certified cowpea seed, and replacing total germination rate by germination on day 2.

Table 4. Two multiple regression models predicting germination rate at day 2 and field emergence with input, production and storage factors. The second and fourth column shows the explained variance per factor as percentage of the total variance in the model. The regression estimates of all factors are presented in columns 3 and 5.

	Model Germ 2			Model Field emergence		
$R^2_{adj.}$	63.1			70.9		
Akaike Information Criterion (AIC)	364			357		
Independent variables:	Explained variance	Regression estimates		Explained variance	Regression estimates	
constant		99.6			16.6	
storage PICS		0	a		0	a
storage double bag room		1.6	a		-2.8	a
storage double bag store		-2.4	a		-18.6	b
storage polybag room	20.7	-2.3	a	25.4	-17.6	b
storage polybag store		-0.2	a		-11.9	b
storage drum		-14.9	b		-20.6	b
broken seeds	2.4	-0.48		4.4	-0.96	
bruchid damage	12.0	-0.53		11.8	-0.65	
other damage	9.6	-0.76		8.6	-0.88	
multiplication 1		0	a		0	ab
multiplication 2		0.7	a		-1.1	a
multiplication 3		-0.9	a		2.9	ab
multiplication 4	11.7	2.9	a	11.6	6.8	bc
multiplication 5		0.7	a		15.7	c
multiplication 6&7		4.7	a		4.2	ab
multiplication 8&9		20.6	b		26.6	d
year 2010	4.7	7.0		5.0	13.6	
variety IT89KD 288		0.0	a			
variety IT89KD 391	8.0	1.1	a			
variety IT93K 452-1		-16.3	b			
inspection	2.6	-9.82				
100 seed weight				7.3	3.5	
state				3.6	-11.0	
Total explained variance	71.7			77.7		

Regression analysis

Field emergence among farmer produced seed samples was poor, considering that 49% of the samples had a field emergence below 50%. Additional analyses were carried out to identify options for improvement. Stepwise multiple linear regression models were analysed predicting germ2 and field emergence with input, production and storage factors. The Akaike Information Criterion (AIC) was used to select the best model for germ2, and the best model for field emergence. The AIC enables to compare models based on the goodness of fit, but penalizes for over fitting by adding more parameters (Burnham & Anderson, 2002). The selected field emergence model had an R^2_{adj} of 70.9, while the selected Germ2 model had an R^2_{adj} of 63.1 (Table 4).

The single most important factor in the two models was storage, explaining 20.7% of the variance in germ2 and 25.4% of the variance in the field emergence model. The influence of storage might even be underestimated by the model, because the effect of storage methods that suppress bruchid damage was captured by the category bruchid damage. The traditional cowpea storage in low density polyethylene bags frequently results in severe seed damage due to storage pests, adversely affecting seed viability. Cowpea bruchid beetles (*Callosobruchus maculatus* F.) can be killed effectively by insecticides applied during seed storage. However these chemicals have a negative effect on human health if consumed, and the application is therefore limited. Some farmers stored their cowpeas in metal drums that are tightly sealed to create a low oxygen environment suppressing bruchids. The double bagging system applies an inner, high density polyethylene bag to create an airtight environment, with an outer polybag to protect the inner bag from damages. Use of only one inner bag might result in penetration of the bag during filling or movement of the bag, allowing oxygen to enter (Moussa et al., 2011). The Purdue Improved Cowpea Storage (PICS) project introduced an airtight storage technology of two high density polyethylene bags, tightly sealed and placed in a nylon bag. The PICS bags effectively arrest insect development, limiting any seed damage while having no impact on germination rates (Sanon et al., 2011). Drums, double bagging and PICS bags are all supposed to be airtight, but drums are more expensive, need to be completely filled to limit the volume of oxygen, and are difficult to transport when filled. Samples stored in drums underperformed in both models with a 14.9%-point lower germ2 and 20.6%-point lower field emergence compared with PICS bags. These samples had significantly ($P=0.05$) lower germ2 than all other storage methods. PICS bags and double bag room outperformed all other methods on field emergence. The superiority of PICS bags over polybags was expected, because the airtight bag layers avoid the entry of oxygen from outside. The significant difference between double bag room and double bag store in

field emergence was more remarkable. Storage in room was superior over store for double bag storage, but store was a better location for polybags, although the difference was not significant (P=0.05). On top of that, store was supposed to be more suitable for storage than a room, which is also used for other activities. Additional research is recommended to investigate interaction between storage location and storage method. Farmers are advised to store their cowpeas in PICS bags, because of the poor performance of double bag in store.

Seed damage was represented in the model by the categories broken seeds, bruchid damage and other damage. Altogether, they explained 24-25% of the variance in both models. Bruchid damage was the most important factor among them, followed by other damage and broken seeds. A 1%-point increase of bruchid damage leads to 0.53%-point lower germ2 and 0.65%-point lower field emergence. Remarkably, the effect of 1%-point other damage was bigger with a regression coefficient of -0.76 for germ2 and a regression coefficient of -0.88 for field emergence.

Broken seeds explained 2.4% of the variance in the germ2 model, and even 4.4% in the field emergence model. Broken seeds had a devastating effect on field emergence with a regression coefficient of -0.96. Therefore, broken seeds might be an indication for poor seed processing in general. Seed damage had more effect on field emergence than on germ2, considering that the regression coefficients for all three damage components were lower for germ2 compared with field emergence. Germ2 is mostly depending on the vitality of the embryo, and was tested in paper towels. Field emergence requires the embryo to emerge from the soil, a process that takes several days in which damaged seeds are not protected by the seed skin.

Variety explained 8.0% of the variance in the germ2 model. Variety IT93K452-1 had with 16.3% significantly (P=0.05) lower germ2 than variety IT89KD288. The underperformance is partly compensated by multiplication 8-9, which only contained samples of variety IT93K452-1. The difference between planting season 2009 and 2010 explained 4.7-5.0% of the variance in the two models. The 2010 season had a 7.0%-point higher germ2, and 13.6%-point higher field emergence than season 2009. The difference in field emergence might be explained by superior field and weather conditions in 2010.

PROSAB implemented a certification system to ensure seed quality. Seed producers were visited by extension agents to observe production conditions and field isolation, and received a certificate at harvest time. Neither germination rate nor physical purity

was measured, so certificates were rewarded solely based on field observations. Although extension agents only rewarded non-legume crops with certificates, field inspections of cowpea were still carried out to check seed production conditions. Only one farmer in Borno was not inspected, while eight farmers in Kaduna State were not visited. Field inspection by extension agents was included in the germ2 model as a factor explaining 2.6% of the variance, where visited farmers had 9.8% lower germ2 than non-visited farmers. The implemented certification system of PROSAB failed to guarantee cowpea seed quality. Personal observation during seed collection showed that farmers were not willing to pay for certification either. Moreover, farmers' perception of the certificate was that they were certified as farmers, not for a specific crop or a season. It is unknown whether users of seed requested a certificate, or that they merely relied on the reputation of the farmer, physical observation of the seed, or experience with the seed producer during previous years.

Seed weight and State were only selected in the field emergence model. Hundred-seed weight among farmer produced samples varied from 12.1-20.7 g with a mean of 15.3 g. The average 100 seed weight of IT93K452-1 was with 14.4 almost 2 g lower than IT89KD288 (16.3 g) and IT89KD391 (16.7 g). One gram increase in hundred-seed weight led to 3.5%-point increase in field emergence. These results were consistent with the results of cowpea seed processing with a gravity separator in Ghana. Cleaned cowpea seed had a higher 100 seed weight, higher germination rate and a higher field emergence (Asiedu et al., 2003).

Regression model 2 showed that farmers in Borno State had 11%-point higher field emergence than farmers from Kaduna State. State was a minor factor in the regression model with only 3.6% of the explained variance. In contrast with Borno State, farmers in Kaduna did not benefit from the support and training in seed production. So the differences between States are an indication that the PROSAB project had a positive influence on seed quality. Unfortunately, a baseline study of seed quality in Borno State prior to PROSAB was not available to confirm that PROSAB had a positive effect on seed quality.
The effects from State and variety are partly intertwined. Variety IT93K452-1 is significantly (P=0.05) different from the other varieties in the germ2 model, but absent in the field emergence model. However, variety IT93K452-1 could only be collected in Kaduna State, which performed significantly worse than Borno State in the field emergence model.

Amaza et al. (2010) considered seed recycling to be one of the problems causing low yields in Borno State, describing that the farmer SS delivered seeds that were "exhausted" after generations of recycling, because the system does not replace the seed frequently with foundation or certified seed. Although the concept of seed recycling is mostly referring to genetics or pathology, it might also affect seed viability and field emergence. Multiplication was the third most important factor, explaining 11.6-11.7% of the variance. Multiplications 6-7 and 8-9 were grouped, because of the small number of samples in these multiplications. In contrast with the expectation that subsequent multiplications would lead to reduced germination speed and field emergence, multiplication 8-9 had a significantly higher germ2 and field emergence than multiplication 1. Multiplication 5 had a significantly (P=0.05) higher field emergence of 15.7% compared with multiplication 1, but this difference did not appear in germ2. Therefore, the results show no evidence for a negative effect of seed recycling effect on seed viability or field emergence.

Multiplication 8-9 was significantly (P=0.05) different from the other multiplications in both models, with a field emergence of 26.6%-point higher than multiplication 1. The three samples of multiplication 8 were collected in 2009. In 2010, the same three farmers multiplied their seed one more season, resulting in samples of multiplication 9. Multiplication 8-9 could only be collected from variety IT93K452-1 from the same village in Kaduna State, which does not belong to the PROSAB area. Multiplication 8-9 had a 20.6%-point higher germ2 than the reference level multiplication 1, but this was partly compensated by the negative regression coefficient (-16.3) of variety IT93K452-1. In the field emergence model, the superiority of multiplication 8-9 is partly compensated by 100 seed weight. Variety 452-1 had an average 100 seed weight of 14.4 g, while varieties IT89KD288 and IT89KD391 had a 100 seed weight of 16.3 and 16.7 g, respectively. Two grams lower seed weight corresponds to 7.0%-point lower field emergence for variety IT93K452-1, but field emergence of multiplication 8-9 was 26.6%-point higher than multiplication 1.

CONCLUSIONS

The PROSAB project trained and supported cowpea farmers to boost the availability of high quality seeds. Further investments in the informal seed system can only be justified if farmers can deliver high quality seed.

Farmers' produced seed on average had significantly more off-types (t-test, P=0.0003) compared with foundation seed, but on average germination (t-test, P=0.4684) and

field emergence (t-test, P=0.7106) were not significantly different. In comparison with seed company samples, there were no significant differences on these four criteria for farmers produced samples.

The major problem is that 97% of the tested samples failed to meet the NASC certification standards, including all seed company samples. Seed quality is therefore a problem in both the formal and informal sector. The regression analysis indicates that a project like PROSAB can effectively improve seed quality. The regression model showed that farmers in Borno had 11%-point higher field emergence, ignoring other effects like seed damage and storage. Field inspection for certification purposes did not have a positive effect on germ2 or field emergence. Reducing seed damage and proper storage had more success. Ten percent more bruchid damage led to 6.5% lower field emergence, and ten percent more other damage reduced field emergence by as much as 8.8%. Storing cowpea seeds in PICS bags increased field emergence up to 17.6% compared to polybags in a room. These results indicate that investments in informal cowpea seed systems yield significant benefits. Seed quality can be improved significantly by training farmers in seed production, emphasising strict seed cleaning and introducing appropriate storage methodologies. Other benefits of projects like PROSAB include the introduction of improved varieties and a dramatic increase of seed production in the region (Amaza et al., 2010).

Further research is required to verify the results for other crops and areas. Our recommendation would be for the NASC to review the standards for foundation and certified seed and to enhance testing or certification. Germ2 is a better predictor of field emergence then germination after 7 days, and is faster and therefore cheaper to determine. This could be cost effectively and easily implemented in testing protocols. Further research to assess the effect of multiplication on field emergence in other crops and regions is recommended.

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CHAPTER 3

Does the informal seed system threaten cowpea seed health?*

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Abstract

Most smallholder farmers in developing countries depend on an informal Seed System (SS) for their seed. The informal SS is often criticized because farmer-produced seed samples are not tested for seed health, thus accepting the risk of planting infected seeds. Here we assessed the quality of seeds acquired from the informal SS, and compared this with the quality of seeds obtained from the formal SS. Cowpea seed production in northern Nigeria was used as a case study to evaluate the seed health of samples from farmers, seed companies, and foundation seed producers. In 2 years, a total of 45.500 seeds from 91 seed samples from 43 sources (farmers, seed companies and research) were tested for seed-borne bacteria and fungi by plating disinfested seed onto an agar medium. The most commonly isolated plant pathogens were *Fusarium oxysporum* (69% of the samples), *Macrophomina phaseolina* (76%) and *Pseudomonas syringae* pv. *phaseolicola* (48%). The infection incidence, the percentage of seeds infected per sample, varied from 0.2 to75.6%. *F. oxysporum* had a median infection incidence of 9% in 2009 and 25% in 2010, while *M. phaseolina* had a median infection between 4 and 10%. On average, 8.8 species per sample were isolated from foundation seed, 9.2 from farmer-produced seed and 9.8 from seed companies' seed. No evidence was found that seed recycling in the informal SS did lead to increased levels of seed-borne pathogens. In contrast to farmers, seed companies distribute seed over large distances, and therefore form a potential threat for spreading diseases at relatively large scale. Responsible authorities are recommended to make seed dressing mandatory for all seeds sold by seed companies.

Keywords: Vigna unguiculata, seed-borne diseases, germination, Nigeria, seed systems, seed pathology.

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INTRODUCTION

Over 80% of smallholder farmers in developing countries depend on the informal Seed System (SS) for their seed supply (Louwaars & De Boef, 2012). The informal SS is defined as a system in which farmers are involved in selection, production and dissemination of seed, whereby sales, exchanges or donations of seed occur in the local community. In contrast, the formal SS is defined as all public institutions and private seed companies involved in breeding, seed production, quality control and dissemination of seed. Farmers in the informal SS use the formal SS from time to time to access new varieties (Almekinders & Louwaars, 2002). Another reason to frequently replace seed is to avoid seed recycling, which is supposed to lead to low yields through decline of seed quality (Amaza et al., 2010). The formal SS aims at regulating the seed sector in an attempt to guarantee sufficient supply of high quality seed. In contrast, the informal SS excludes seed testing, which leads to substantially lower prices than the formal SS, thereby accepting the presumed risk of reduced seed quality (van Gastel et al., 2002).

Plant diseases are a major threat to food security, contributing to the malnutrition of over 800 million people worldwide (Strange & Scott, 2005). Many plant diseases are seed-borne, i.e. they are transmitted by the seed. Planting infected seeds increases germination failure, seedling mortality, and diseased plants, all leading to lower yields. Moreover, infected crops may lead to increased levels of seed infection in the progeny. Since various soil-borne pathogens can be seed-borne, trade of infected seeds can facilitate the introduction of soil-borne pathogens to hitherto uninfested soils. Therefore most countries put phytosanitary regulation in place to ensure that only healthy seeds are traded (Maddox, 1998). However, an infrastructure for seed health testing is required to enforce these regulations. Although the formal SS has these institutions in place, their performance in developing countries is unknown, despite efforts of the International Seed Testing Association (ISTA) to improve and standardize seed testing in these countries. This study assesses the performance of both SSs for cowpea (*Vigna unguiculata* (L.) Walp.) in northern Nigeria.

Cowpea is a widely grown legume in Nigeria, providing vital proteins to millions of people (Langyintuo et al., 2003). Cowpea fields can suffer significant yield losses from plant diseases, including seed-borne diseases (Bankole & Adebanjo, 1996). The role of the informal SS in transmitting cowpea diseases was previously analysed in Zimbabwe. Manyangarirwa et al. (2009) tested 20 samples of farmer-retained cowpea seeds on seed-borne fungi and bacteria, and investigated seed to plant transmission.

The results showed that the samples were heavily infected with seed-borne fungi and bacteria, including *Bipolaris* sp. (present in 25% of the samples), *Fusarium oxysporum* (60%), *Phoma* sp. (75%), and *Macrophomina phaseolina* (25%). *F. oxysporum* and *M. phaseolina* were also observed on cowpea seeds that had been produced in northern Nigeria (Emechebe and McDonald, 1979).

This research compares the seed health status of cowpea samples from the formal (seed companies and foundation seed producers) and informal (farmers) SS in northern Nigeria. Since the informal SS does not have any quality control in contrast to the formal SS, the hypothesis is that farmer-produced seed (= informal SS) are infected with relatively high levels of seed-borne pathogens, while seed company and foundation seed samples (= formal SS) are relatively free of seed-borne pathogens. This study also assesses the relation between seed recycling and seed health, expecting increased seed health risks after more seasons of seed recycling. Furthermore, the study assesses which pathogens occurred in the collected samples and whether infection of the various pathogens encountered showed correlations. We also tested the effect of seed infection on cowpea germination for all pathogens in the infected samples.

MATERIAL AND METHODS

Seed samples

Seed samples of 2.5 kg each were collected from farmers and seed companies in Borno and Kaduna states in northern Nigeria. Borno was a focus state for the project "Promoting Sustainable Agriculture in Borno state" (PROSAB), which trained and supported farmers in seed production to enhance seed availability and improve seed quality. Kaduna state is situated in the centre of northern Nigeria, comprising the Southern and Northern Guinea savannah agro-ecologies. Borno state is the most north-eastern state of Nigeria, containing both Guinea savannah zones and the Sudan savannah zone. Three improved cowpea varieties were selected based on their maturity type and wide adoption among farmers. The late-maturing variety IT89KD288 and medium-maturing IT89KD391 were most popular in Borno State. The very-early-maturing variety IT93K452-1 was the most preferred variety in Kaduna State.

A total of 91 cowpea seed samples were collected, 45 in 2009 and 46 in 2010 (Table 1). Eighty-one samples originated from 40 seed producing farmers, five samples from two seed companies and five samples from the International Institute of Tropical

Agriculture (IITA). Twenty-seven farmers contributed one sample in 2009 and one from the same variety in 2010, and four farmers delivered samples from two varieties in both years. One farmer delivered three samples, and eight farmers only one sample. The farmers were recorded by name and village. To calculate the number of on-farm multiplications of the seed, farmers were asked which year they received foundation seed. Farmers were also asked whether they applied the insecticide phostoxin prior to storage, a common way to prevent seed damage from the storage pest like the bruchid beetle *Callosobruchus maculatus* (Fabricius).

Table 1. Overview of cowpea seed samples collected from farmers, seed companies and foundation seed producers. Farmers received foundation seed between 2001 and 2009, and multiplied their seed for 1-9 seasons until our sampling in 2009 or 2010. The number of seasons the farmer multiplied the seed on-farm is referred to as "on-farm multiplication".

Source	State	Number of on-farm multiplications	Number of samples	
			2009	2010
Foundation seed	[a]	0	3	2
Seed company	[a]	0	1	4
Farmers	Borno	1	3	3
		2	2	3
		3	1	2
		4	2	1
		5	2	0
		6	0	1
	Kaduna	1	5	0
		2	6	5
		3	7	6
		4	5	7
		5	3	4
		6	2	3
		7	0	2
		8	3	0
		9	0	3
Total			45	46

[a] outlet and production location may not be the same state.

Samples from commercial seed companies were purchased from the company outlets in Borno, Kaduna, and Kano states, one sample in 2009 and four samples in 2010. IITA delivered five foundation seed samples; one for each variety in 2009, and one for varieties IT89KD288 and IT89KD391 in 2010. In contrast with IITA policy for seed delivery, the foundation seed samples had not been tested and selected for being free from diseases. All seed samples were stored at room temperature between collection

and planting time to mimic storage conditions of farmers buying seed from their colleagues. Prior to the storage period, samples with bruchid damage were treated with Degesch phostoxin with 56% aluminium phosphide, produced by Detia Freyberg GmbH from Germany, to stop the insect from spreading through the seed sample.

Seed health testing

Five hundred seeds from each sample were analyzed for seed-borne pathogens. Seeds in each sample were visually inspected to select undamaged seeds (e.g. insect damage, discolorations, malformation), because damaged cowpea seeds are less likely to germinate (Biemond et al., 2012). The root of germinating seeds physically opens the seed, increasing the chance that pathogens inside the seed escape and invade the agar medium of the petri-dish. Seeds were surface-sterilized by soaking them in a 10% (v/v) sodium hypochlorite solution for 1 min. followed by washing with three changes of sterile distilled water and blotting dry on paper towels. Seeds were then planted on nutrient broth yeast (NBY) agar media plates (10 seeds per plate) and incubated at 27°C for 4 days. Any fungal / bacterial growth was transferred and purified using the hyphal tip / single spore technique. Fungal cultures were identified based on the morphological characters (Barnett & Hunter, 1998) and bacterial cultures were identified based on Gram reaction or aerobic and anaerobic reactions (Klement et al., 1990).

Data analysis

The seed health testing results were used to calculate the infection frequency and infection incidence (Ghiasian et al., 2004), which were calculated as follows:

$$\text{Infection frequency (\%)} = \frac{\text{number of samples in which the bacteria/fungi occurred}}{\text{total number of samples}} \times 100\%$$

$$\text{Infection incidence (\%)} = \frac{\text{number of seeds infected by a bacteria/fungi}}{\text{total number of seeds}} \times 100\%$$

The infection frequency was calculated for all bacteria and fungi, and presented separately for samples produced by farmers in 2009, by farmers in 2010, by seed companies and by foundation seed producers. For a comparison between the informal and formal SS, the results for farmers in 2009 and 2010 were combined into the category informal SS, and the results for seed company and foundation seed samples

from the two years formed the category formal SS. The infection incidence was calculated with the farmer produced samples in which the organism occurred. The median and maximum infection incidence were presented for optimal representation of the binomially distributed data.

To test if individual pathogens were associated with each other, a Pearson Chi-square test for independence was carried out with Genstat 14th edition (VSN-International, 2011). The assumption to apply the Pearson chi square test to a 2-by-2 table requires a minimum score of 5 for all expected values. The expected values were calculated by multiplying the infection frequency of one pathogen with the ratio of another pathogen. Combinations with at least one organism with a frequency close to 0% or to 100% are likely to have an expected value below 5. From the 300 unique combinations of the 25 organisms, only 85 combinations had no expected value below 5 based on the infection frequencies of both organisms.

The effect of seed recycling on seed health was analysed in two ways. After planting foundation seed, farmers multiplied the seed on-farm for 1-9 seasons. The first method determined the number of different pathogens identified per sample versus the number of on-farm multiplications. A t-test ($P<0.05$) was applied using Microsoft Excel to determine which multiplications were significantly different from each other. The second method determined if the infection incidence increased with an increasing number of on-farm multiplications.
The effect of pathogen infection on germination was determined by comparing the number of germinated seeds versus the infection incidence of each pathogen. Eight categories of germination were formed, ranging from 3 out of 10 till 10 out of 10 germinated seeds per Petri dish. The average incidence was determined for each category, and each pathogen. The effect of phostoxin treatment prior to storage by farmers on germination and seed-borne fungi and bacteria was analyzed with a GLM regression with binomial distribution. Average infection incidence included samples in which the organism did not occur. Interaction between phostoxin treatment by farmers and season was analysed with a stepwise regression with binomial distribution with Genstat 14th edition.

Table 2. Overview of bacteria and fungi identified in the seed samples obtained from farmers, seed company outlets and IITA (foundation seed). Columns 3-4 indicate whether the organism is a seed- and/or soil-borne pathogen. Columns 5-8 show the % of samples with at least 1 infected seed, separated for farmers in 2009 and 2010, foundation and seed company samples. Columns 9-10 compare the % samples infested from farmers in 2009 and 2010, representing the Informal Seed System (Informal), with seed companies and foundation seed samples combined, representing the formal SS (Formal). Columns 11-14 show the median and maximum (max) % of seeds infected per sample of 500 seeds, for infested samples of farmers in 2009 and 2010.

				% of samples infested						% seeds infected per sample (for infested samples of farmers)			
				Farmers		Foundation	Company	Informal	Formal	Median	Max	Median	Max
Bacteria	Disease *	Seed-borne	Soil-borne	2009	2010	2009/2010	2009/2010	2009/2010		2009		2010	
Bacillus cereus		N	Y	98	100	100	100	99	100	12.1	51.8	30.9	59.2
Bacillus subtilis		N		90	98	100	100	94	100	4.9	34.7	7.6	21.0
Pseudomonas syringae pv. phaseolicola	bacterial halo blight	Y		39	50	80	80	44	80	1.0	41.0	1.8	6.1
Xanthomonas axonopodis pv. vignicola	bacterial blight /leaf spot	Y		32	35	40	20	33	30	0.8	2.0	1.6	20.6
Clavibacter sp.				2	0	0	0	1	0	0.2	0.2		
Fungi													
Fusarium oxysporum	fusarium wilt	Y	Y	90	100	100	100	95	100	9.0	53.5	25.2	75.6
Aspergillus flavus		N		88	80	40	100	84	70	1.9	53.5	1.3	16.6
Aspergillus niger		N		85	88	80	60	86	70	2.4	12.5	1.6	11.6
Macrophomina phaseolina	pod mould	Y		71	80	80	80	75	80	4.4	61.0	10.2	72.6
Penicillium oxalicum		N		49	65	60	100	57	80	0.8	19.0	4.2	12.6
Botryodiplodia theobromae	seed rot	Y		44	33	40	60	38	50	1.6	17.5	2.6	8.0
Curvularia lunata	leaf spots	Y		34	43	60	40	38	50	0.5	3.3	2.7	8.4
Cladosporium sp.	pod mould	N		34	5	40	20	20	30	1.7	4.1	1.2	1.8
Fusarium verticillioides	wilting	Y		32	23	0	20	27	10	0.6	48.7	0.8	1.0
Aspergillus sp.		N		29	30	0	60	30	30	0.5	4.7	1.6	3.4
Alternaria sp.	alternaria leaf spot	Y		17	30	20	20	23	20	0.4	1.0	4.8	11.6
Colletotrichum truncatum	brown blotch	Y		17	13	20	0	15	10	1.6	6.7	0.8	21.4
Rhizopus sp.		N		15	28	20	60	21	40	1.1	3.4	2.8	10.6
Fusarium solani	collar rot	Y	Y	10	8	0	0	9	0	0.3	0.6	1.6	4.5
Phoma sp.	stem rot	Y		7	3	0	40	5	20	0.8	1.0	0.6	0.6
Cochliobolus sativus				5	3	0	0	4	0	0.2	0.2	0.2	0.2
Bipolaris sp.	leaf spots	Y		5	0	0	0	2	0	0.7	1.0		
Corynespora cassiicola	target leaf spot	Y		5	0	0	0	2	0	0.2	0.2		
Rhizoctonia solani	web blight	Y	Y	5	0	0	0	2	0	0.2	0.2		
Nigrospora sp.		N		2	13	0	20	7	10	4.3	4.3	1.8	2.4

* based on expert knowledge

RESULTS

Seed health

Five bacterial and 20 fungal species were isolated from the cowpea seed samples (Table 2). Nearly all samples were infected with the bacteria *Bacillus cereus* and *Bacillus subtilis*. The seed-borne bacterial pathogens *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *vignicola* were present in 30-50% of the 81 farmer-produced samples. The most frequently occurring seed-borne fungal pathogens were *Fusarium oxysporum* with infection frequencies of 90-100%, and *Macrophomina phaseolina* (70-80%). *Botryodiplodia theobromae* and *Curvularia lunata* were identified in 33-44% of the farmer-produced seed samples. *Cladosporium* sp. occurred in 34% of the farmer produced seed samples in 2009, but only in 5% in 2010. *F. verticillioides* appeared slightly more frequently in 2009 (32%) than in 2010 (23%), but the opposite was true for *Alternaria* sp. with 17% in 2009 and 30% in 2010. *Colletotrichum truncatum* occurred in 13-17% of the farmer produced samples. *Corynespora cassiicola*, *Bipolaris* sp. and *Rhizoctonia solani* appeared each in 5% of the samples in 2009, but were not identified in 2010.

All formal SS samples appeared to be infected with *F. oxysporum*, 8 out of 10 infected with *M. phaseolina* and 8 out of 10 with *P. syringae*. Infection with *B. theobromae* and *C. lunata* were observed in 5 out of 10 samples. On average, fewer pathogen species were identified in foundation seed samples than in samples from seed companies: 8.8 versus 9.8 pathogen species per sample, respectively.

Infection frequencies for formal and informal seed sources differed less than 10% for 4 bacteria and 12 fungi (Table 2, columns 6-7). Farmer-produced seed samples had infection frequencies which were at least 10%-point lower than the formal SS for seven pathogens: *P. syringae*, *Penicillium* sp., *B. theobromae*, *C. lunata*, *Cladosporium* sp., *Rhizopus* sp. and *Phoma* sp. Only *Aspergillus flavus*, *A. niger* and *F. verticillioides* had over 10%-point higher infection frequencies in farmer-produced samples compared to the formal SS. Except for the numerical outperformance of informal SS over the formal SS, the seven organisms that occurred more often in seed company and foundation seed samples also cause more serious diseases like bacterial halo blight, leaf spots, pod mold and stem rot. The seed health status of the formal SS samples was therefore worse than that of the samples of the informal SS.

The infection incidence ranged from 0.2 to 75.6% (Table 2, columns 11-14). The highest infection incidence was reported for *F. oxysporum*. The average infection incidence across all organisms in 2010 was with 6.1% slightly higher than in 2009 with 4.2%. The bacteria with the highest median were the two (non-pathogenic) *Bacillus* species, while *F. oxysporum* and *M. phaseolina* had the highest median infection incidence rates among farmer-produced samples. *B. cereus* had a median infection incidence of 30.9% in 2010, more than twice as high as the 12.1% in 2009. The maximum infection incidence of *P. syringae* was 41%, while the median was only 1.0%. Similar patterns were observed for *F. verticillioides* in 2009 and *C. truncatum* in 2010. Most bacteria and fungi had a median infection incidence below 3%. In general, organisms with high infection frequencies also had high infection incidences.

Table 3. Pearson Chi-Square testing independence between occurrences of different organisms. Only combinations of pathogens with a significant (P<0.05) dependence, meeting the assumption of expected value ≥5, are shown. Mutually excluding pathogens occurred significantly (P<0.05) less often together than statistically expected.

Pathogen 1	**Pathogen 2**	**Pearson Chi-Square p-value**
Xanthomonas axonopodis pv. vignicola	*Aspergillus flavus*	0.012
	Alternaria sp.	0.031
	Botryodiplodia theobromae	0.001
	Macrophomina phaseolina	0.027
	Penicillium oxalicum	0.001
	Pseudomonas syringae pv. phaseolicola	0.004
Botryodiplodia theobromae	*Aspergillus flavus*	0.015
	Macrophomina phaseolina	0.004
	Pseudomonas syringae pv. phaseolicola	0.016
Pseudomonas syringae pv. phaseolicola	*Curvularia lunata*	0.001
	Rhizopus sp.	0.016
Curvularia lunata	*Aspergillus niger*	0.036
Penicillium oxalicum	*Alternaria sp.*	0.022
Exclusive pathogens		
Botryodiplodia theobromae	*Colletotrichum truncatum*	0.011
Macrophomina phaseolina	*Penicillium oxalicum*	0.049

Association between organisms

The cowpea seed samples contained between 2 and 17 different organisms per sample. A total of 15 out of 85 potentially significant combinations showed significant ($P<0.05$) dependence at the Pearson chi square test (Table 3). The most predominant pathogens with significant associations were *X. axonopodis*, *B. theobromae* and *M. phaseolina*. Significant ($P<0.05$) associations were shown for *X. axonopodis* with 6 out of 12 organisms, for *B. theobromae* with 5 out of 12 other possible combinations, for *P. syringae* with 4 out of 12, and for *M. phaseolina* with 3 out of 14 combinations. From the non-pathogenic organisms, *Penicillium* sp. had the most significant associations with 3 out of 13 combinations. From two associations, the organisms were observed less than expected indicating the organisms were mutually excluding. These were *B. theobromae* versus *C. truncatum* and *Penicillium sp.* versus *M. phaseolina.*

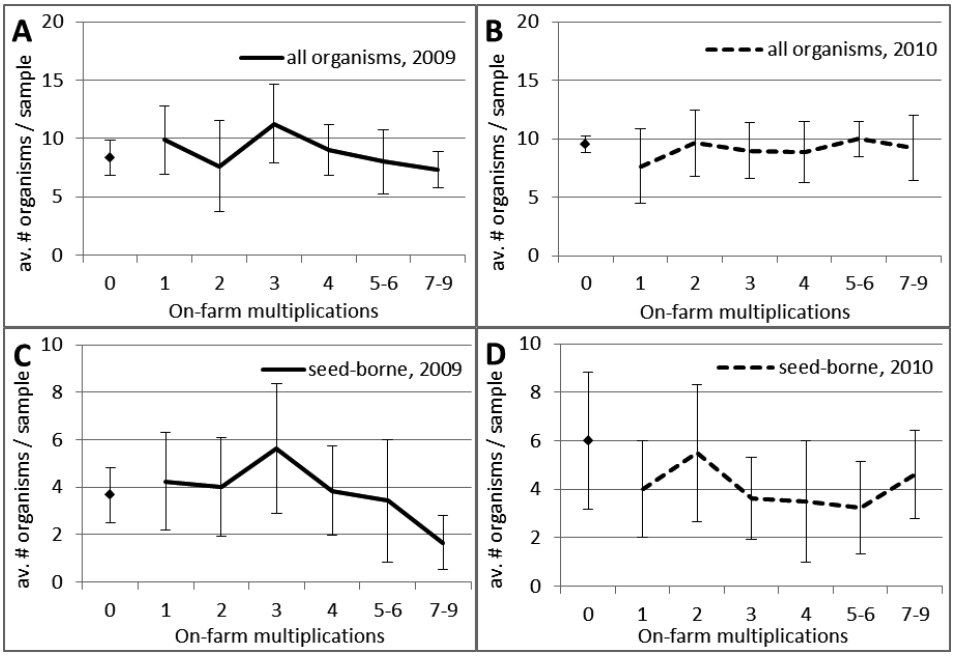

Fig. 1. Average number of species of bacterial and fungal organisms per sample versus the number of on-farm multiplications. Bars indicate the standard deviation per multiplication category. Multiplication 0 is untreated foundation seed. Panel A shows the average number of all bacteria and fungi for 2009, and Panel B for 2010. Panel C shows the average number of seed-borne pathogens for 2009, and Panel D for 2010.

To test if seed-borne pathogens as a group mutually exclude non-pathogenic organisms, a Pearson chi square test was performed as well. There was no relation between the number of seed-borne pathogens and the number of non-pathogenic organisms that infected a sample in 2009 (P=0.12) and in 2010 (P=0.22). Note that one of the test assumptions, all minimum expected values should be at least 5, was not met in 2010 for this test.

The relation between on-farm multiplication and infection incidence in 2009 and 2010 was tested for four pathogens (Fig. 2). If seed recycling would increase infection incidence, multiplication 1 is expected to have the lowest incidence, followed by a steady increase after more on-farm multiplications. The two bacteria and the two seed-borne fungi showed different infection incidence patterns for 2009 and 2010. There were no significant (t-test, $P<0.05$) differences between multiplications for any of the four organisms in 2009. In 2010, only multiplications 3 and 7-9 differed significantly (t-test, P=0.0444) from each other in infection incidence with *B. cereus*.
For *B. subtilis*, multiplication 1 was significantly different from multiplication 4 (t-test, P=0.0019) and multiplication 5-6 (t-test, P=0.0054). Multiplication 4 had significantly (t-test, P=0.0199) higher infection incidence of *B. subtilis* compared to multiplication 2. The on-farm multiplications of *F. oxysporum* were not significantly ($0.19<P<0.85$) different from each other in 2010. Despite the large differences in *M. phaseolina* infection incidence, none of the multiplications were significantly ($0.32<P<0.97$) different from each other in 2010. These results showed no evidence that continued seed recycling leads to increased levels of seed infection by pathogens.

Germination

The average infection incidence over classes of cowpea germination is shown in Fig. 3. Only the results of eight pathogens are shown, including the four most frequently observed seed-borne pathogens (*B. theobromae*, *F. oxysporum*, *M. phaseolina*, and *P. syringae*), and the four most frequently observed non-pathogens (*A. flavus*, *A. niger*, *B. cereus* and *B. subtilis*). Higher infection rate with *M. phaseolina* occurred mostly in Petri-dishes with less germinated seeds. Petri dishes with only 3-5 out of 10 seeds germinated had infection incidence of *M. phaseolina* of up to 50% in 2010. Petri dishes with >6-7 germinated seeds had infection incidence <17%. Low germination rates were also observed in association with presence of *F. oxysporum* (both years) and *B. theobromae* (2009). *P. syringae* was identified more in petri dishes with high germination in 2009. From the non-pathogenic organisms, only *A. flavus* was observed more in dishes with low percentage germination compared to dishes with high

germination in 2009. In both years the two *Bacillus* species occurred most in dishes with >9 out of 10 seeds germinated, suggesting a positive influence of *Bacillus* infection on germination.

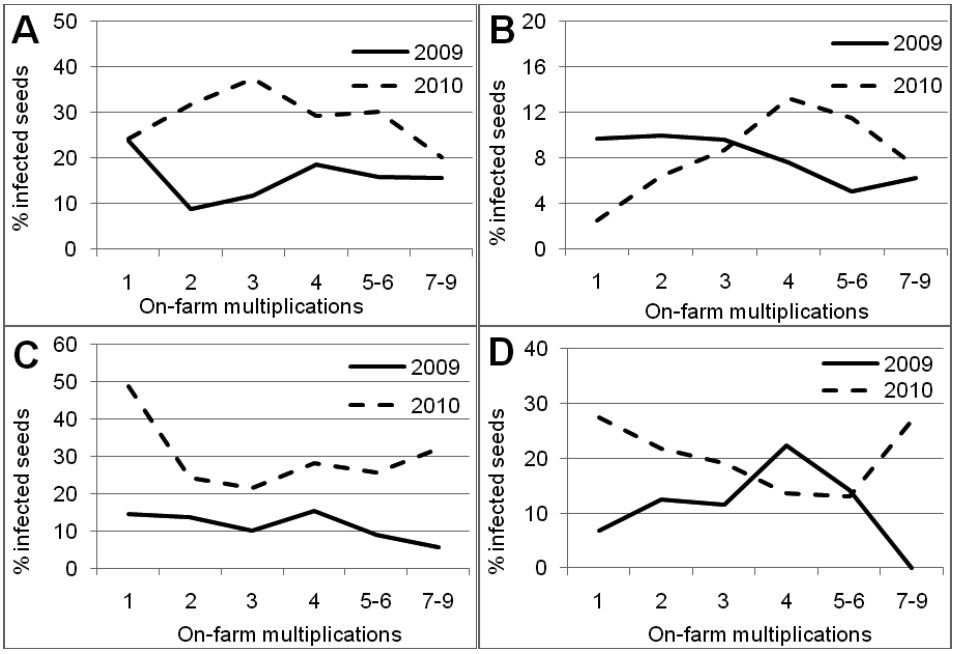

Fig. 2. Percentage of seeds infected in samples showing infection of specific species versus the number of on-farm multiplications. Panel A: Bacillus cereus, Panel B: Bacillus subtilis, Panel C: Fusarium oxysporum, Panel D: Macrophomina phaseolina. Please note the differences in scales between the panels.

The majority of the farmers, 83% in 2009 and 79% in 2010, indicated at the survey that they applied phostoxin prior to storage. Germination of phostoxin treated samples was significantly ($P<0.01$) higher (89.1%) than the germination of untreated samples (72.3%) (Table 4). The average infection incidence of all 20 fungi was 2.7% for phostoxin treated samples compared to 2.2% for untreated samples, but the difference was not significant ($P=0.17$). The average infection incidence of treated and untreated samples was not significantly ($P=0.87$) different for the five bacteria either. The stepwise regression showed that the average infection incidence for all fungi and all bacteria was only significantly ($P<0.01$) different between seasons, not between treated and untreated samples. From the four seed decay and four seed-borne organisms, only *B. subtilis* ($P=0.05$) and *M. phaseolina* ($P=0.02$) showed significant difference between treated and untreated samples, while season had no significant

(P>0.05) effect for either of them. Untreated samples had two times higher infection incidence of *M. phaseolina* compared to phostoxin treated samples. Remarkably, the phostoxin treated samples had higher infection of *B. subtilis* compared to untreated samples.

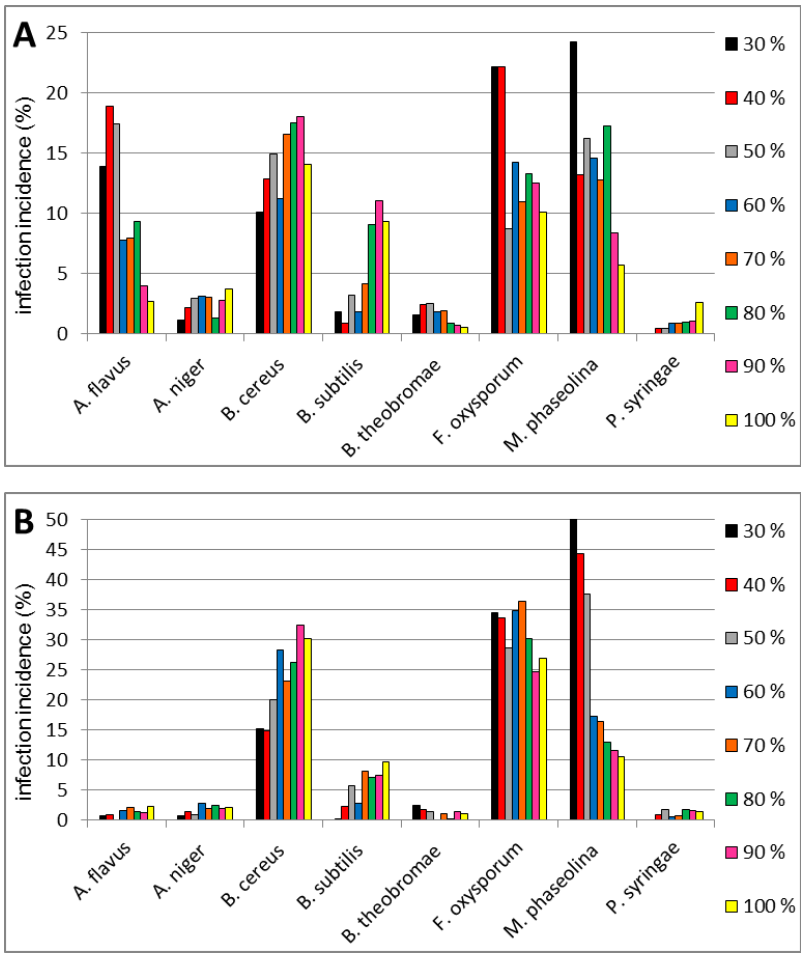

Fig. 3. Pathogen infection incidence versus germination of surface-disinfected cowpea seeds in 2009 (panel A; 22,500 seeds plated) and in 2010 (panel B; 23.000 seeds plated). Infection incidence (%) is the number of seeds infected by the pathogen, divided by the total number of seeds x 100%. Each petri-dish contained 10 seeds. The number of germinated seeds per petri-dish formed eight categories, ranging from 30% (3 out of 10) till 100% (10 out of 10) seeds germinated. The average infection incidence was calculated for all petri-dishes of the same germination category, regardless to which sample the petri-dish belonged.

Table 4. Effect phostoxin treatment prior to seed storage on germination, bacteria and fungi infection in 2009 and 2010. Columns 2-3 and 5-6 show average infection incidence (%) or germination (%) for respectively phostoxin treatment and season. Columns 4 and 7 shows the F-probability for a Generalized Linear Model (GLM) regression based on binomial distribution of a single factor, phostoxin treatment and season, respectively. Columns 8-10 show the stepwise regression in GLM with binomial distribution of the two factors phostoxin treatment and season, whereby the dashes represent non-significant (P>0.05) relations. Average infection incidence was calculated for all organisms observed (see Table 2), and infection incidence was calculated for the eight organisms (selected organisms) shown in this table.

	Phostoxin treatment			Season			GLM stepwise regression		
Parameter	untreated	treated	F.pr.	2009	2010	F.pr.	phostoxin	season	interaction
Germination (%)	72.3	89.1	< 0.01	81.6	90.4	0.02	< 0.01	< 0.01	-
Average all organisms	3.4	3.1	0.21	2.5	3.9	< 0.01	-	< 0.01	-
Average selected organisms	9.2	7.7	0.06	6.4	9.7	< 0.01	0.04	< 0.01	-
Aspergillus flavus	3.8	5	0.59	7.5	1.9	< 0.01	-	< 0.01	-
Aspergillus niger	2.2	2.5	0.61	2.9	2	0.13	-	-	-
Bacillus cereus	26.1	21.7	0.32	15.8	29.7	< 0.01	-	< 0.01	-
Bacillus subtilis	4.9	8.9	0.05	7.8	8.5	0.69	0.05	-	-
Botryodiplodia theobromae	1.4	0.8	0.18	0.8	1	0.61	-	-	-
Fusarium oxysporum	21.2	19	0.65	11.3	28	< 0.01	-	< 0.01	-
Macrophoma phaseolina	21.4	9.6	0.02	9.2	14.6	0.14	0.02	-	-
Pseudomonas syringae pv. phaseolicola	1.0	1.7	0.38	1.8	1.3	0.44	-	-	-

DISCUSSION

Formal and informal seed systems

The informal SS outperformed the formal SS concerning cowpea seed health. Foundation seed samples and seed company samples had high infection frequencies for seed-borne pathogens, including some species that are notorious soil-borne pathogens. These infection frequencies were higher than those of farmer-produced samples. Seed producers of the formal SS are expected to deliver samples free of seed-borne pathogens based on their seed health testing. One possible explanation for the high infection frequencies could be that seed companies may use out-growers for their seed multiplication. Out-growers may use similar agronomic practices compared to farmers producing seed, but lack an incentive to remove diseased plants. Another possible explanation might be that seed companies do not check for seed health.
The high infection incidence of foundation seed emphasizes how difficult it can be to produce seed free of pathogens, but it is not considered a problem given the IITA policy for seed shipment. Before shipment, all seed samples have to be tested for seed health. Only samples free of seed- and soil-borne pathogens are cleared for transportation and distribution. This does not apply to the seed company samples, which were purchased in retail outlets where farmers also buy their seed. Despite the regulations concerning seed quality in the formal SS, retail outlets from seed companies failed to deliver samples free of seed-borne pathogens. Application of fungicides during the growing season may limit yield reductions, but negatively impact the environment, and raise production costs for farmers.

Seed health

The results showed that farmer-produced cowpea seeds were heavily infected with a range of seed- and soil-borne pathogens. Manyangarirwa et al. (2009) came to the same conclusion for the informal SS of cowpea in Zimbabwe, emphasizing the negative influence on germination and potential crop losses. In the research from Zimbabwe, *P. syringae*, *X. axonopodis* and over 12 fungal pathogens were identified in only 20 samples. *Phoma* spp. occurred in 75% of the samples in Zimbabwe, while the current research identified the fungus only 7 and 3% in 2009 and 2010, respectively. *F. oxysporum* was identified in 60% of the samples in Zimbabwe, against 95% in Nigeria.

The consequences of the high infection frequencies of samples differ among the pathogens. For solely seed-borne pathogens, it threatens the plant, and also other plants during the growing season may become diseased. Soil-borne pathogens such as *F. oxysporum*, *Fusarium solani*, *M. phaseolina* and *R. solani* may lead to soil infestations of hitherto uninfested fields, thereby also affecting the following seasons and different crops. This can be especially problematic for pathogens having a wide host range, such as *M. phaseolina*. *F. oxysporum* appears to be very frequent in Nigeria, and the majority of the fields might be infested already (Onyike & Nelson, 1993).

A further comparison was made with the data from Emechebe and McDonald (1979), who collected cowpea samples from markets, and from fields with disease symptoms across northern Nigeria. The seed-borne pathogens *C. truncatum*, *R. solani*, *F. oxysporum*, *F. solani*, *M. phaseolina* and *Xanthomonas* sp. were identified in both studies, but *Ascochyta* sp., *C. lindemuthianum*, *Cercospora canescens*, *Sclerotium rolfsii* (= *Corticium rolfsii*) and *Septoria vignae* were only identified by Emechebe and McDonald (1979). Fields infected with bacterial blight, caused by *Xanthomonas* sp. resulted in an average infection incidence of 16%. This statistic was 30% for fields infected with *M. phaseolina*. In our study, we found less infection for both pathogens, which is at least in part due to the fact that Emechebe and McDonald (1979) deliberately selected samples from infested fields. The current research collected samples from farmers, regardless the disease pressure in their fields, in the same way seeds would be entering the seed system.

The Pearson chi square test for independence proved that 15 out of 85 possible associations were significant ($P<0.05$). The reason that some organisms occur more frequently together might indicate a similar seed infection strategy or seed infection time (Neergaard, 1977). Bacteria and fungi might be antagonists for other organisms in the case of mutually exclusive organisms, or compete for the same niche. Organisms infecting the same seed might interact, positively or negatively, affecting seed viability and disease transmission. Further research is needed to analyse the dynamics of seed infection by multiple organisms, and the way organisms interact affecting seed viability and disease transmission.

Seed recycling

If continued seed recycling was responsible for increasing infection, a gradual and significant increase in number of pathogens would be expected at increasing number

of multiplications. There was however no relation between number of on-farm multiplications and number of pathogens. The second method to test for any effect of seed recycling on seed health was based on the infection incidence. If seed recycling would have increased pathogen infection, on-going on-farm multiplications was supposed to result in higher infection incidences. The data collected in 2009 and 2010 did not show any evidence for this relation for the four selected and most prevalent organisms. These results are in line with the effect of seed recycling on seed viability, using the same samples. Seed recycling in cowpea did not reduce germination or field emergence (Biemond et al., 2012).

Germination

Fawole et al. (2006) analysed the effect of seed-borne fungi infection of cowpea seed on germination rate and seedling height. All nine fungi tested reduced germination rate and seedling height in the four varieties tested. Infection was achieved by coating the seeds with *A. flavus*, *A. niger*, *Alternaria* sp., *Cladosporium* sp., *F. oxysporum*, *F. solani*, *F. semitectum*, or *Penicillium* sp. This method led to high inoculum densities outside the seed, while in the current study germination was determined as function of natural infection. Naturally infected seeds contain the pathogen inside the seed, and seeds may have been simultaneously infected with multiple organisms. Among the seed-borne pathogens, *M. phaseolina* co-occurred most consistently with low germination, followed by *B. theobromae* and *F. oxysporum*. High infection rates of *A. flavus* were found to be associated with low germination rates, but only in 2009. These results are in line with those of Fawole et al. (2006) concerning *A. flavus* and *F. oxysporum*. The negative effect of *A. niger* on germination as described by Fawole et al. (2006) was however not confirmed. According to Manyangarirwa et al. (2009), cowpea seed infected with *M. phaseolina* does not germinate at all. This research confirmed that *M. phaseolina* has a devastating effect on cowpea germination, although we did not find complete germination failure. *P. syringae* pv. *phaseolicola* is a seed-borne pathogen causing bacterial halo blight in cowpea fields, but strangely, it rather had a positive influence on cowpea germination in 2009. To support cowpea breeding, further research is recommended to analyse whether pathogens differently affect cowpea seed viability among varieties.

The two *Bacillus* species rather seemed to have had a positive effect on germination in both 2009 and 2010. Various papers proved the effectiveness of *Bacillus* sp. for biological control. *B. cereus* and *B. subtilis* found in compost reduced the mycelial growth of *F. oxysporum* and *R. solani* (Muhammed & Amusa, 2003). Seed dressing with *B. cereus* and *B. subtilis* proved to be effective against *Fusarium spp.*, *R.*

solani and *M. phaseolina* (Dawar et al., 2010). The results of the current experiment indicated that *Bacillus spp.* may also suppress the negative effects of other pathogens. Further research is required to analyse the dynamics of natural infection with *Bacillus* spp. on germination and on either infection with or functioning of other micro-organisms.

The majority of the farmers applied phostoxin prior to storage to protect their seed from bruchids, but phostoxin treatment also increased germination by 17%-point. The storage chemical did not appear to be a straight bactericide or fungicide, because average infection incidence with bacteria and fungi was not significantly($P=0.05$) different between treated and untreated samples. Treatment led to a 55% reduction of *M. phaseolina* infection incidence, the pathogen associated with low germination. Remarkably, phostoxin treated samples had 82% higher infection incidence with *B. subtilis* compared to untreated samples. Figure 3 showed that *B. subtilis* is associated with high germination rates. Note that application of phostoxin constitutes a human health hazard since unsold seed is mostly consumed by farmers, and that 47% of the farmers store seed in their own house. Farmers are recommended to apply phostoxin prior to storage under the condition that they can assure the seeds will not be used for consumption, for example by proper labelling of the seed bags to avoid confusion. Instead of phostoxin, quality can also be raised by applying the triple bagging storage technology, which avoids chemical application (Biemond et al., 2012).

CONCLUSIONS

Farmer-produced seed samples had less frequent infection of seed- and soil-borne pathogens than seed company and foundation seed samples. Both the formal and the informal seed system failed to produce seed-borne pathogen free samples. Considering that seed companies distribute seed over large distances in contrast to the informal SS, the seed companies form a larger threat for spreading diseases and potentially even introduction of diseases to new areas, as long as current flaws in the control on seed quality are not addressed.

This research provided an extensive list of prevailing fungi and bacteria in cowpea samples in northern Nigeria. The Pearson-chi square test gave insight which organisms occur simultaneously under the condition of natural infection. We did not find any evidence for a negative effect of seed recycling on seed health. The results also showed that natural infection of cowpea seeds with the fungi *A. flavus*, *F. oxysporum* and *M. phaseolina* may reduce germination, while infection with *Bacillus spp.* may rather increase germination. To support cowpea breeding, further research is

recommended to test the single and combined effect of pathogens on germination for different cowpea varieties. Responsible authorities are recommended to make seed dressing mandatory for all seeds sold by seed companies, and accompany this with the relevant measures to make the rule effective.

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CHAPTER 4

Seed health of maize in Nigeria*

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Abstract

Many Nigerian farmers depend for their seed on seed producing farmers, the so-called informal Seed System (SS), but seed quality remains unknown. Farmers planting low quality seed risk poor field emergence and low plant vigour as a result of low physiological quality or infection with seed-borne pathogens. The objective of this research was to test seed quality of seed from the informal SS in Northern Nigeria, and to compare it with seed company and foundation seed samples (formal SS). A total of 49,500 seeds (99 samples of 500 seeds each) were tested for germination, off-types and seed health. Seed pathology was quantified by plating disinfected seeds onto agar, and identifying all bacteria and fungi present after three days. Twelve seed-borne pathogens were identified including *Bipolaris maydis* (found in 45% of the farmer-produced samples), *Botryodiplodia theobromae* (97%) and *Curvularia lunata* (38%). All samples were infected with *Fusarium verticillioides*, with a median infection incidence of 59.3% (2009) and 51.2% (2010). None of the 99 samples tested passed the demands for certified seed of the National Agriculture Seed Council (NASC) in Nigeria, in particular the limit of maximum five off-types per kg seed sample. The seed company samples had significantly (t-test, $P < 0.01$) higher germination (99.3%) than farmer-produced seed (97.7%). Both seed producing farmers and seed companies are recommended to improve disease control and seed cleaning. The NASC is recommended to revise the strict norms for off-type seeds, since removing off-types is labour intensive.

Keywords: seed system, seed pathology, *Zea mays*, seed-borne diseases, Nigeria.

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INTRODUCTION

Maize is an important cereal in Nigeria with an annual production of 7.3 million metric tonnes in 2009, larger than that of sorghum, millet or rice. The Nigerian population consumed 29.4 kg of maize/capita/year in 2009 (FAO, 2012). Maize is also used for livestock feed and as raw material for industrial production (Tambo & Abdoulaye, 2012). Improving the availability of high quality maize seed of well adapted varieties is important to boost agricultural productivity, leading to higher farmers' income, reduced poverty and improved food security (Abdoulaye et al., 2009). The physiological quality of maize seed is a serious concern in Nigeria. Results from south-west Nigeria showed that farmer-produced seed lots had on average 91% germination, but low vigour and large variability within seed lots and between farmers (Odeyemi et al., 2010). Forty-seven percent of the maize seed in Nigeria is produced by the formal Seed System (SS), while the majority of smallholder farmers depend on the informal SS (Abdoulaye et al., 2009).

The informal SS consists of all farmers involved in on-farm selection, seed production and dissemination of seed. Public institutions and private seed companies involved in breeding, variety selection, seed production, dissemination and quality control form the formal SS (Biemond et al., 2012). Both systems have their strengths and weaknesses. The formal SS has extended breeding programmes to develop new varieties and can control seed quality through seed testing facilities. Unfortunately, the formal SS usually fails to produce seed of a wide range of varieties and to distribute adequate quantities to remote areas for prices affordable to smallholder farmers. The informal SS fills this gap, because it lacks the overhead that comes with institutions and large companies. Within this system, farmers produce their own seed, and sell, exchange or give away excess seed in the local community. Seed production methods range from farmers using grain as seed, to farmers that have special fields for seed production and apply strict seed cleaning, all to maintain quality and purity. Since farmers do not check the quality, the actual quality remains unknown to producer and buyer. So the buyer accepts the risk of planting poor quality seed (Biemond et al., 2013). Farmers may decide to risk poor field emergence or increase sowing density, resulting in a trade-off between risk and additional costs (van Gastel et al., 2002).

Seeds may contain seed- or soil-borne pathogens that lead to reduced germination, seedling mortality and diseased plants (Maddox, 1998). Diseases are an important concern to smallholder maize farmers. A survey among farmers in the humid southwest part of Nigeria showed that disease resistance is the second most important motivation to choose for improved maize varieties (Daniel & Adetumbi, 2006). Thirty-

nine per cent of the farmers adopted improved varieties for their high yielding properties, while 24% mentioned disease resistance as the most important characteristic (Daniel & Adetumbi, 2006). Planting disease resistant varieties is the most effective method of avoiding maize diseases, but there is no general resistance against all diseases and pests (White, 1999). Currently, local maize breeding programs focus mainly on varieties that can cope with more important constraints like low soil fertility, drought (Badu-Apraku & Akinwale, 2011) and the parasitic weed *Striga hermonthica* (Del.) Benth (Menkir et al., 2012). On top of that, some diseases occur more often under environmental stress conditions or when seed quality has been compromised (Solorzano & Malvick, 2011).

Research of maize seed quality in South-West Nigeria showed that physiological quality is a problem in farmers-produced seed lots. Seed samples appeared physiologically non-uniform with high viability, but low vigour. There was also high variability in seed quality between farmers, and even between states (Odeyemi et al., 2010). Seed health of farmer-produced maize seed samples was analysed in Burkina Faso. The samples were heavily infected with a total of ten different pathogenic fungi. Seed to seedling transmission was proven at greenhouse condition for nine out of ten pathogens, showing the importance to control seed health in order to deal with seed-borne maize diseases (Somda et al., 2008). The project Promoting Sustainable Agriculture in Borno state (PROSAB) identified the availability of high-quality seed as a key factor to boost agricultural production in Borno state, northern Nigeria. Farmers received training and support to produce high-quality seed, while improved varieties of cowpea, maize and soybean were introduced (Amaza et al., 2010). Seed health analysis of farmer-produced cowpea seeds showed that samples were infected with a range of seed-borne and soil-borne pathogens. Despite the low seed health quality, farmer-produced seed samples did not underperform compared to samples from seed companies (Biemond et al., 2013). Here, we evaluate the results of the project in terms of seed quality of maize. We tested the hypothesis that maize seed health, germination and physical purity of farmer-produced seed is not significantly different from seed company samples.

MATERIAL AND METHODS

Sample collection

A total of 99 maize (*Zea mays* L.) seed samples was collected from 51 seed producing farmers, from a seed company and from the International Institute of Tropical Agriculture (IITA) in Nigeria. Seventeen farmers provided a single sample. Thirty-

three farmers delivered one sample in 2009 and one in 2010, of whom 32 had the same variety in both years. One farmer provided seed of two varieties in both years. All farmers were situated in Borno state, the most north-eastern state of Nigeria. Three Open Pollinated Varieties (OPV) introduced by IITA were selected for this research based on their popularity among farmers. The late-maturing and *S. hermonthica* resistant Sammaz-11, the drought tolerant and early-maturing Sammaz-20 and the early-maturing and *S. hermonthica* tolerant Sammaz-21 (Table 1). Before official release, Sammaz-11, Sammaz-20 and Sammaz-21 were known as TZL-Comp1-SYN, TZE-Comp3-DT and TZE-Comp5, respectively.

Table 1 Overview of maize seed samples collected from IITA, a seed company, and seed producing farmers. Farmers received foundation seed between 2003 and 2009, and multiplied their seed for 1-6 seasons until sampling in 2009 and 2010.

Variety	Source	No. of multiplications by farmer	No. of samples taken	
			2009	2010
Sammaz-11	IITA	0	1	1
	seed company	0	1	1
	farmers	1	3	3
		2	3	3
		3	3	3
		4	3	3
		5	3	3
		6	0	3
Sammaz-20	IITA	0	1	1
	seed company	0	1	1
	farmers	1	3	2
		2	3	1
		3	3	3
		4	3	3
		5	3	3
		6	0	3
Sammaz-21	IITA	0	1	1
	seed company	0	1	1
	farmers	1	3	3
		2	2	3
		3	2	3
		4	2	1
		5	3	1
		6	0	1
Total			48	51

All three varieties were initially provided to the farmers by the PROSAB project. Farmers received foundation seed between 2003 and 2009, and were trained to multiply the seed on-farm. To calculate the number of on-farm multiplications, the farmers were asked which year they received foundation seed. In total, 87 samples were collected from farmers, six samples were purchased from a seed company, and six foundation seed samples were collected from IITA to serve as control.

Physical purity and germination

Physical purity and germination were assessed according to protocols described in Biemond et al. (2013), which are an extension to guidelines of the International Seed Testing Association (ISTA) (Jones, 2008). In short, the composition of 1 kg of each seed sample was divided into the categories inert matter, other crop and weed seeds, off-types, damaged seeds and pure seed. Inert matter included all non-seed material and broken seeds. Off-types are seeds from a different variety based on visual differences in color or shape. Maize seeds with any form of damage were classified as seed damage, as well as seeds that were very small or shriveled. Off-type seeds with seed damage were classified as off-type. The remaining maize seeds were classified as pure seed. Hundred-seed weight was measured after excluding inert matter and other crop seeds, but including off-types.

Germination rate was analysed with the paper towel method according to the ISTA guidelines (Jones, 2008). In short, four-hundred seeds (broken seeds excluded) per sample were rolled in 8 paper towels, each holding 50 seeds. The paper towels were vertically placed in a cup with 1 cm water and placed in a Sanyo incubator MIR-253 at 27°C. Germinated seeds were counted and removed every 24 h, up to 7 days after initiation of the test. Only the results of total germination at day 7 were used in this paper.

Seed health

Five hundred undamaged seeds from each sample were analysed for the presence of seed-borne pathogens. Seeds were surface-sterilized by soaking them in a 10% (v/v) NaOHCl solution for 1 min followed by washing with three changes of sterile distilled water and blotting dry on sterile paper towels. Seeds were placed on nutrient broth yeast (NBY) agar media plates (10 seeds per plate) and incubated at 27°C for 4 days. Any fungal or bacterial growth was transferred and purified using the hyphal tip and/or single spore technique. Fungal cultures were identified based on morphological characteristics (Barnett & Hunter, 1998) and bacterial cultures were identified based

on Gram reaction or aerobic and anaerobic reactions (Klement et al., 1990). Damaged seeds were excluded from seed health testing for two reasons. Damaged seeds are less likely to germinate, while the root tip of a germinating seed breaks the seed open, facilitating pathogens to exit the seed and invade the agar medium. The second reason is that disinfection liquid can enter damaged seeds, and disinfect the inner tissue of the seed instead of only sterilizing the seed surface.

The infection frequency and infection incidence (Ghiasian et al., 2004) were calculated as follows:

$$\text{Infection frequency (\%)} = \frac{\text{number of samples in which the bacteria/fungi occurred}}{\text{total number of samples}} \times 100\%$$

$$\text{Infection incidence (\%)} = \frac{\text{number of seeds infected by a bacteria/fungi}}{\text{total number of seeds}} \times 100\%$$

Statistics

The variation in 100-seed weight, inert matter, number of off-types, and germination of all samples was visualized with frequency distribution histograms. From the 24 organisms identified, the four most devastating maize pathogens in Nigeria were selected based on expertise of the authors for detailed analysis. Average infection incidences of the farmer-produced samples were compared with seed company samples for each variety. An one-sample Poisson test was applied to test if the seed company sample was significantly (2-sided, $P<0.05$) different from the average farmer-produced samples of the same variety and season. Data are presented in histograms to visualize the number of farmer-produced samples which had similar infection incidence as the seed company and foundation seed samples for the four selected pathogens. To test if continued on-farm multiplication would lead to increased number of off-types and increased infection, average infection incidences were calculated for each multiplication. One way ANOVA of square root transformed data was used to test for significant differences between the multiplications. GenStat 14th edition was used for all statistics (Genstat, 2011).

RESULTS

The average percentage of inert matter was 0.9% and 1.6% in 2009 and 2010, respectively. Fifteen out of 87 of the farmer-produced seed samples had more than 2%

inert matter, which is the limit for certified and foundation seed of the National Agricultural Seed Council (NASC) of Nigeria (Fig. 1b). None of the seed company and foundation seed samples exceeded this threshold. The six seed company samples and three out of six foundation seed samples had less than 1% inert matter. On average farmer-produced seed had significantly (t-test, $P < 0.01$) more inert matter than seed company samples, but not in comparison with foundation seed (t-test, $P = 0.37$).

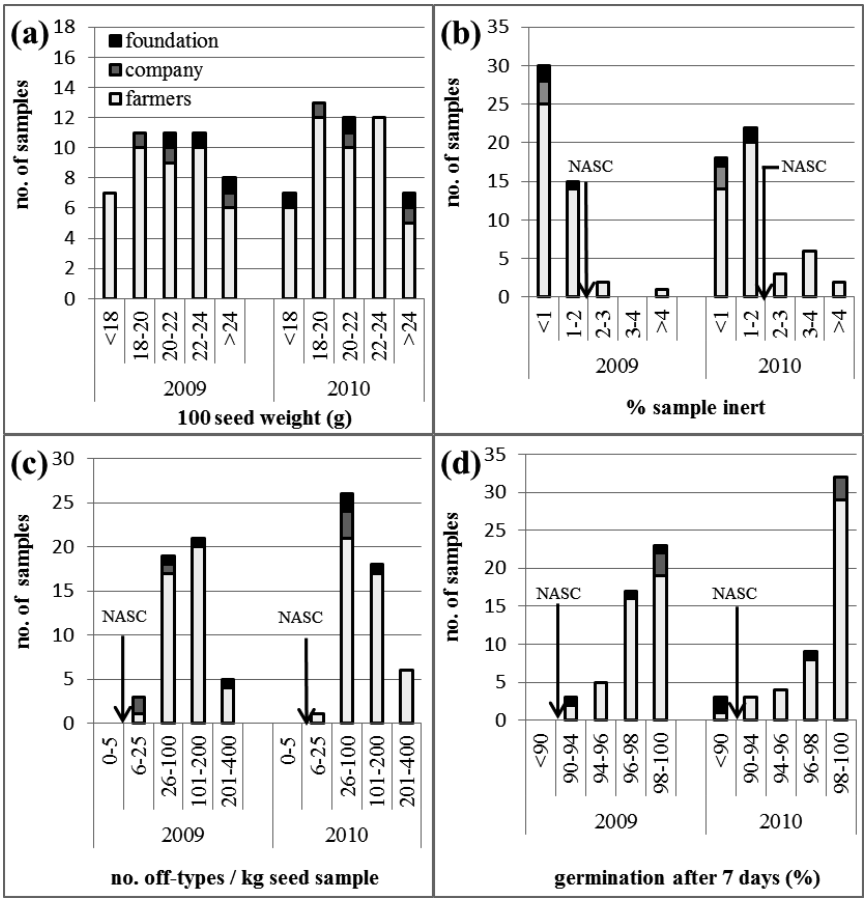

Fig. 1 Distribution of farmer-produced seed lots based on 100-seed weight, inert matter, off-types and germination. The three seed company (in grey) and three foundation seed samples (in black) were added on top of the farmer-produced seed sample bars of 2009 and 2010. Inert matter is weight-based percentage of broken seeds and non-seed material in 1 kg seed. Arrows show standards set by the National Agricultural Seed Council (NASC) of Nigeria for maximum number of off-types, maximum percentage inert matter, and minimum germination rate.

The number of off-type seeds per kg sample varied from 14-380, with an average of 115 and 120 in 2009 and 2010, respectively (Fig. 1c). Over the two seasons, seed company samples had with an average of 43.3 significantly fewer off-types than the 125.4 off-types found on average in foundation seed (t-test, $P = 0.04$) and the average of 122.0 off-types of farmer-produced seed samples (t-test, $P < 0.01$). The difference in average off-types between foundation and farmer-produced seed samples was not significant (t-test, $P = 0.90$).

Germination at day 7 varied from 92.8-100% in 2009 and from 37.3-100% in 2010 (Fig. 1d). The lowest germination rate in 2010 was from a foundation seed sample. The other five foundation seed samples varied from 89.8-99.3%. The seed company samples had between 98.5-99.8% germination across both years. The average germination of farmer-produced samples was with 97.7% significantly (t-test, $P < 0.01$) lower than the 99.3% of seed company samples, but not significantly (t-test, $P = 0.27$) different from foundation seed samples. Twenty out of 87 farmer-produced seed samples had ≥99.3% germination, the average germination of seed company samples.

Pseudomonas syringae pv. *syringae* appeared in 5 and 16% of the farmer-produced samples in 2009 and 2010, respectively (Table 2). Other seed-borne bacteria were rare. The most frequently encountered seed-borne fungus was *Fusarium verticillioides* (isolated from all samples), followed by *Botryodiplodia theobromae* and *Aspergillus niger*. Other seed-borne fungi with infection frequencies over 25% of the farmer-produced samples were *Bipolaris maydis*, *Curvularia lunata*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Nigrospora* sp. and *Penicillium oxalicum*.

The fungus with the highest infection incidence was *F. verticillioides* with 59.3% and 51.2% in 2009 and 2010, respectively. *Fusarium solani* infected only one sample in 2009 with an infection incidence of 64%, while the maximum infection incidence of 2010 was only 1%. In 2009, only one sample was infected with *Phomopsis* sp., which had an infection incidence of 32.6%. *B. theobromae* had a median infection incidence of 8.0-14.1% while the maximum was 67.4%. *B. cereus* and *M. phaseolina* had median infection incidences between 4 and 7% in 2009 and 2010. The bacterium *Bacillus subtilis* and the fungi *B. maydis*, *C. lunata*, *F. oxysporum* and *Nigrospora* sp. had median infection incidences between 1 and 3% in both years. All other bacteria and fungi had median infection incidences below 1%, except the sample infected with *Drechslera* sp.

Table 2. Overview of bacteria and fungi identified in the seed samples obtained from farmers, a seed company outlet and foundation seed. Column 2 shows the potential of each organism to cause a disease in maize, or to serve as biocontrol against another organism. Columns 3-4 indicate whether the organism is a seed- or soil-borne pathogen. Columns 5-8 show the infection frequency, the percentage samples with at least 1 infected seed, separated for farmers in 2009 and 2010, foundation and seed company samples. Columns 9-12 show the median and maximum (max) infection incidence, the percentage of seeds infected per sample of 500 seeds, of farmers in 2009 and 2010.

				Infection frequency (%)				Infection incidence (%)			
				Farmer	Farmer	Foundation	Company	Median	Max	Median	Max
Bacteria	Disease[a] / biocontrol	Seed-borne	Soil-borne	2009	2010	2009 / 2010	2009 / 2010	2009	2009	2010	2010
Bacillus cereus	Biocontrol of e.g. B. maydis	U[b]	U	100	98	100	100	7.7	41	4.2	24.9
Bacillus subtilis	Biocontrol of e.g. F. verticillioides	U	U	100	69	100	100	2.3	24.2	1.6	21.6
Clavibacter sp.	Goss's bacterial wilt and blight	Y1	U	0	2	0	0	*	*	0.4	0.4
Pseudomonas syringae pv. syringae	Holcus spot	U	Y1	5	16	50	17	0.8	1.4	0.4	6.8
Xanthomonas sp.	Bacterial leaf spots	Y1	U	0	7	17	0	*	*	0.6	0.6
Fungi											
Aspergillus flavus	Aspergillus ear rot	U	Y1	45	31	83	17	0.4	7.2	0.8	2.2
Aspergillus niger	Black mold, Aspergillus ear rot	Y1	U	55	67	100	83	0.6	3.6	0.8	4.3
Aspergillus termari		U	U	17	0	33	17	0.4	1.6	*	*
Bipolaris maydis	Southern corn leaf blight	Y1	U	69	22	17	33	2.2	7.2	1.6	4.4
Botryodiplodia theobromae	Black kernel rot	Y1	Y1	95	98	67	100	8	67.4	14.1	51.2
Cladosporium sp.	Cladosporium ear rot	U	Y1	7	2	17	17	0.9	7.2	0.5	0.6
Colletrotrichum gloeosporioides	Anthracnose leaf blight	U	U	5	0	0	0	0.3	0.4	*	*
Curvularia lunata	Curvularia leaf spot	Y2	Y2	31	44	33	17	1.8	6.4	1	3.5
Drechslera sp.	Helminthosporium leaf disease	U	U	2	0	0	0	2.4	2.4	*	*
Fusarium oxysporum	Fusarium stalk and root rot	Y1	Y1	29	76	33	67	1.1	15.2	1.8	11.8
Fusarium verticillioides[c]	Fusarium stalk and ear rot	Y1	Y3	100	100	100	100	59.3	77.4	51.2	91.6
Fusarium solani	Fusarium stalk and root rot	Y1	Y1	2	7	17	0	64	64	0.8	1
Macrophomina phaseolina	Charcoal rot	Y4	Y4	60	89	33	67	4.8	13.2	4.4	26
Melanospora zamiae		U	U	12	0	17	0	0.8	1.4	*	*
Nigrospora sp.	Nigrospora ear rot	Y1	U	43	18	0	50	1.7	7.4	1.2	1.9
Penicillium oxalicum	Penicillium ear rot / Blue eye	Y1	Y1	33	24	67	50	0.6	2.6	0.5	8.5
Phoma sp.	Red root rot	Y5	Y6	12	0	0	0	0.8	1.6	*	*
Phomopsis sp.	Seed decay	U	U	2	0	0	0	32.6	32.6	*	*
Rhizopus sp.	Rhizopus ear rot	Y1	U	14	2	17	0	0.8	6	0.9	1.2

a Based on expert opinion

b Y = yes, U = unlikely, 1 = MacGee 1988 , 2 = Akinbode 2010, 3 = Cavaglieri et al. 2005, 4 = Shekhar and Kumar 2010, 5 = Somda et al. 2008 , 6 = White 1999.

c Synonym : *Fusarium moniliforme*

* Organism not identified in year

Table 3 Off-type seeds and infection incidence (%) of seed company samples versus average farmer-produced seed samples, for variety 11 (=Sammaz-11), 20 (=Sammaz-20) and 21 (=Sammaz-21). Plus/minus sign shows farmer-produced sample had significantly (Poisson one sample test, P<0.05) more/less infected or off-type seeds compared to seed company seed. Differences without plus or minus sign were not significant (P>0.05).

Season	Variety	Source	Bipolaris maydis		Botryodiplodia theobromae		Curvularia lunata		Macrophomina phaseolina		Off-type (no. kg^{-1})	
2009	11	Farmers	1.6	+	7.4		0.1	+	3.6	+	152	+
	11	Seed company	0.0		7.2		0.0		0.0		21	
	20	Farmers	1.7		19.3	–	1.7	+	4.1	+	84	+
	20	Seed company	1.6		67.4		0.0		0.0		60	
	21	Farmers	3.0	+	15.4	+	0.0		1.8	–	121	+
	21	Seed company	1.0		11.8		0.0		9.0		15	
2010	11	Farmers	0.2	+	12.3	+	0.9	+	5.3		143	+
	11	Seed company	0.0		0.8		0.0		5.4		50	
	20	Farmers	0.7	+	25.7	+	0.4		6.2		135	+
	20	Seed company	0.0		14.1		0.8		5.5		50	
	21	Farmers	0.6	+	34.2	+	0.3	+	3.7	–	85	+
	21	Seed company	0.0		1.0		0.0		6.6		64	

Farmer-produced seed had significantly (P<0.05) higher average infection incidences of *B. maydis* compared with seed company samples for five out of six varieties (Table 3). *B. theobromae* and *C. lunata* infection of farmer-produced samples was significantly (P<0.05) higher compared to seed company samples for four out of six varieties. The seed company sample of Sammaz-20 in 2009 was an exception with an infection incidence of 67.4% for *B. theobromae*, over three times higher than the average of the farmer-produced samples. The seed company samples of Sammaz-11 and Sammaz-20 were free from *M. phaseolina* infection in 2009, but Sammaz-21 had significantly (P<0.05) more infection with the fungus in both 2009 and 2010. On average farmer-produced seed had significantly (P<0.05) more off-types per kg seed than seed company samples for all varieties in 2009 and 2010. The difference between farmer-produced seed and seed company samples varied from 33% (64 versus 85) for variety Sammaz-21 in 2010 until 807% (15 versus 121) for the same variety in 2009.

For most pathogens and varieties, farmer-produced samples had on average higher infection incidences than seed company samples and foundation seed samples. However, the majority of the farmer-produced seed samples had similar infection incidences as the seed company and foundation seed samples due to the variation

among farmer-produced seed samples. All seed company and foundation seed samples had less than 2% infection incidence of *B. maydis*, while 74% for farmer-produced samples had less than 2% infection incidence in both years (Fig. 2). The infection incidence of *B. theobromae* was less than 10% for 41% of the farmer-produced samples, 50% of the seed company samples, and 100% of the foundation seed samples. Sixty-two percent of the farmer-produced samples were free from *C. lunata* infection, against 83% and 67% of the seed company samples and foundation seed samples, respectively. Only 33% of the seed company samples had less than 5% *M. phaseolina* infection incidence over two years, far less than the farmer-produced samples (66%) and foundation seed samples (83%).

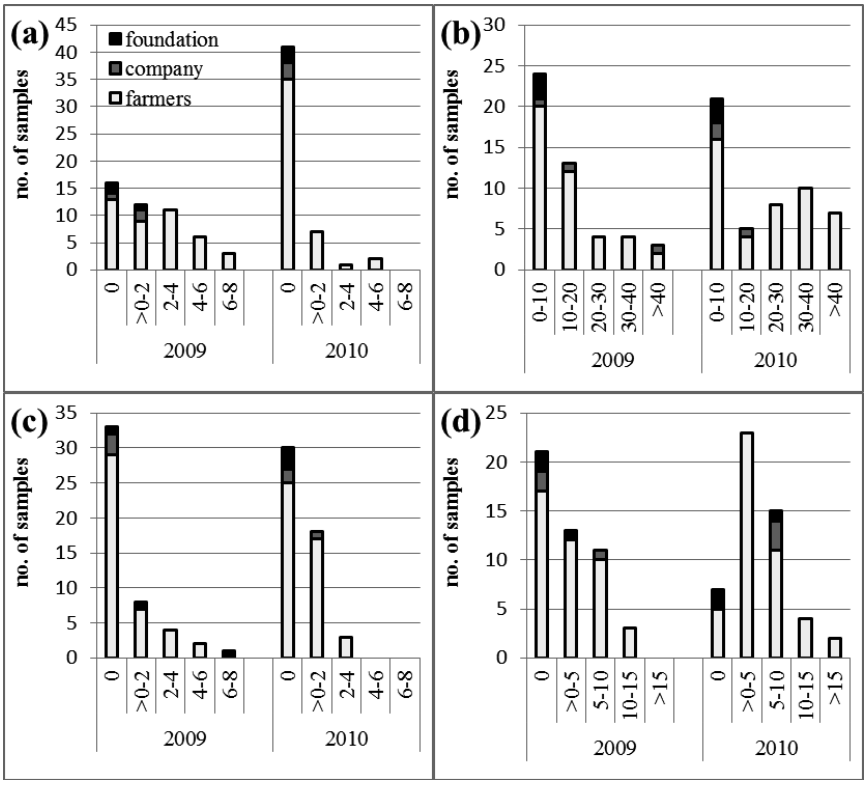

Fig. 2 Distribution of farmer-produced seed lots, seed company samples and foundation seed for infection incidence of *Bipolaris maydis* (panel a), *Botryodiplodia theobromae* (b), *Curvularia lunata* (c) and *Macrophomina phaseolina* (d). Total number of farmer-produced seed lots was 42 in 2009 and 45 in 2010, with three foundation seed samples and three seed company samples in each year.

Table 4 Effect of on-farm multiplications on infection incidence (%) with seed-borne or soil-borne fungi, and on the number of off-type seeds per kg seed sample. Fisher probabilities (F.Pr.) are based on ANOVA of square root transformed data.

Season	No. on-farm multiplications	*Bipolaris maydis*	*Botryodiplodia theobromae*	*Curvularia lunata*	*Macrophomina phaseolina*	Off-types (no. kg^{-1})
2009	1	1.7	16.8	0.9	2.8	98
	2	1.2	11.5	0.5	5.7	133
	3	2.0	11.8	0.8	3.5	123
	4	2.8	15.9	0.4	2.4	136
	5	2.5	13.5	0.4	2.3	108
F.Pr.		0.350	0.767	0.992	0.273	0.612
2010	1	0.5	15.0	0.5	6.3	105
	2	0.0	21.0	0.5	2.6	122
	3	0.0	29.7	0.4	4.2	107
	4	0.3	13.7	0.4	6.5	110
	5	0.6	28.2	0.3	7.0	149
	6	1.3	27.0	1.1	4.5	165
F.Pr.		0.212	0.154	0.639	0.471	0.505

Continued on-farm multiplication was expected to lead to higher levels of infection and off-types. For the four fungi, there were no significant (P=0.15-0.77) differences in infection incidences between numbers of on-farm multiplications (Table 4). *B. maydis* showed an upward trend of infection incidence in 2009 and 2010, and *B. theobromae* in 2010. *C. lunata* showed a downward trend between multiplication 1-5 in both years, with an exception for multiplication 6 in 2010. The infection incidences of *M. phaseolina* did not show an upward or downward trend. The number of off-types per kg sample showed an inconsistent trend between multiplications 1 and 5 in 2009, while multiplication 5-6 had the highest number of off-types in 2010.

DISCUSSION

Most smallholder farmers depend on the informal SS for their maize seed, but seed companies are an alternative source. The ability to provide small quantities of seed in remote areas at affordable prices has contributed to the success of the informal SS, but seed quality remained unknown. This research tested the seed quality of farmer-produced seed on physical purity, germination and seed health, and compared the

quality with foundation seed and seed company samples. Since variety may interact with seed health, only seed company samples from the selected varieties were included. Unfortunately, only one seed company could deliver seeds of the requested varieties, so the results may not be representative for all seed companies in Nigeria.

Physical purity

The National Agricultural Seed Council of Nigeria defined standards for foundation and certified maize seed, allowing < 2% inert matter and requiring > 90% germination. Seventeen percent of the farmer-produced seed samples exceeded the inert matter threshold, while all foundation samples and seed company samples were within the limit. Germination requirement was only a problem for the foundation seed samples of which two out of six samples failed. Odeyemi et al. (2010) analysed farmer-produced seed from the more humid southwest part of Nigeria, resulting in a germination rate of only 91.5%. Odeyemi et al. tested germination by sowing seeds under a thin layer of sand, requiring seeds to emerge through the sand, possibly explaining the 6.2%-point less germination compared to the paper towel method used in this research. However, both experiments confirmed the larger variability within and between farmer-produced seed samples. The NASC has set the standard for off-types at maximum 5 off-types per kg seed sample, corresponding to 0.11% at a 100-seed weight of 21.0 g. All samples tested, including the foundation seed and seed company samples, had more than 5 off-types per kg. Ninety-five out of 99 samples, including all foundation seed samples and 4 out of 6 seed company samples, had even more than 25 off-types per kg. The NASC would have rejected all samples, suggesting that seed quality is a problem in both formal and informal SSs of maize. Especially the high number of off-types for foundation seed is of big concern, since foundation seed is used as input for seed multiplication. IITA, seed companies and farmers are recommended to put more effort in seed cleaning to reduce the number of off-types in their seed lots, as well as remove inert matter like broken seed and non-seed material. On top of that, a basic germination test is recommended to assure sufficient germination. The NASC is recommended to revise the strict standard for off-types, especially for certified seed, because removing off-types is a very time consuming job.

Seed health

The second quality aspect analysed was the infection with seed-borne bacteria and fungi. A total of 20 pathogens were isolated from the surface-sterilized seeds, of which 12 species are able to incite disease in maize. All samples tested were severely (51-

59%) infected with *F. verticillioides*, which can cause stalk and ear rot in maize. The fungus can produce fumonisins, which can be toxic for humans and livestock (Wilke et al., 2007). Seed-borne *B. maydis*, present in 45% of the farmer-produced samples, can cause southern corn leaf blight. An outbreak in the US corn belt in 1970 had devastating effects on maize yields, while the disease is proposed to be the most damaging fungal maize disease in Burkina Faso, another West-African country (Somda et al., 2008). Another damaging fungus is *C. lunata*. This causal agent of Curvularia leaf spots in maize appeared in 38% of the farmer-produced samples. The necrotic leaf spots of this seed- and soil-borne fungus reduce the photosynthetic area of the plants, which can lead to considerable yield reductions (Akinbode, 2010). Over 50% of the samples was infected with *M. phaseolina*, a polyphagous pathogen able to persist for multiple years in soil (Islam et al., 2012). Once established soilborne pathogens like *M. phaseolina* are difficult to manage (Ndiaye et al., 2007), stressing the importance of seed health. The median infection incidence was below 3% for most seed-borne pathogens. The large number of potentially harmful pathogens identified show that seed health is a concern for the SS. Although seed health testing reveals potential risks, the high costs for the testing method used in this research would require large, uniform quantities of seed to spread the costs thinly per kg seed. Testing every seed lot would force companies to raise their prices substantially, while seed producing farmers simply lack the infrastructure. The current results emphasize that both seed-producing farmers and seed companies have to emphasize more on removal of diseased plants in the field, proper storage to avoid infection, and apply seed dressing to reduce disease transmission to the plants. Seed companies are recommended to test for seed health from time to time to check if their seed health efforts pay off.

Formal versus informal seed system

This research compared seed samples from seed-producing farmers, the informal SS, with seed company and foundation seed samples, part of the formal SS. The quality of farmer-produced samples was on average not significantly different from foundation seed concerning inert matter ($P = 0.37$), off-types ($P = 0.90$) and germination ($P = 0.27$). However, seed company samples outperformed average farmer-produced seed with significantly less inert matter ($P = 0.37$), less off-types ($P < 0.01$), and higher germination ($P < 0.01$). Seed company samples also had lower infection incidences than average farmer-produced seed for three out of four selected pathogens. *B. maydis*, *B. theobromae*, *C. lunata* and *M. phaseolina* were selected by the authors as the four most devastating pathogens in Nigeria. Seed company samples had significantly

($P<0.05$) less infection incidence of *B. maydis*, *B. theobromae*, *C. lunata* and *M. phaseolina* for two out of three varieties in 2009, and outperformed farmer-produced samples on average for *B. maydis* and *B. theobromae* in 2010 for all three varieties. It can be concluded that seed quality of seed companies is higher compared to the average farmer-produced seed for both physical purity and seed health. There are two important points to be made. First of all, seed health was also a problem in the seed company samples tested. Secondly, the variation in seed quality among farmer-produced seed samples is large. Many farmer-produced seed samples had similar or better performance for each of the seed quality parameters in comparison with seed company samples. Despite the variation in farmer-produced seed samples, seed quality can be one of the reasons to justify higher effort and costs of buying seed from a seed company rather than from seed-producing farmers. However, the farmer's decision to choose for the formal or informal SS also depends on other factors like the seed price, travel expenses to the retail office, available budget, varietal preferences and the choice for hybrids or OPVs.

Seed recycling

Seed recycling or on-farm multiplication may lead to lower seed quality compared with seed production in the formal SS (Amaza et al., 2010). Seed-producing farmers may pay less attention to remove diseased or off-type plants compared to seed companies, and apply less or lower quality inputs like agrochemicals. Nevertheless no increase in infection incidences from multiplication 1-6 occurred in any of the four selected pathogens (Table 4). Despite the differences between on-farm multiplications, average infection incidences of all four fungi were not significantly (ANOVA, $P<0.05$) different from each other within season and fungi. So, overall, there was no evidence for increased infection after on-going on-farm multiplications. This conclusion is consistent with the seed recycling research in cowpea carried out in the same area (Biemond et al., 2012).

CONCLUSIONS AND RECOMMENDATIONS

All samples tested failed the NASC guidelines for certified seed due to the standard for off-type seeds. Seed health is a problem in both SSs with high infection frequencies for potentially harmful pathogens. Overall seed quality of seed company samples was higher than for farmer-produced seed, and could therefore be one of the reasons for farmers to prefer seed from seed companies over seed producing farmers. However, the variation among samples is large, leaving many farmer-produced samples with

equal or better quality than seed company samples. All seed producers are recommended to improve seed cleaning and disease control. Seed companies could consider testing for seed health from time to time to evaluate efficacy of their efforts to reduce seed infection. The NASC is recommended to revise the limit for off-type seeds, because removal of off-types is very time consuming.

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CHAPTER 5

How maize seed systems can contribute to the control of infection by mycotoxigenic fungi: a perspective*

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Abstract

Mycotoxins are fungal produced toxins threating human health in developing countries. Consumption of contaminated maize can cause cancer and even sudden death. Infection by mycotoxigenic fungi is a prerequisite for mycotoxin production, which can occur by seed-to-seedling transmission of infected seeds. This perspective assessed opportunities to prevent infection by mycotoxigenic fungi in maize seed. A case study in Nigeria showed that all farmer-produced, seed company and foundation seed samples were heavily infected. A schematic overview of the formal and informal seed system is presented to analyse seed system contribution to fungi infection and mycotoxin contamination in the maize value chain, and to set criteria for successful control. We recommend an integrated approach to control mycotoxigenic fungal infection, including resistant varieties and other control methods, with an important role for seed systems.

Keywords: Mycotoxins, *Zea mays*, Nigeria, informal seed system, *Fusarium verticillioides*.

* Submitted

BACKGROUND

Mycotoxins, fungal produced toxins, are a largely ignored human health issue in developing countries. Chronic exposure to contaminated maize can cause cancer, while acute exposure can lead to sudden death (Wild & Gong, 2010). Mycotoxin exposure is most common in developing countries with poor food handling, inadequate food storage, malnutrition and weak governments (Bennett & Klich, 2003). Ironically, the population of these countries also lack access to healthcare to treat complex diseases (e.g. cancer, kidney toxicity) resulting from chronic mycotoxin exposure.

High income countries control the mycotoxin hazard with a combination of regulation and certification (Figure 1A). The Food and Drugs Authority (FDA) (Egmond, 2002) and the European Commission (2006, 2007) determined maximum food contamination levels for specific mycotoxins to protect consumers from mycotoxin exposure. Certification, like Hazard Assessment and Critical Control Point (HACCP), assists companies in the food value chain to prevent mycotoxin contamination within acceptable levels (Magan, 2006). However, certification and enforcing food regulation are not compatible with smallholder farmers in low income countries. Farmers consume part of their own produce without market interference, making food safety control difficult to execute (Wild & Gong, 2010). Food safety controls would increase food prices where the majority of the population already spend up to 50% of their income on food (Bryngelsson et al., 2012).

An important control strategy to avoid mycotoxin contamination is to prevent infection by toxigenic fungi (Munkvold, 2003), which can occur by seed-to-seedling transmission from infected seeds (Bacon et al., 2001). This research analysed how seed health can be controlled to avoid mycotoxigenic fungal infection in developing countries. A case study in northern Nigeria demonstrates the high infection levels of locally available maize seed. A schematic overview of the seed system (SS) is presented to analyse the problem of mycotoxigenic fungal infection of seed. Potential control methods are discussed in relation to sustainable implementation in the formal and informal SS.

Mycotoxins

Mycotoxins are defined as natural products with low molecular weight, produced as secondary metabolites by filamentous fungi that are toxic to vertebrates at low concentrations. They differ widely in biosynthetic origin, chemical structure and toxicity (Bennett & Klich, 2003).

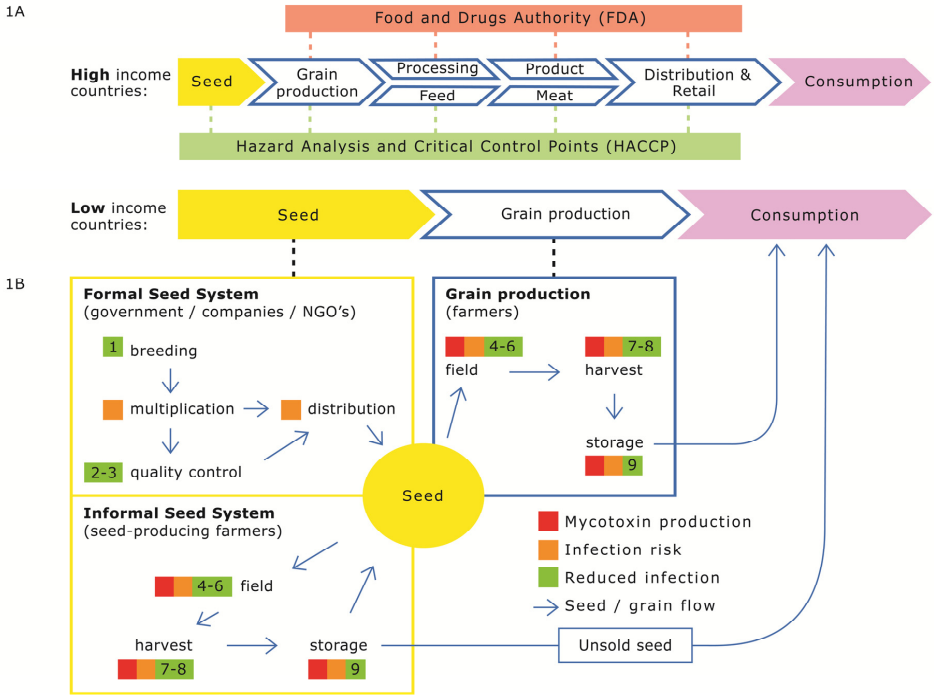

Figure 1A. Maize value chain in high and low income countries. Regulation by e.g. the FDA and certification like HACCP avoid mycotoxin contamination in maize in high income countries. Low income countries lack government regulation and certification to avoid mycotoxin contamination in maize. A relatively large share of the maize production is consumed without industrial processing, resulting in a shorter value chain compared with high income countries.

Figure 1B. The effect of formal and informal Seed Systems on infection by mycotoxigenic fungi, mycotoxin production and control methods in low income countries. The formal Seed System consists of government bodies, NGOs and private companies involved in breeding, seed multiplication, external quality control and dissemination of seed, while the informal SS only consists of seed producing farmers. Arrows show flows of maize seed or grain. Control methods to avoid or reduce mycotoxigenic fungal infection include breeding insect and mycotoxigenic fungi resistant varieties (1), seed health testing (2), seed treatment with fungicides (3), fungicide and insecticide application (4), atoxigenic strains (5), biological control with antagonistic organisms (6), avoid seed damage (7), fast drying to <14% moisture (8) and clean and dry storage (9).

The symptoms of mycotoxin poisoning depend on the type and amount of mycotoxin, duration of exposure, and the age, sex, health and nutritional status of the victim (Bennett & Klich, 2003). An example of fatal mycotoxin poisoning was the aflatoxicosis outbreak in Kenya in 2004. Over 300 people suffered from acute hepatic failure after consumption of aflatoxin contaminated maize, which eventually killed 125 people (Azziz-Baumgartner et al., 2005).
The most prevalent fungi that can produce mycotoxins belong to the genera *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* (Tsitsigiannis et al., 2012). Control measurements differ in effectiveness between genera. Avoiding grain damage during harvest, fast drying to low moisture levels and adequate storage methods can be effective against *Alternaria* and *Aspergillus* species. These 'storage' fungi produce mycotoxins primarily in storage, in contrast to *Fusarium* and *Penicillium* species which produce mycotoxins mostly under field conditions (Tsitsigiannis et al., 2012). Optimal storage conditions do not solve the problem of pre-harvest mycotoxin production, and are difficult to implement in developing countries. Agronomic practices like crop rotation, tillage, irrigation and fertilizer application do not provide sufficient protection either, and available varieties do not have sufficient resistance against all mycotoxigenic organisms. Infection by mycotoxigenic fungi in maize is a persistent problem in developing countries (Munkvold, 2003), especially *Fusarium verticillioides* is problematic. The fungus is a seed-borne disease in maize causing systemic infection in the plant. Neither seed treatments with fungicides nor chemical control in the field are effective. Biological control strategies are developed involving pre- or post-harvest applications (Bacon et al., 2001), but it is unlikely that resource poor farmers will pay for an extra treatment without benefits such as improved yield or increased market price for mycotoxin free maize. Seed health is not only an important aspect in the control of *F. verticillioides*, but can also contribute to the control of other seed-borne, mycotoxigenic fungi like *Fusarium oxysporum*, *Fusarium solani*, and *Penicillium oxalicum (MacGee, 1988)*.

Seed systems

Seed health can only be controlled through the source of seed, the SS. The formal SS consists of government bodies, NGOs and private companies involved in breeding, seed multiplication, external quality control and dissemination of seed (Figure 1B). Government bodies and NGO's run public breeding programs, while some seed companies also develop new varieties. Responsible authorities test varieties for Distinctness, Uniformity and Stability (DUS) criteria for varietal registration. The new varieties are multiplied, tested for seed quality and sold to farmers.

Table 1. Overview of mycotoxigenic fungi identified in maize seed samples obtained from farmers (N=87), a seed company outlet (N=6) and a foundation seed producer (N=6). Samples were collected in 2009 and 2010 in Nigeria. Fungal growth in each sample was determined for 500 undamaged, surface sterilized seeds after 4 days incubation on agar. Column 1 shows the mycotoxigenic fungi identified, column 2 indicates one of the mycotoxins it can produce according to literature, and column 3 indicates whether the organism is a seed- or soil-borne pathogen. The infection frequency, i.e. the percentage samples with at least 1 infected seed, is shown for seed samples from farmers (column 4), a seed company (6) and foundation seed producer (8). The median infection incidence, i.e. the median of the percentage of seeds infected per sample based on infected samples only, is shown for farmer-produced (column 5), seed company (7) and foundation seed (9) samples.

Mycotoxigenic fungi	Mycotoxins produced**	Seed-borne	Farmers		Company		Foundation	
			Infection frequency (%)	Infection incidence (%)	Infection frequency (%)	Infection incidence (%)	Infection frequency (%)	Infection incidence (%)
Aspergillus flavus	Aflatoxin[a]	U	38	0.6	17	0.8	83	0.4
Aspergillus niger	Ochratoxin A[b]	Y[e]	61	0.6	83	0.6	100	1
Fusarium oxysporum	Deoxynivalenol[c]	Y[e]	53	1.4	67	5.1	33	3.8
Fusarium solani	Deoxynivalenol[c]	Y[e]	5	0.9	0	***	17	0.8
*Fusarium verticillioides**	Fumonisins[a]	Y[e]	100	55.2	100	48.7	100	45.1
Penicillium oxalicum	Secalonic acid D[d]	Y[e]	29	0.6	50	0.2	67	1.4

* synonym: *Fusarium moniliforme*

** a = Desjardins et al., 1998, b = Windham & Williams, 2012, c = Richard et al., 2007, d = Balasubramanian et al., 2000, e = MacGee, 1988, U = unlikely, and Y = yes.

*** organism not identified in company samples

Source: Chapter 4

Table 2. Control measures of infection by mycotoxigenic fungi in relation to criteria for successful adoption in the seed system.

	Criteria for successful adoption			
Control measures	Effectiveness against mycotoxigenic fungi	Additional financial benefits	Easy implementation in seed system	Number of criteria met
1. Breeding resistant varieties	Y	Y	Y	3
2. Seed health testing	Y	N	N	1
3. Seed treatment	Y	Y	N	2
4. Atoxigenic strains	Y	N	N	1
5. Agrochemical application	Y	Y	N	2
6. Biological control agents	Y	N	N	1
7. Avoid seed damage	Y	Y	N	2
8. Fast drying <14% moisture	Y	Y	N	2
9. Optimal storage	Y	Y	N	2

The informal SS consists of farmers producing seed for their own production, and often selling or exchanging seed within their local community. In general, farmers try to maintain varietal purity and seed quality, but are not able to test it. Excess seed is used for own consumption or sold as grain on the market. Both SSs interact. The informal SS depends on the formal SS to access new varieties and occasional seed renewal, while the latter uses the informal SS for farmer participatory breeding, varietal testing or seed multiplication. Farmers involved in grain production can buy seed from the formal SS at a relatively high price, or purchase seed locally from the informal SS at a relatively low price (Almekinders & Louwaars, 2002).

MAIZE SEED HEALTH IN NIGERIA

Since the informal SS lacks access to the required infrastructure, seed-producing farmers do not test seed health. Recent research results from Nigeria showed that farmer-produced seed samples were heavily infected with a range of mycotoxigenic fungi (Table 1). All samples were infected with *Fusarium verticillioides* with a median infection incidence of 55.2%. The fungus can produce fumonisins that are classified as potential carcinogenic to humans causing oesophageal cancer. An evaluation of data

from the World Health Organisation and the Food and Agricultural Organisation revealed a relation between the consumption of mycotoxin-prone food and HIV transmission. Avoiding fumonisin contamination may prevent 1,000,000 HIV transmissions in Sub-Saharan Africa annually (Williams et al., 2010). The most frequently occurring *Aspergillus* species was *Aspergillus niger*, occurring in 61% of the samples, which can produce the mycotoxins ochratoxin A (OTA) and fumonisin B2 (Windham & Williams, 2012). Ochratoxin A is classified as possible human carcinogen and shows immunotoxic properties (Di Giuseppe et al., 2012). *Penicillium oxalicum* was identified in 29% of farmer-produced seed samples. The fungus can produce the mycotoxin secalonic acid D (Balasubramanian et al., 2000), which dramatically increases the chance of Cleft palate, a common birth defect (Dhulipala et al., 2005). Soil-borne *Aspergillus flavus*, producing aflatoxins, occurred in 38% of the farmer-produced seed samples. Chronic aflatoxin exposure can lead to stunted growth of children, liver cancer among adults and reduced life expectance, while acute aflatoxicose can be lethal within weeks (Wild & Gong, 2010).

The seed company and foundation seed samples were also heavily infected with various mycotoxigenic fungi (Table 1). In contrast with the International Institute of Tropical Agriculture (IITA) policy for seed deliveries, the foundation seed samples were not tested or treated for seed-borne diseases in order to illustrate how difficult it is to produce healthy seeds. The fact that mycotoxigenic fungi appear in seed company samples raises the question whether they test for mycotoxigenic fungi. This has serious implications for the seed-producing farmers in the informal SS that use seed companies to access new varieties or replace poor quality seed. If their input seed is already heavily infected, it gives farmers virtually no chance to produce healthy seeds.

Our results of maize seed were compared with maize grain destined for consumption. Several studies collected maize samples from local markets in African countries, and analysed fungi infection. The dominance of *F. verticillioides* infection in Nigerian maize is confirmed in these reports (Adejumo et al., 2007). Evidence from south-west Nigeria showed maize grain infection frequency of 89.3% and infection incidence of 49.4% for *F. verticillioides*, while infection incidences of other identified fungi varied from 1.3-14.7% (Bankole & Mabekoje, 2004). The seed samples of the current research showed similar results with high infection frequency (100% of farmer produced seed) and incidence (55.2%) for *F. verticillioides*, in combination with low infection incidences for all other identified fungi. The list of identified fungi differed between south-west and northern Nigeria, probably due to environmental differences. Maize grain samples in South Africa showed similar infection levels of *F.*

verticillioides (identified in 87.5% of the samples), *A. flavus* (42.5%), *A. niger* (25.0%), and *P. oxalicum* (27.5%) (Chilaka et al., 2012). It appears that seeds have similar infection levels to maize grain for human consumption.

Relevance fungi

Although the seed samples were not tested for mycotoxins, it is highly likely that the identified fungi contain toxigenic strains. Toxigenic strains are able to produce mycotoxins, in contrast with atoxigenic strains. Results from Portuguese maize showed that 14% of the *A. niger* isolates could produce ochratoxin A and 39% fumonisin B (Soares et al., 2012), while a report of maize in Argentina showed that 25% of the *A. niger* strains was toxigenic (Magnoli et al., 2006). A search for atoxigenic *A. flavus* strains in Kenya showed that only 33% of the analysed isolates were atoxigenic, while the majority could produce aflatoxins (Probst et al., 2011). An experiment in Kansas, USA, could not identify any atoxigenic strains of *F. verticillioides*, and isolates with relatively low fumonisin production in vitro did not show a consistently low level of fumonisin production in vivo (Desjardins et al., 1998). All four cases have in common that atoxigenic strains were absent, or accompanied by toxigenic strains, leaving the risk of mycotoxin production. Another aspect to judge the relevance of the identified fungi is their ability to transmit the fungus from seed-to-seedling, the so called seed-borne pathogens. Seed-borne infections are an important infection source for *F. verticillioides* (Bacon et al., 2001). The other two *Fusarium* species, *A. niger* and *P. oxalicum*, are also seed-borne diseases, but *A. flavus* is most likely only a soil-borne disease (MacGee, 1988).

CONTROL

Control methods

The case study of maize seed in Nigeria showed that both formal and informal SSs struggle with infection by mycotoxigenic fungi (Table 1), but there are several control methods. Important control methods of the formal SS are seed health testing and breeding. Seed companies and foundation seed producers should test seed health in order to avoid that infected seeds are sold to farmers. However, seed companies may not have the infrastructure themselves, or cannot charge higher seed prices to cover seed health testing costs. Breeding programmes have reported sources of resistance against *A. flavus* preventing aflatoxin contamination (Kelley et al., 2012) and against *F. verticillioides* preventing fumonisin production, but varieties resistant to all

mycotoxigenic fungi are currently not available (Small et al., 2012). Insect resistance is also a valuable trait to avoid infection, because insect damage facilitates fungi to enter and infect maize plants (Munkvold, 2003). Farmers may easily adopt these varieties when combined with other beneficial traits like tolerance to low soil fertility and drought, resistance to the parasitic weed *Striga* spp., or higher market value of the product. Research is already going on to optimize the dissemination of improved varieties by the formal and informal SSs (Almekinders & Louwaars, 2002), and to increase adoption of improved maize varieties by smallholder farmers (Amaza et al., 2010) (Daniel & Adetumbi, 2006).

The growing season offers ample opportunities to farmers, seed companies and foundation seed producers to avoid infection by mycotoxigenic fungi. Seed treatment with fungicides might reduce infection with *A. flavus* and *A. niger*, and simultaneously raise germination (Saleem et al., 2012), but whether it can stop seed-to-seedling transmission of *F. verticillioides* is debated (Bacon et al., 2001). Another control method is foliar agrochemical application. Insecticides reduce insect damage to the plant, avoiding airborne spores of mycotoxigenic fungi like *F. verticillioides* to enter and infect the plant. A combination of insecticide and fungicide can reduce both infection with *F. verticillioides*, *Fusarium* ear rot incidence and fumonisin contamination (De Curtis et al., 2011). Although agrochemicals can raise yields and production quality, high costs and adverse health effects for farmers can hamper the adoption. Biological control agents form an alternative to agrochemicals. Pre-harvest application of *Bacillus subtilis* avoids fumonisin production, because the bio-control agent competes for the same niche as *F. verticillioides* (Bacon et al., 2001). Another biological control method is the application of atoxigenic strains of *A. flavus* to the soil surface. The atoxigenic strains colonise the soil, infect the plant and compete for the same niche with toxic *A. flavus* strains (Probst et al., 2011). A similar strategy might be feasible with atoxigenic *Fusarium* spp. strains to combat mycotoxigenic *Fusarium* spp. (Mogensen et al., 2011). Besides effectiveness against mycotoxins, biological controls lack additional benefits that lead to a financial incentive for farmers, which will most likely hamper adoption. Harvest and storage practices also form potential control options. Avoiding seed damage during harvest can avoid further fungi infection. Fast drying of maize after harvest to final moisture content < 14% is important to prevent fungal growth in storage, but can be difficult to achieve in humid areas with labour scarcity at harvest time. Creating optimal storage conditions with low humidity, protection against insects, and free of infected plant material can avoid new infection (Chulze, 2010).

Adoption constraints

Adoption of these control strategies is constrained by a lack of financial incentive. Farmers and consumers cannot discriminate between mycotoxin contaminated maize samples and a safe product by visible inspection (Abbas et al., 2004), making it highly unlikely that contaminated maize samples are sold for lower prices. So there is no financial incentive for farmers to invest in avoiding or reducing mycotoxin contaminations. Even if there was a clear financial incentive, socio-economic constraints could still hamper adoption by smallholder farmers, like happened for fertilizer application (Dimithe et al., 2002). Another aspect is the sustainable implementation of control measurements in the SSs. To inform millions of smallholder farmers in remote areas, and convince them to make financial investments in control methods might be the biggest constraint of the informal SS. The formal SS consists of a relatively small number of actors, but adoption may be constraint by weak government institutions, development and enforcement of regulation, and market failure.

Successful control strategies against infection by mycotoxigenic fungi combine effectiveness, additional financial benefits beyond fungal infection prevention, and easy and sustainable implementation in SSs. Breeding resistant varieties is the only control method that meets these three criteria (Table 2). Seed treatments, agrochemical spraying, avoiding seed damage at harvest and storage technologies only meet the two criteria effectiveness and additional financial benefits, but encounter important implementation problems in the SSs. Application of biological control or atoxigenic strains are only effective, but are difficult to implement in the SS and lack financial benefits to the farmers.

Integrated approach

We recommend an integrated approach to develop optimal control of infection by mycotoxigenic fungi. This approach consists of mycotoxigenic fungi and insect resistant varieties in combination with effective control measures that also have additional financial benefits beyond mycotoxin control. If mycotoxigenic fungi control is still insufficient, effective control measures without financial benefits can be applied too. The optimal mix may differ between regions and countries as a result of agro-ecological and socio-economic differences, in particular the type of mycotoxins, and the level of food contamination. Aflatoxin contamination might require the application of atoxigenic strains, for example the product Aflasafe, while fumonisin contamination might be controlled with the application of insecticides and fungicides. This strategy

might also be relevant for mycotoxin contamination in other crops. Further research is recommended to test combinations of control measurements for different climatic zones, crops and countries, and test their effectiveness, additional financial benefits, and sustainable implementation in the SS. Public research programs are recommended to breed mycotoxigenic fungi resistance into varieties popular among farmers, and to develop new methods to control infection by mycotoxigenic fungi.

CONCLUSIONS AND RECOMMENDATIONS

Mycotoxin contaminated maize is a known human health hazard in developing countries, but the potential of SSs to control this hazard seems neglected. Maize seed samples are heavily infected with mycotoxigenic fungi as illustrated by the Nigerian case study. A schematic overview of the formal and informal SS was presented to visualize the risks of mycotoxigenic fungi infection and mycotoxin contamination, as well as potential control measures (Figure 1). We recommend an integrated approach combining resistant varieties with other methods to prevent mycotoxigenic fungi infection, with special attention to sustainable integration in formal and informal SS. Further research is recommended to determine the optimal combination of control measures for different climate zones, socio-economic conditions and crops, and to breed varieties with resistance to all mycotoxigenic fungi.

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CHAPTER 6

General Discussion

The general discussion of this thesis starts with some remarks about the research design, followed by answers to the research questions. Subsequently I will discuss in depth the bottlenecks of seed quality in the informal seed systems, and methods of seed quality assurance. The discussion ends with some final conclusions and recommendations for further research.

RESEARCH DESIGN

The collection of seed samples in Borno and Kaduna states of Nigeria enabled the analysis of seed recycling. A selection of farmers in both states received foundation seed of improved varieties from the International Institute of Tropical Agriculture (IITA) between 2001 and 2009. Some farmers stopped seed production or changed variety, but most of these farmers continued with their own on-farm multiplication. The comparison between both states also allowed an evaluation of the project Promoting Sustainable Agriculture in Borno state. PROSAB provided foundation seed of improved varieties, trained farmers in seed production, and carried out field inspections at farmers' fields, while farmers in Kaduna state only received foundation seed.

Seed samples were collected from seed producing farmers, from seed companies and from IITA. In contrast to the IITA policy for seed delivery, samples obtained from IITA were not tested and treated for seed health. The performance of untreated foundation seed highlighted the difficulty of producing high-quality seed, even on an international research institute. The varieties were selected on the basis of availability of foundation seed and popularity among farmers. Unfortunately, only two retail outlets of seed companies offered the requested cowpea varieties, and only one outlet for maize, resulting in a poor representation of the private sector. As a consequence, the results of this thesis should not be used to judge the formal seed system, but rather as an evaluation of seed quality in the informal seed system. It shows the reality of the situation of smallholder farmers, who have different options: produce their own seed, buy from several neighbours, or buy from maximum one or two seed companies within one day travel distance.

The two crops selected for this research were cowpea and maize, representing two extremes in formal seed system involvement. The formal seed system (SS) primarily

produces seeds of hybrids and high value crops, because minor crops generate insufficient profit margins and turnover to cover all costs and realise a profit (Louwaars & De Boef, 2012). The reluctance of the private sector to produce seed of minor crops is not limited to Nigeria. A seed project in Malawi revealed that many smallholder farmers were actually willing to pay for seed of new bean varieties, but the private sector was still not eager to enter the market (Chirwa et al., 2007).

This research analysed seed health in the informal seed system for cowpea and maize. Although literature provides sufficient evidence of yield suppression due to seed-borne bacteria and fungi in cowpea (Bankole & Adebanjo, 1996) and maize (MacGee, 1988), the importance of seed health for smallholder farming can be debated. Final yield may be constrained more by physical and physiological quality of seed, genetic quality, or environmental constraints like poor soil fertility or inadequate rainfall. The original research setup intended to analyse the importance of different seed quality parameters on yield. Field experiments would determine the yield of the seed samples, which would also be tested for physical purity, physiological quality, infection with seed-borne bacteria, fungi and viruses. DNA fingerprinting with SSR markers would have given an indication of genetic quality. Unfortunately, virology testing, DNA fingerprinting and the field experiments of both crops failed due to equipment break downs and management problems, leaving me with a more restraint research objective.

RESEARCH QUESTIONS

Does the informal seed system underperform compared to the formal seed system in delivering high-quality seed?

Chapters 2-4 compared seed quality of the informal and formal seed systems for cowpea and maize. The results were not consistent between the two crops. The informal seed system did not underperform compared with the formal SS for cowpea. Germination and field emergence of farmer produced seed were not significantly ($P>0.05$) different compared to foundation and seed company samples. The informal SS even outperformed the formal SS for cowpea seed health. Seed company and foundation seed samples had on average higher infection frequencies for harmful seed-borne pathogens like *Pseudomonas syringae* pv. *phaseolicola*, *Botryodiplodia theobromae* and *Curvularia lunata*. Chapter 4 tested seed quality of the formal and informal seed systems of maize. The informal seed system had similar seed quality compared with foundation seed, but underperformed in comparison with seed company samples. Company samples had fewer off-types ($P < 0.01$) and higher

germination ($P < 0.01$) than farmer-produced seed. Seed company samples also had significantly ($P<0.05$) lower infection incidences for *Bipolaris maydis*, *Botryodiplodia theobromae* and *Curvularia lunata* compared to farmer-produced seed.

However, seed quality is a problem in both formal and informal seed systems. The National Agriculture Seed Council (NASC) of Nigeria set crop specific standards for certified seed. At least one of the NASC requirements concerning inert matter, off-types and germination was violated by 97% of the cowpea samples, including all seed company samples. All the maize seed samples from the formal and informal seed systems failed the NASC guidelines for certified maize seed, mostly due to the strict norms for off-types. Cowpea and maize seed samples were heavily infected with seed- and soil-borne pathogens, risking low germination, seedling mortality and high disease pressure after planting. Distribution of infected seeds over large distances can also facilitate the introduction of diseases to hitherto uninfested fields.

Does continued seed recycling affect physiological quality or seed health?

Chapters 2-4 analysed if seed recycling negatively affected physiological quality and seed health. Seed-producing farmers multiply their seed on-farm for several seasons without seed renewal, referred to as "seed recycling" or "on-farm multiplication". Seed recycling contributes to accumulation of seed-borne diseases in potato, resulting in yield losses (Gildemacher et al., 2009). Some farmers in Borno and Kaduna state received foundation seed between 2001 and 2009. Most farmers continued with on-farm multiplication of the seed, which enabled this research to investigate seed recycling effects up to nine subsequent seasons. Amaza et al. (2010) suggested that seed recycling was responsible for low seed quality, which led to the hypothesis that seed quality gradually decreases after every season of on-farm multiplication. However, continued on-farm multiplication had no negative effect on seed viability or field emergence of cowpea. There was also no relation between the number of on-farm multiplications and the number of pathogens per sample for cowpea. The effect of seed recycling on the percentage of infected seeds per sample was determined for the major seed-borne fungi of cowpea and maize. There was no relation between the number of on-farm multiplication and the percentage of infected seeds per sample for *Fusarium oxysporum* and *Macrophomina phaseolina* in cowpea. The seed health results of maize were consistent with cowpea. There were no significant ($P=0.15-0.99$) effects of on-farm multiplication on the infection incidences of *B. maydis*, *B. theobromae*, *C. lunata*, and *M. phaseolina* of maize seed. The seed health results were not consistent with seed potatoes in Eastern Africa, where seed-recycling led to higher infection incidences and

a higher number of different viruses identified per sample (Gildemacher et al., 2011). Difference in crop, pathogen and agro ecology between the two reports is an obvious explanation. An important aspect may be the success rate of seed-to-seedling transmission of the pathogen in a crop. Potato viruses may be more successful in infecting plants through infected seed potatoes in comparison to bacteria and fungi in infecting plants from cowpea and maize seeds.

From this research I conclude that seed recycling has no effect on physiological quality and seed health of cowpea and maize, but the effect on genetic quality was not analysed. Especially an outcrossing crop such as maize may change gradually after each season of on-farm multiplication. Farmers isolating seed production plots from other varieties experience an outcrossing rate of 0.4% per season at 35 m, while 100 m distance between the fields reduces this risk to 0.05% per season (Goggi et al., 2006). Seed production fields of smallholder farmers are in general smaller than 1 ha, which is too small to realize isolation with 100 m borders on their own field. So they have to convince farmers of neighbouring fields to plant other crops, or the same variety of the same crop. There are two remarks to be made about the importance of seed recycling in relation to genetic quality. First of all, the only data collected about genetic quality is the number of off-types in maize seed samples, which showed no significant (P=0.50-0.61) effect of seed recycling on the number of off-types. The high level of off-types in farmer-produced seed was explained by the fact that foundation seed also contained on average 2.6% (w/w) off-types. If farmers can sell seed with 2.6% off-types, it is unlikely that they bother to isolate their fields to avoid an outcrossing rate of 0.4%. Secondly, some seed-producing farmers use on-farm selection to maintain or even improve varieties. Mexican farmers selected maize plants in their field to maintain varietal characteristics that are important to them (Louette & Smale, 2000). Another report from Mexico described that improved maize varieties were “creolized”. Farmers planted the improved variety alongside local varieties to stimulate outcrossing with local varieties. The creolized varieties contain beneficial traits of improved varieties, but are better adapted to the low input levels of poor farmers (Bellon & Risopoulos, 2001). Overall, seed recycling appeared to be unimportant for seed quality of cowpea and maize. There is no evidence that seed renewal after a limited number of on-farm multiplications can raise seed quality.

Can seed systems contribute to the control of mycotoxigenic fungi infection?

Chapter 3 revealed that maize seed samples from the formal and informal SS were heavily infected with seed-borne fungi. Literature showed that some of these fungi can

produce mycotoxins. Mycotoxins are fungal-produced toxins that threat human and livestock health through contamination of maize or other commodities (Wild & Gong, 2010). All tested maize samples from the formal and informal seed systems were severely infected with the seed-borne fungus *Fusarium verticillioides*, with median infection incidences of 51 and 59% in 2009 and 2010, respectively. Seed-to-seedling transmission is an important infection source for this fumonisin-producing fungus (Bacon et al., 2001). Other mycotoxigenic fungi identified were *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium oxalicum*, which can produce aflatoxins (Wild & Gong, 2010), ochratoxin A (Di Giuseppe et al., 2012) and secalonic acid D (Balasubramanian et al., 2000), respectively. Chapter 4 argues that both the formal and informal seed systems have great potential to contribute to the control of mycotoxigenic fungi infection. The formal SS can breed mycotoxigenic fungi and insect resistant varieties, and can test their seed to avoid sale of infected seeds to farmers. Seed-producing farmers can choose a range of agronomic measures to avoid or reduce infection, including atoxigenic strains, agrochemical application at various stages, and optimal harvest and storage methodologies.
However, farmers lack a financial incentive to invest in mycotoxin control, because local markets cannot distinguish mycotoxin-contaminated from unaffected maize. Successful control strategies against mycotoxigenic fungi infection combine effectiveness, additional financial benefits beyond mycotoxigenic fungi prevention, and easy and sustainable implementation in SSs. Only breeding mycotoxigenic fungi resistance and insect resistance in already popular varieties meets all these three criteria. Agrochemical application and optimal harvest methodologies are both effective and provide additional financial benefits, but encounter implementation problems in the informal seed system. Application of atoxigenic *Aspergillus flavus* strains can avoid aflatoxin contamination of maize, but has no financial benefit to smallholder farmers. An integrated approach to develop optimal control to mycotoxigenic fungal infection is recommended. It should combine resistant varieties with control measures like agrochemical spraying and optimal harvest and storage methodologies.

Mycotoxigenic fungal infection in seed illustrates the main problems of seed quality in the informal SS; awareness, detection, production and storage methods, and incentive. Farmers may not be aware that seeds can contain mycotoxigenic fungi, and the health risks involved in mycotoxin contamination of their product (Wild & Gong, 2010). Farmers are unable to measure seed quality and detect either mycotoxins (Abbas et al., 2004) or mycotoxigenic fungi infection by visible observation. Farmers do not apply optimal production and storage methods to avoid infection (Wild & Gong, 2010).

Furthermore, farmers lack incentives to invest in control, because mycotoxigenic fungi infected seed or mycotoxin contaminated grain can still be sold for the same price as unaffected product.

A similar story is valid for other seed quality aspects, for example physiological quality of cowpea. Farmers are not aware that high quality seed enables them to reduce seeding rates substantially. Chapter 2 showed that germination after 2 days correlates well with field emergence, but farmers are not used to test that. Farmers do not know that production methods like seed cleaning enhance germination substantially (Asiedu et al., 2003), and that Purdue Improved Cowpea storage (PICS) bags are the best way to store seeds. There is also no incentive, because seed cleaning reduces the total amount of seed without a price increase, and in fact, low quality seed can be sold too.

BOTTLENECKS FOR SEED QUALITY IN THE INFORMAL SEED SYSTEM

Chapter 2-4 revealed that the informal seed system struggles with low seed quality, but that seed-recycling is not responsible for that. There are five major bottlenecks of seed quality in the informal seed system: awareness, detection, production methods, storage, and incentives. These elements are discussed in detail below.

Awareness

Farmers are not fully aware of all seed quality aspects, or the benefits of high seed quality. Sorghum farmers in Ethiopia planted 3-6 times the seeding rates recommended by agricultural research organisations (McGuire, 2007). High seeding rates compensate for low field emergence, which in fact is a response to low physical and physiological quality and poor seedbed conditions. Chapter 2 showed that germination speed of cowpea correlates well with field emergence. Low physical and physiological quality can be partly compensated by increasing seeding rates, but requires more seed. On top of that, heterogeneous seed lots also have more intraspecific competition leading to lower yields compared with homogeneous seed lots, as was reported for maize in Brazil (Mondo et al., 2013). An experiment in Greece showed that fast emergence of maize seeds and vigorous growth are also advantages in the competition with weeds (Travlos et al., 2012). Many seed-producing farmers are not aware of the importance of seed health. Planting cowpea seeds infected with seed-borne pathogens reduces germination, increases seedling mortality, and causes high disease pressure in the growing season (Zaidi, 2012). Soil-borne pathogens may even infect fields that have not been infested before, resulting in higher

disease pressure in the subsequent seasons. The importance of seed- and soil-borne pathogens varies widely between agro-ecologies, crops and the level of resistance in varieties, as illustrated by the following examples. Improving seed health of seed potatoes in Kenya increased yields by 30%. Instead of post-harvest selection, seed potatoes were selected pre-harvest from healthy looking mother plants (Schulte-Geldermann et al., 2012). Seed-borne fungi infection of millet samples was hardly a problem in the drought ecology of Niger (Ndjeunga, 2002).

Incentives

The best incentive for seed-producing farmers is a higher profit of seed production compared with grain production, which requires a higher seed price compared with grain. Hybrid maize seed from American seed companies cost 438% more compared to average grain prices (van Roekel & Coulter, 2012). The situation for the informal seed systems in African countries is different. Prices for millet seed equalled grain prices in Niger (Ndjeunga, 2002). In Kenya, seed potatoes were 67-124% more expensive than ware potatoes, while the price of seed potatoes in Uganda was 145-229% higher compared to output prices. These seed prices were still too high for smallholder farmers. Economic analysis, based on expected yield increase of high quality potato seed, only justified a price difference up to 54% (Gildemacher et al., 2009).

Furthermore, measures to achieve high seed quality increase the seed production costs. Additional costs can include higher input costs for foundation seed and agrochemicals, additional labour, and seed testing costs. The production losses originating from removing diseased and off-type plants in the field, seed cleaning, and rejection after seed testing should also be considered as costs. Furthermore, many seed producers fail to sell all seed produced. Individual farmers report that up to 75% of their seed production is eventually consumed by their own family, or sold as grain. On top of that, social circumstances force farmers to give considerable amounts of seed for free to neighbours, relatives or friends. Smallholder farmers in Ethiopia received on average 20% of their sorghum seed for free, which came almost entirely from the informal seed system (McGuire, 2007). That may explain why seed producers are very reluctant to make additional investments or to accept production losses to raise seed quality.

Low seed prices have three major advantages to smallholder farmers. First, low seed costs reduce the financial loss of seed failure. Environmental conditions can lead to crop failure, forcing farmers to acquire new seed to plant again (McGuire, 2007). The

risk of losing seed increases when farmers plant very early in the rainy season in an attempt to get an early harvest, and benefit from higher prices. Secondly, low seed prices enable poor farmers to buy sufficient seed. Most smallholder farmers have very low purchasing power to buy inputs and additional labour, and have to deal with cash-flow problems. Out-of-pocket costs are limited as much as possible, whereby investments for seed have to compete with household expenses. The third advantage is that low seed costs enable farmers to allocate more money for investments in other inputs, and create an optimal combination of inputs to maximize yield at their own field conditions (Vanlauwe et al., 2010), as illustrated by an economic analysis in Zimbabwe. Smallholder maize farmers, struggling with high prices for hybrid seed, were advised to buy Open Pollinated Variety seeds and to invest the savings in additional fertilizer or labour (Pixley & Bänziger, 2002). Purchasing seed instead of planting farmer-produced seed should not only lead to higher benefits than the additional costs, but it should also result in a higher return on investment than on fertilizer and labour investments.

Detection

The difficulty for farmers to detect seed quality differs strongly between the different aspects. Physical quality can be determined with visible observation, while most aspects of physiological quality can be determined with simple germination tests. Chapter 2 showed that cowpea field emergence correlated well with germination after 2 days, which can be easily determined by farmers themselves. Seed health and genetic purity require more advanced techniques. The International Seed Testing Association (ISTA) described a range of seed testing methods in detail. Seed health and genetic purity testing require access to equipped laboratories, chemicals and qualified staff (Jones, 2008). However, smallholder farmers do not have access to seed testing infrastructure, and have to rely on simple methods like seed cleaning and germination tests. This has severe implications for the informal SS. Seed-producing farmers are unable to claim a higher seed price for better seed quality without any proof for that. Potential seed buyers can only rely on the reputation of the seller to avoid purchase of poor quality seed.

Storage

Storage is important to maintain seed quality achieved in seed production, and can affect all aspects of seed quality. High moisture content of the seed, storage temperature and storage time contribute to seed ageing, which reduces the viability of

the seed (Ellis & Auma, 1980). Chapter 2 showed that cowpea stored in the PICS bags had significantly ($P<0.05$) higher field emergence than traditional polybags. PICS bags provide airtight storage, preventing storage pests to damage cowpea seeds or grains (Moussa et al., 2011). Storage is also important for seed health and mycotoxin contamination. Storage conditions should be clean to avoid carry-over infection from infected plant or seed material from previous seasons. High moisture content and high relative humidity enhance fungi growth and mycotoxin contamination in stored maize (Bennett & Klich, 2003).

Storage is also important in relation to seed testing, when testing date and planting date are far apart. Seed samples may lose viability in storage, for example due to the combination of high temperature and high relative humidity. This can also affect seed samples bought from the formal SS, which farmers store in their own house between purchasing and planting time. External seed testing results may not be accurate if carried out months before planting, except for genetic quality.

Production methods

Instead of paying for output quality, farmers could also rely on efforts made for seed production. That is in fact the method used by the Promoting Sustainable Agriculture in Borno state (PROSAB) project. PROSAB trained and supported farmers to produce high quality seed, but did not test the actual seed quality. To maintain varietal purity, farmers received seed of improved varieties, and were advised to select an appropriate plot to avoid outcrossing, and to remove off-type plants (Amaza et al., 2010). To control seed health, farmers were advised to remove diseased plant and apply agrochemicals for disease control. However, Chapters 3 and 4 showed that most farmer-produced seed samples were heavily infected with seed-borne pathogens. An alternative method is positive selection. Kenyan farmers were advised to take seed potatoes from healthy looking mother plants, which were identified pre-harvest. These seed potatoes had 30% higher yield compared to post-harvest selection due to lower virus infections (Schulte-Geldermann et al., 2012). However, the high plant density and creeping plant stature of cowpea makes it difficult to separate and mark individual cowpea plants for positive selection. Furthermore, farmers cannot identify non-diseased plants if they cannot distinguish disease symptoms from water stress symptoms, which also turn leaves yellow.

Extension workers carried out field inspection for PROSAB to check if farmers followed the seed production protocols, and gave farmers a certificate which they could show to customers. Chapter 2 revealed that seed-producing farmers from Borno

state produced higher quality seed samples than farmers in Kaduna state, who did not benefit from the project. The research also showed that the physiological quality of cowpea can be further increased with sufficient seed cleaning and proper storage technologies. However, it remains unclear if farmers can cover field inspection costs after project funding stops, and if seed buyers value a certificate at seed purchase.

SEED QUALITY ASSURANCE

The best assurance of high seed quality is a combination of production guidelines and seed testing. Governments and NGOs developed four methods to protect farmers from low-quality seed, which are seed laws, certification, quality declared seed (QDS) and Truth-in-Labelling (TIL). Seed laws set standards for minimum quality, but most governments in developing countries do not have sufficient resources for law enforcement. Seed certification mostly co-exists with seed laws, but imposes additional requirements in terms of seed quality (van Gastel et al., 2002). Certification schemes have strict regulations about the seed production process and the final seed quality. Production protocols often include land requirements, use of input seed e.g. foundation seed, removal of off-type plants, processing, storage, packaging and labelling. Seed quality has to meet minimum standards concerning physical purity, germination and seed-borne infections of major pathogens (Louwaars, 2007). An independent not-for-profit organisation controls the seed production with field inspections and seed testing. Seed certification is the best way to assure high seed quality, but is not compatible with seed systems in developing countries (van Gastel et al., 2002).

Two alternative control systems were developed to assure seed quality in developing countries; QDS and TIL (van Gastel et al., 2002). QDS requires that seed producers are nationally registered, only produce seed of officially released varieties, and meet the standards for seed production and seed quality of the government. In contrast to seed law, the government agrees to test at least 10% of the production fields and 10% of the seeds lots sold (FAO, 2006). Law enforcement of seed laws is not bounded to a minimum number of field inspections and seed testing. TIL has no minimum quality standards for seed, but only requires external seed testing and labelling of the test results (van Gastel et al., 2002). Changes in the Seed policy regulations in Bangladesh in 2013 recognized “truthfully labelled seed” as a special class of seed, whereby seed producers test and certify their own seed. New rules in 1998 still considered options for market inspections of seed quality, but responsible authorities did not have the manpower to execute nationwide controls of seed quality (Huda & Smolders, 2002).

The informal seed system in developing countries struggles with three aspects of these seed quality assurance systems. First of all, governments in developing countries fail to carry out their tasks properly, due to insufficient funds and qualified staff. Secondly, seed-producing farmers can't pay for external seed testing. Third, poor storage conditions can reduce seed quality after seed testing.

CONCLUSIONS AND RECOMMENDATIONS

For cowpea and maize in Nigeria it is recommended that seed quality in the informal SS is improved through a combination of the PROSAB approach and TIL, without permanent government interference and external seed testing. All farmers, seed sellers and buyers, have to learn the importance of seed quality, have to learn basic seed testing skills like physical purity and germination speed, and learn to interpret the results. Seed-producing farmers are trained in the production and storage of high quality seed. They have to test for germination and physical quality themselves, and label the seed bag with the test results and date. Farmers buying seed can re-test it to verify the results.

The formal seed system should be supportive to the informal SS. NGOs, agricultural research institutes and extension workers can teach the informal SS to adopt this system, and provide foundation seed of new varieties for seed renewal. Seed companies can also offer new varieties, try to offer better quality seed, or sell hybrids. Further research is required to develop and implement this system in different countries, agro-ecologies and crops. Minimum standards for seed quality have to be developed per crop and agro-ecology, while different countries and crops require different methods to reach and teach farmers. Furthermore, methods have to be developed that enable farmers to test for seed health themselves, or to raise seed health quality in production.

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SUMMARY

Seed is a crucial external input for agricultural production. Improving the availability of high-quality seed of well-adapted varieties can boost agricultural productivity, leading to higher farmers' income, reduced poverty and improved food security. Improved varieties are introduced through the formal seed system, which consists of all formal institutions and private companies involved in breeding, varietal registration, seed multiplication, quality control and seed dissemination. The formal seed system fails to provide small quantities of seed to remote areas at affordable prices. Approximately 80% of the smallholder farmers in Africa depend for their seed on the informal seed system, consisting of farmers involved in selection, production and dissemination of seed. The lack of overhead, distribution and seed testing costs enables seed-producing farmers to offer seed for relatively low prices compared to seed companies, but the flip side is the risk of poor quality seed. Seed-producing farmers multiply their seed on-farm without frequent seed renewal, referred to as seed recycling. Seed recycling may lead to low seed quality, which negatively effects yield. Favourable traits of improved varieties are lost due to outcrossing with local varieties, but seed recycling may also reduce physiological quality or seed health. Literature shows that seed recycling in potato leads to low seed tuber quality, while open pollinated maize varieties could be multiplied on-farm without yield depression. This research analysed the effect of seed recycling on physiological quality and seed health of cowpea and maize.

In this research seed samples of cowpea and maize were collected from the formal and informal seed systems in Nigeria, i.e. from seed-producing farmers, from seed companies, and from the International Institute of Tropical Agriculture (IITA). A survey among the seed-producing farmers delivered information about the number of on-farm multiplications, a parameter for seed-recycling. Physical quality, physiological quality and seed health of the seed samples were determined to answer the following research questions:

1. Does the informal seed system underperform compared to the formal seed system in delivering high-quality seed?
2. Does continued seed recycling affect physiological quality or seed health?
3. Can seed systems contribute to the control of mycotoxigenic fungi infection?

Chapter 2 analysed the physical and physiological quality of cowpea seeds produced by the formal and informal seed system, and the effect of seed recycling on these quality parameters. We carried out a survey among seed-producing farmers about their production and storage practices, and tested seed quality of samples from these farmers, from seed companies, and compared these to foundation seed. Field emergence of farmers' produced seed was not significantly different from that of foundation seed or seed company samples. Cowpea seed quality, however, was inadequate in both the formal and informal seed systems. Five out of six foundation seed samples, 79 out of 81 samples of farmers' seed, and six out of six seed company samples failed to meet standards for foundation and certified seeds of the National Agriculture Seed Council (NASC), the seed industry regulatory agency in Nigeria. Our findings suggest that it is worthwhile to invest in the informal seed system of cowpea.

Chapter 3 compared cowpea seed health of the formal and informal seed systems, and determined if seed recycling in the informal seed system affects cowpea seed health. A total of 45,500 surface sterilized seeds from 91 seed samples (farmers, seed companies and research) were tested for seed-borne bacteria and fungi by plating disinfected seeds onto an agar medium. The most commonly isolated plant pathogens were *Fusarium oxysporum* (69% of the samples), *Macrophomina phaseolina* (76%) and *Pseudomonas syringae* pv. *phaseolicola* (48%). The percentage of seeds infected per sample varied from 0.2 to 75.6%. On average, 8.8 species per sample were isolated from foundation seed, 9.2 from farmer-produced seed and 9.8 from seed companies' seed. No evidence was found that seed recycling in the informal seed system did lead to increased levels of seed-borne pathogens. In contrast to farmers, seed companies distribute seed over large distances, and therefore, potentially, form a larger threat for spreading diseases at large scale. Seed dressing should therefore be made mandatory for all seeds sold by seed companies.

Chapter 4 analysed seed quality of the informal seed system of maize to compare it with foundation and seed company samples, and to assess the effect of seed recycling on seed quality. A total of 49,500 seeds (99 samples of 500 seeds each) were tested for germination, off-types and seed health. The seed company samples had significantly higher germination (99.3%) than farmer-produced seed (97.7%), but not a single sample passed the requirements for certified seed of the NASC. Twelve seed-borne pathogens were identified including *Bipolaris maydis* (found in 45% of the farmer-produced samples), *Botryodiplodia theobromae* (97%) and *Curvularia lunata* (38%). All samples were infected with *Fusarium verticillioides*, with a median infection incidence of 59.3% (2009) and 51.2% (2010). Formal and informal seed system actors are recommended to improve seed cleaning and disease control.

Chapter 5 assessed opportunities to prevent mycotoxigenic fungi infection in maize seeds. A schematic overview of the formal and informal seed systems was presented to analyse their contribution to fungal infection and mycotoxin contamination in the maize value chain. Literature showed a range of control measures including resistant varieties, agrochemicals, atoxigenic strains, quick drying after harvest, and optimal storage methods. These control methods have to fit the situation of smallholder farmers in developing countries. Control methods should meet three criteria: effectiveness against mycotoxigenic fungi infection, additional financial benefits beyond mycotoxin control, and easy and sustainable implementation. Based on these criteria, an integrated approach is recommended including resistant varieties and other control methods, with an important role for seed systems.

Chapter 6 discussed the major findings of this thesis. The informal seed system did not underperform compared to the formal seed system for cowpea. For maize, the informal seed systems had similar seed quality compared to foundation seed, but underperformed in comparison to seed company samples. Yet, more than 97% of all tested samples failed to meet the Nigerian requirements for certified seed, showing that seed quality is problematic in both formal and informal seed systems. There was no evidence that seed recycling reduces seed quality of cowpea and maize seed samples, so frequent seed renewal will not improve seed quality of the informal seed system, while it will increase costs. Five major bottlenecks were identified that prohibit seed quality improvement in the informal seed system; awareness of seed quality, detection of low seed quality, production and storage methods, and incentive to invest in seed quality. I recommend a new quality assurance system based on capacity building in the informal seed system, which does not depend on external seed testing or permanent government interference. All farmers have to become more aware of the importance of seed quality, and learn basic seed testing skills and interpretation of seed testing results. Seed-producing farmers are trained in the production and storage of high quality seed. They should test germination and physical quality of their seed, and label the testing results on the seed bag. Buyers can re-test the seed to verify the results. Further research is required to develop and implement this system in different countries, agro-ecologies and crops. Furthermore, methods have to be developed that enable farmers to test for seed health themselves or to enhance seed health quality.

SAMENVATTING

Zaaizaad is een essentieel element voor agrarische productie. Een betere beschikbaarheid van zaaizaad van hoge kwaliteit en van verbeterde rassen leidt tot hogere opbrengsten, hogere inkomens voor de boeren en meer voedselzekerheid. Verbeterde rassen worden geïntroduceerd door het formele zaaizaadsysteem, dat bestaat uit instituten en private bedrijven gericht op veredeling, rassenonderzoek en registratie, vermeerdering, kwaliteitscontrole en distributie. Het formele zaaizaadsysteem is niet in staat om, voor lage prijzen, boeren in afgelegen gebieden van kleine hoeveelheden zaad te voorzien. Ongeveer 80% van de kleinschalige boeren in Afrika is afhankelijk van het informele zaaizaadsysteem, waarbij de selectie, productie en distributie van zaad door boeren wordt uitgevoerd. De afwezigheid van overhead, distributie en kwaliteitscontrole stelt boeren in staat om tegen veel lagere kosten zaaizaad te verkopen dan bedrijven dat kunnen. De kwaliteit van boerenzaad is echter onbekend. Boeren vermeerderen het zaaizaad verscheidene seizoenen achtereen zonder basiszaad te kopen; dit wordt zaaizaadrecycling genoemd. Basiszaad wordt doorgaans gebruikt voor de productie van gecertificeerd zaaizaad, en derhalve zijn de kwaliteitseisen voor basiszaad hoger dan die van gecertificeerd zaaizaad. Zaaizaadrecycling kan leiden tot lagere kwaliteit, hetgeen de opbrengst verlaagt. Raseigenschappen kunnen verloren gaan door kruisbestuiving; kruisbestuiving kan ook leiden tot een lagere fytosanitaire of fysiologische kwaliteit van het zaaizaad. Wetenschappelijke literatuur wijst uit dat zaaizaadrecycling in aardappel leidt tot lagere kwaliteit, terwijl de vermeerdering van niet-hybride maïsrassen door boeren niet met kwaliteitsverlies gepaard hoeft te gaan. Dit onderzoek analyseert het effect van zaaizaadrecycling op fytosanitaire en fysiologische kwaliteit van ogenboon en maïs.

Voor dit onderzoek zijn monsters van ogenboon en maïs verzameld van het formele en informele zaaizaadsysteem in Nigeria, namelijk van boeren, van bedrijven en van het "International Institute of Tropical Agriculture" (IITA). Het aantal seizoenen dat boeren hun zaaizaad hebben vermeerderd zonder nieuw zaaizaad te kopen is vastgesteld via interviews. Fysieke, fysiologische en fytosanitaire kwaliteit van de monsters zijn gemeten om de volgende onderzoeksvragen te beantwoorden:

1. Presteert het informele zaaizaadsysteem slechter dan het formele zaaizaadsysteem met betrekking tot de kwaliteit van zaaizaad?
2. Verlaagt zaaizaadrecycling de fysiologische en fytosanitaire kwaliteit van zaaizaad?
3. Kunnen zaaizaadsystemen bijdragen aan de bestrijding van mycotoxinen producerende schimmels?

Hoofdstuk 2 analyseert de fysieke en fysiologische kwaliteit van zaden van de ogenboon afkomstig van het formele en informele zaaizaadsysteem, alsmede het effect van zaaizaadrecycling op deze kwaliteitskenmerken. Middels interviews werden de specifieke productie- en opslagmethoden van de zaaizaad-producerende boeren in kaart gebracht. De zaaizaadkwaliteit werd getest en vergeleken met de kwaliteit van zaaizaad van bedrijven en basiszaad. De veldopkomst, het aantal zaden dat binnen 14 dagen na planten een kiemplantje ontwikkelt, was niet significant verschillend tussen door boeren geproduceerd zaaizaad enerzijds, en zaad van bedrijven en basiszaad anderzijds. De zaaizaadkwaliteit van de ogenboon was echter onvoldoende in zowel het formele als het informele zaaizaadsysteem. Vijf van de zes IITA monsters, 79 van de 81 monsters afkomstig van boeren, en alle zes monsters afkomstig van het zaaizaadbedrijven voldeden niet aan de kwaliteitsnormen van de "National Agricultural Seed Council" (NASC), het Nigeriaanse overheidsinstituut verantwoordelijk voor de zaaizaadmarkt. De resultaten uit dit onderzoek wijzen uit dat investeringen in het informele zaaizaadsysteem voor de ogenboon effectief kunnen zijn.

In **Hoofdstuk 3** wordt de fytosanitaire kwaliteit van zaaizaad van de ogenboon afkomstig van het formele of het informele zaaizaadsysteem vergeleken, en wordt vastgesteld of zaaizaadrecycling in het informele zaaizaadsysteem de fytosanitaire kwaliteit beïnvloedt. In totaal werden er 45.500 zaden van 91 zaaizaadmonsters (afkomstig van boeren, bedrijven en een onderzoeksinstituut) getest op de aanwezigheid van zaadgebonden schimmels en bacteriën. De meest voorkomende ziekteverwekkers waren *Fusarium oxysporum* (69% van de zaaizaadmonsters), *Macrophomina phaseolina* (76%) en *Pseudomonas syringae* pv. *phaseolicola* (48%). Het aantal zaden per monster dat geïnfecteerd was, varieerde van 0,2 tot 75,6%. Gemiddeld werden er 8,8 verschillende soorten ziekteverwekkers aangetroffen in monsters van basiszaad, tegen 9,2 en 9,8 voor respectievelijk boeren en bedrijven. Zaaizaadrecycling bleek niet te leiden tot meer infectie van zaadgebonden ziekteverwekkers. In tegenstelling tot boeren distribueren bedrijven zaaizaad over grote afstanden, waardoor de kans op verspreiding van plantenziekten door besmet zaaizaad toeneemt. Derhalve dient het preventief behandelen van zaaizaad verplicht te worden voor zaaizaad-producerende bedrijven.

In **Hoofdstuk 4** wordt de zaaizaadkwaliteit van maïs uit het informele zaaizaadsysteem geanalyseerd en vergeleken met zaaizaad van bedrijven en onderzoeksinstituten, om het effect van zaaizaadrecycling op de kwaliteit te onderzoeken. In totaal zijn 49.500 zaden getest op kieming, aanwezigheid van

afwijkende typen en fytosanitaire kwaliteit. De monsters van zaaizaadbedrijven hadden significant hogere kieming (99.3%) in vergelijking met zaad geproduceerd door boeren (97.7%), maar geen enkel monster voldeed aan de kwaliteitsnorm voor gecertificeerd zaaizaad. Twaalf zaad-gebonden ziekteverwekkers werden aangetroffen, waaronder *Bipolaris maydis* (gedetecteerd in 45% van de monsters geproduceerd door boeren), *Botryodiplodia theobromae* (97%) en *Curvularia lunata* (38%). Alle monsters waren geïnfecteerd met *Fusarium verticillioides*. Het percentage zaden per monster dat geïnfecteerd was met deze schimmel had een mediaan van 59.3% in 2009 en 51.2% in 2010. Alle partijen in het formele en informele zaaizaadsysteem worden geadviseerd om de fysieke en fytosanitaire kwaliteit van maiszaad te verbeteren middels ziektebestrijding en betere zaaizaadverwerking.

In **Hoofdstuk 5** worden de mogelijkheden geanalyseerd om infectie van maïs door mycotoxinen producerende schimmels te voorkomen. Een schematisch overzicht laat zien hoe het formele en informele zaaizaadsysteem bijdragen aan schimmelinfectie en mycotoxinen productie in zaaizaad van maïs. Wetenschappelijke literatuur beschrijft verschillende maatregelen om infectie te voorkomen, waaronder resistente rassen, biologische bestrijding, gewasbeschermingsmiddelen, snel drogen na de oogst, en optimale opslagcondities. Niet alle maatregelen zijn geschikt voor kleinschalige boeren in ontwikkelingslanden. Drie criteria zijn cruciaal voor het succes van de maatregel, te weten: effectiviteit tegen mycotoxinen producerende schimmels, financieel voordeel voor de boer, en eenvoudige en duurzame implementatie. Op basis van deze criteria wordt een pakket maatregelen geadviseerd, bestaande uit resistente rassen en andere maatregelen, waarbij rekening dient te worden gehouden met de lokale zaaizaadsystemen.

Hoofdstuk 6 vormt de algemene discussie van dit proefschrift, waarin de belangrijkste resultaten worden besproken. Het informele zaaizaadsysteem presteert niet slechter dan het formele zaaizaadsysteem met betrekking tot de ogenboon. Voor de zaaizaadkwaliteit van mais ligt dit anders. Hoewel het informele zaaizaadsysteem vergelijkbare kwaliteit heeft ten op zichte van basiszaad, is de kwaliteit van maïszaad van bedrijven beter. Echter, de kwaliteit van 97% van de geteste monsters voldeed niet aan de Nigeriaanse richtlijnen voor gecertificeerd zaaizaad. Hiermee wordt aangetoond dat zaaizaadkwaliteit een probleem is in het formele en het informele zaaizaadsysteem. Dit onderzoek heeft geen bewijs gevonden voor een negatief effect van zaaizaadrecycling op de kwaliteit van zaad van de ogenboon en van maïs. Derhalve is het onwaarschijnlijk dat het regelmatig vervangen van zaaizaad in het informele zaaizaadsysteem leidt tot een hogere zaadkwaliteit, terwijl het wel de kosten verhoogt. De discussie benoemt vijf elementen die wel invloed hebben op de kwaliteit, te weten:

kennis over zaaizaadkwaliteit bij boeren, detectie van lage kwaliteit door boeren, productie en opslag methoden van zaaizaad, en financiële prikkels om te investeren in zaaizaadkwaliteit. Ik adviseer een nieuw kwaliteitssysteem gebaseerd op kennisoverdracht in het informele zaaizaadsysteem, onafhankelijk van externe laboratoria en overheidsbemoeienis. Boeren testen zelf de kwaliteit van hun zaaizaad, en vermelden de resultaten op de verpakking. Afnemers kunnen het zaaizaad opnieuw testen om de informatie op de label te controleren. Hiervoor moeten boeren bewust worden van het belang van zaaizaadkwaliteit, leren welke maatregelen de kwaliteit kunnen verbeteren, en getraind worden om de kwaliteit zelfstandig te testen. Vervolgonderzoek is nodig om dit kwaliteitssysteem te kunnen implementeren in verschillende landen, klimaatzones en gewassen. Daarnaast is onderzoek gewenst naar methoden waarmee boeren eigenhandig de fytosanitaire kwaliteit kunnen bepalen, en naar methoden om deze kwaliteit te verbeteren.

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LIST OF PUBLICATIONS

Biemond P.C, Oguntade O, Stomph T.J, Lava-Kumar P, Termorshuizen A.J, Struik P.C, 2013. Health of farmer-saved seed of maize in north-east Nigeria.
Accepted by the *European Journal of Plant Pathology*.

Biemond P.C, Oguntade O, Lava-Kumar P, Stomph T.J, Termorshuizen A.J, Struik P.C, 2013. Does the informal seed system threaten cowpea seed health?
Crop Protection **43**, 166-74.

Biemond P.C, Stomph T.J, Kamara A, Abdoulaye T, Hearne S, Struik P.C, 2012. Are investments in an informal seed system for cowpea a worthwhile endeavour? *International Journal of Plant Production* **6**, 367-86.

PE&RC PhD Training Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- "Profitability and quality of farmer's seed production of cowpea and maize" (introduction part)

Writing of project proposal (4.5 ECTS)

- "Profitability and quality of farmer's seed production of cowpea and maize" (research setup part)

Post-graduate courses (4.2 ECTS)

- SAS course: applying SAS for diverse statistical methods (2010)
- DSSAT course on crop modelling software (2011)

Laboratory training and working visits (4.5 ECTS)

- DNA fingerprinting; CIMMYT (2011)

Invited review of (unpublished) journal manuscript (1 ECTS)

- Asian Journal of Agricultural Extension, Economics & Sociology: Salient factors in the slow utilization of PICS Bags (triple bagging) in Kano, Nigeria (2012)

Deficiency, refresh, brush-up courses (0.3 ECTS)

- Lab safety course (2010)

Competence strengthening / skills courses (1.5 ECTS)

- Career Orientation (CO) (2012)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.1 ECTS)

- PE&RC Seminar: Modelling photosynthesis and growth from cells to climate (2012)
- PE&RC Weekend (2013)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

- IITA R4D week (2008)
- IITA R4D week (2009)
- IITA R4D week (2010)

International symposia, workshops and conferences (5 ECTS)

- 5th World Cowpea Conference; oral presentation; Saly, Senegal
- 10th Int. Congress on Plant Pathology; Beijing, China (2013)

Lecturing / supervision of practical's/ tutorials; (2.4 ECTS)

- Course "Data collection in agricultural science"

CURRICULUM VITAE

Pieter Christiaan Biemond was born in Dordrecht, the Netherlands, on 8th October 1981. He completed his secondary school at the "Norbertus College" in Roosendaal in 2000, after which he started the programme Plant sciences of Wageningen University, Wageningen, the Netherlands. His master, with specialisation Plant breeding, included a four-months internship at the flower breeding department of Syngenta, and a six-months master thesis at the Laboratory of Plant Breeding of Wageningen University. The latter was titled "Starch of Maltose-O-acetyl transferase transformants as an alternative for chemical derivatives of starch". Furthermore, he did a traineeship in development economics to investigate the relation between real exchange rates and commodity prices, which included a six-weeks internship at the International Rubber Study Group (IRSG) and the International CoCoa Institute (ICCO), both in London, United Kingdom. After receiving his master degree in 2006, he joined the "Nederlandse Fruittelers Organisatie" (NFO) as a staff member for applied research. He was project manager of an EU-funded project on integrated pest management in fruit production. In October 2008, he accepted an "Associated Professional Officer" (APO) position to work as "seed systems specialist" at the International Institute of Tropical Agriculture (IITA) in Kano, Nigeria. His research focussed on seed quality of cowpea and maize, with special emphasis on farmer-produced seed. The APO project ended after three years, after which he joined the Crop Systems Analysis (CSA) group as an external PhD student to analyse the data collected in Nigeria, publish the results, and write this PhD thesis.

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